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## 1–01

### Regeneration of the rat tibialis anterior muscle is impaired despite induction of the SPARC-beta-catenin pathway during post-immobilization recovery

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**Background and aims:** The immobilization-induced tibialis anterior (TA) muscle atrophy worsens after cast removal concomitantly with changes in the extracellular matrix composition. SPARC is a matricellular glycoprotein involved in tissue response to injury and in stabilization of *f*nbeta-catenin, which induces muscle regulatory factors (MRFs) controlling muscle regeneration. We hypothesized that SPARC expression changed upon immobilization and could be involved in the worsening of TA muscle atrophy by altering muscle regeneration processes pending cast removal.

**Methods:** Wistar rats were subjected to hindlimb immobilization for 8 days (I8) or not (I0), and allowed to recover for 1 to 10 days (R1-10). Expression of SPARC, beta-catenin, and proliferative (i.e. MyoD and MyF5) or differentiation (i.e. myogenin) MRFs were assessed by Western blots and/or RT-qPCR during recovery of previously immobilized TA.

**Results:** SPARC mRNA levels increased only during recovery at R1 (+161 %) and R10 (+200 %), compared to I8 and I0. beta-catenin mRNA levels increased at I8 (+80 %) and R10 (+190 %), while protein levels accumulated from R1 to R10 (+350 to 400 %) in immobilized TA vs. I0. MyoD and MyF5 mRNA levels increased by 2–3 fold only at I8 and R1 in immobilized TA vs. I0. By contrast, myogenin mRNA levels decreased at I8 (−60 %) and R1 (−90 %), and increased at R10 (+100 %).

**Conclusions:** We report an induction of the SPARC-beta-catenin pathway associated with increased mRNAs of the proliferative MRFs (Myf5 and MyoD) in the recovering TA early after cast removal. The differentiation MRF myogenin was first largely repressed, but increased later on, when TA started to recover. Altogether, the data suggest that the TA tended to preserve muscle regeneration potential through induction of proliferative MRFs. However this process was poorly efficient presumably because of an alteration in satellite cell differentiation.

## 1–02

### Upregulation of genes involved in muscle protein breakdown coincides with downregulation of genes involved in the immunoproteasome in a cancer cachectic C26 mouse model

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**Background:** Cachexia is characterized by loss of muscle mass and is associated with complications like a reduced immune response. In cancer patients with low level of immunoproteasome expression, tumour-and infection-derived epitopes have been suggested to have a higher chance of escaping from immune surveillance. Here we present the C26 adenocarcinoma mouse model derived data on transcriptomics analysis of two highly interconnected systems: the muscle protein breakdown, ubiquitin-proteasome and the immunoproteasome pathways.

**Methods:** Male CD2F1 mice, aged 5–6 weeks, were randomly divided into a control (C) or a tumour-bearing group

(TB). At day 20 after tumour inoculation, body composition, muscle-weight and muscle-protein-breakdown was determined by ex-vivo incubation of mTibialis followed by HPLC detection of 3-methyl-histidine in the organ bath. The mGastrocnemicus was used for pooled transcriptomics using Affymetrix chips. Expression of genes that were highly up or downregulated were confirmed with real-time PCR.

**Results:** In TB animals, the proteasome pathway was significantly upregulated as a result of the upregulation of the different genes of this pathway (Ubiquitin(2.0), PSMA2(1.9), PSMA4(2.6), PSMA7(2.4)). The upregulation of muscle specific E3-ligases Murf-1 (3.96) and Atrogin (3.62) indicated elevated muscle-protein specific breakdown occurring. This was confirmed by real-time PCR and by an increased ex-vivo 3-methyl-histidine excretion of the mTibialis. Additionally, we observed that the MHC-class I antigen presentation pathway (immunoproteasome) was significantly downregulated as a result of downregulation of several key regulator genes (BSMB8(−1.9), BSMB10(−1.8), PSME1(−1.9), TAP1(−2.1) and MHC-I(−1.4)). The confirmation of these data with real time PCR is ongoing.

**Conclusions:** In the presented study two phenomena occur at the same time: the ubiquitin-proteasome pathway leading to muscle protein breakdown is upregulated, while at the same time the immunoproteasome pathway is downregulated. It needs further investigation if these two pathways are causally related and generally changed in the same way throughout the body and if this might in part explain the relation of cachexia to impaired immune function.

### 1–03
### The deuterium oxide (D2O) stable isotope tracer is a powerful tool for monitoring short-term changes in skeletal muscle protein synthesis at rest and in response to exercise

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**Background and Aims:** The ability to quantify muscle protein synthesis is key for understanding the aetiology of muscle atrophy (e.g. ageing, cancer) and for defining effective countermeasures. Application of amino-acid (AA) tracers necessitates bed-rest, I.V lines and controlled laboratory settings, restricting studies to <12 h. The reintroduction of D2O tracer has been suggested to overcome these limitations. We tested the efficacy of D2O for measuring muscle anabolism in free-living volunteers over 8-days, a period unfeasible for AA tracer use or to detect changes in mass.

**Methods**: Eight young men (22±3.5y) undertook one-legged knee-extensor resistance exercise over 8-d (4 bouts of 8–10 repetitions at 80 %1-RM every second day to yield internal 'rest' and 'exercise' comparisons) with Vastus Lateralis muscle biopsies taken bi-laterally at 0, 2, 4 and 8-d. After day 0 biopsies, subjects consumed a D2O bolus (150 ml; 70Atom%); saliva was collected daily. Synthesis rates of myofibrillar (MPS), sarcoplasmic (SPS) and collagen (CPS) protein fractions were calculated as FSR(%/d)=[(APEAla)]/[3.7×(APEBW) × t] × 100 (APEAla = deuterium enrichment of protein bound alanine, measured by GC-Pyrolysis-IRMS; APEBW = body water enrichment; measured using TC-EA-IRMS, 3.7=mean number of deuteriums per alanine).

**Results**: Body-water was initially enriched at 0.16–0.24 APE and decayed at ~0.01 %/d. In the rest-leg, MPS was: 1.45± 0.10 %/d, 1.47±0.06 %/d-, 1.28±0.06 %/d at 2, 4 and 8-d respectively (~0.05–0.06 %/h, aligned with AA tracers). MPS was greater in the exercised-leg (2-d 1.97±0.13 %/d, 4-d 1.96 ±0.15 %/d; $P<0.01$), falling slightly at 8-d (1.61±0.08 %/d; $P>0.05$). CPS was slower than MPS, but followed a similar pattern over 8-days, with the exercised-leg showing a trend for greater FSR (2-d; 1.14±0.13 %/d vs. 1.45±0.15 %/d, 4-d; 1.13±0.07 %/d vs. 1.47±0.18 %/d, 8-d; 1.03±0.09 %/d vs. 1.40±0.11 %/d). SPS was significantly greater in the exercised-leg at 2 and 4-d.

**Conclusions**: D2O is a valid, minimally invasive "real-life" approach to quantify anabolism in multiple muscle protein fractions in free-living humans, before any detectable changes in mass.

### 1–04
### USP19 regulates differentiation of muscle cells

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**Background and aims**: Myogenesis is an ongoing process and defects in this process may contribute to muscle wasting or impaired recovery. We previously showed that the USP19 deubiquitinating enzyme is induced in various forms of atrophying muscle and that loss of USP19 protects muscle cells from atrophy. USP19 is expressed as two main isoforms – one ER localized and the other cytoplasmic. We tested whether USP19 isoforms modulate muscle cell differentiation in vitro by examining the effects of modulating USP19

isoform levels on muscle cell fusion and expression of key myogenic proteins.
**Methods:** USP19 was silenced in L6 muscle cells by transfection of siRNA oligonucleotides. Levels of USP19 were raised in L6 and C2C12 muscle cells by adenoviral mediated overexpression. Myogenesis was assessed by measuring the proportion of fused muscle cells following induction of differentiation and myogenic proteins were assessed by qPCR/immunoblotting. Catalytically inactive mutant or non-ER localized forms of USP19 were also tested.
**Results:** Silencing USP19 promoted muscle cell fusion and increased myotube diameter and expression of myogenin and myosin heavy chain. Overexpression of USP19 had the opposite effect of a decrease in both fusion and expression of myogenin and myosin heavy chain. Both catalytic activity and ER localization were required for these effects. A subset of myoblasts undergoes induction of ER stress and this induction promotes myoblast fusion. The ability of overexpressed USP19 to inhibit myoblast fusion coincided with a decrease in the induction of ER stress as assessed by the number of CHOP positive cells. USP19 expression increases during muscle cell differentiation and may serve to prevent excess myoblast fusion.
**Conclusions:** Fine regulation of USP19 expression is required for normal differentiation in vitro, is dependent on catalytic activity at the endoplasmic reticulum and may act through regulation of ER stress during myoblast fusion.

## 1–05
## Identification and characterization of USP19 inhibitors

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Muscle atrophy occurs as a consequence of disuse, cancer, fasting, glucocorticoid treatment and aging. Imbalance between anabolic and catabolic pathways results in loss of muscle mass and muscle atrophy. Therapeutic intervention thereby consists in increasing protein synthesis and slowing down protein degradation. However there are no effective treatments for muscle atrophy. USP19 is an ubiquitin specific protease and its mRNA and protein are increased in skeletal muscle under catabolic stimuli such as fasting, diabetes and tumor bearing. In addition, USP19 genetic depletion decreases protein degradation and increases myofibrillar and myogenin protein levels in L6 myoblast. Thus, USP19 is considered as a therapeutic target for treatment of muscle atrophy. However, there is no report for the inhibitor and screening system of USP19. Therefore, we have been exploring the high-throughput screening assay to identify small molecule inhibitors of USP19. To monitor the inhibitory activity of compounds, we used ubiquitin amino luciferin as a substrate of USP19 and measured luciferase activity by luminescence as an output of deubiquitinase activity. The counter screening assay was conducted to eliminate false-positive compounds having inhibitory activity of luciferase. As the result of screening using our chemical library (30,000 compounds), about 40 compounds were identified as hits of USP19 inhibitor with the criteria based on the results of pIC50 values (> 5.0) and counter screening negative. The derivatives of primary hit compounds improved inhibitory activity up to pIC50 >7. Currently, we have been confirming the cellular activity and deubiquitinase selectivity of those compounds to identify lead structures. In this study, we report the first time that the development of novel high-throughput screening system of USP19 and identification of USP19 inhibitors.

## 1–06
## MEK/ERK-dependent regulation of mTOR activity is mediated through TSC2/Rheb signaling in C2C12 myoblasts

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**Background and Aims:** The enhanced rates of protein synthesis overcoming to the protein degradation resulted in a net increase in cellular protein accumulation that leads to skeletal muscle maintenance. The mitogen-activated protein kinase kinase (MEK)/extracellular signal-regulated kinase (ERK)-dependent regulation of mammalian target of rapamycin (mTOR) activity and subsequent protein synthesis has been suggested, however, the exact molecular mechanisms underlying this regulation are poorly defined. The purpose of this study was to determine the regulatory mechanism in MEK/ERK-dependent pathway leading to mTOR activation in skeletal muscle cells.
**Methods:** C2C12 myoblasts were stimulated with phorbol 12-myristate 13-acetate (PMA) for 20 min (100 nM), co-incubated with/without mTOR inhibitor rapamycin (50 nM), MEK inhibitor U0126 (10 mM) or PKC inhibitor BIM-I (10 μM).
**Results:** Phosphorylation of the p70 ribosomal S6 kinase 1 (S6K1) at both Thr389 and Ser421/Thr424 sites, which show the functional activity of mTOR signaling, were markedly increased (6.21±0.44 fold increase in Thr389 and 12.53±0.59 fold increase in Ser421/Thr424 compared to the control, $p > 0.01$) in response to the PMA treatment. Conversely, inhibition of MEK/ERK-dependent pathway by BIM-I (upstream PKC-inhibitor) completely blocked PMA-induced activation of mTOR signaling. Phosphorylation of tuberous sclerosis complex 2 (TSC2) at S664 site (ERK-specific residue), an upstream regulator of mTOR, was also prevented by MEK/ERK

inhibition. Overexpression of Ras homolog enriched in brain (Rheb), a downstream target of TSC2 and an mTOR activator, was sufficient to activate mTOR signaling. We also identified that, in the absence of Rheb with using siRNA gene knock down, PMA-induced activation of mTOR was significantly prevented.
**Conclusions:** These observations demonstrated that the MEK/ERK-dependent activation of mTOR is mediated through TSC2 phosphorylation and its downstream target Rheb. It was also confirmed that Rheb is essential regulator of mTOR activation and enhanced protein synthesis in skeletal muscle cells.

## 1–07
## Acute endotoxaemia in humans increases muscle PDK4 protein expression and impairs nutrient-mediated increases in muscle PDC activity and whole-body glucose disposal

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Using a rodent model, we have previously demonstrated that lipopolysaccharide (LPS) induced-endotoxaemia is linked to the inhibition of pyruvate dehydrogenase complex (PDC) activity probably via the cytokine mediated up-regulation of pyruvate dehydrogenase kinase isoform 4 (PDK4) expression, resulting in reduced muscle carbohydrate oxidation and increased lactate accumulation (Crossland et al. J Physiol. 2010;588:1333–47). However, muscle fuel use and protein metabolism in rodents differ considerably from humans and therefore we aimed to determine the impact of LPS-induced endotoxaemia on whole-body glucose disposal and muscle PDC regulation in healthy volunteers under fed-state insulin clamp conditions.
Seven healthy volunteers (mean age of 27.8 years (18.1–30.5; 95 % CI) and BMI of 26.4 kg m−2 (22.9–29.9; 95 % CI)) participated in this ethically approved study. On 2 occasions separated by 2 weeks, subjects underwent a 4 h hyperinsulinaemic (40 mU m−2 min−1) euglycaemic clamp in combination with a primed mixed amino-acid (6 g.hr−1; 'fed state') infusion immediately following a bolus saline (control) or LPS (4 ng kg−1 body weight) infusion (order randomised). Arterialised-venous blood glucose concentration was measured at baseline and at 15 min intervals during the fed-state insulin clamp, whilst a vastus lateralis muscle biopsy was obtained at baseline and following the clamp for determination of muscle TNFα and PDK4 protein expression and PDC activity.
The increase in muscle TNFα and PDK4 protein expression during the clamp with LPS infusion was significantly greater relative to control (3-fold; $P<0.01$ and 2-fold; $P<0.05$; respectively). Muscle PDC activity and whole body glucose disposal during the clamp with LPS infusion were significantly lower relative to control (33 %; $P>0.01$ and 34 %; $P<0.001$, respectively).
Acute endotoxaemia in humans increases muscle protein expression of TNFα and its downstream target PDK4. This latter response is likely to be, at least partly, responsible for the impairment of nutrient-mediated increases in muscle PDC activity and whole-body glucose disposal under these conditions.

## 1–08
## The p97/VCP ATPase is critical in muscle atrophy and for the accelerated degradation of most muscle proteins

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The p97/VCP ATPase complex facilitates the extraction and degradation of ubiquitinated proteins from larger structures. We therefore studied if p97 participates to the rapid degradation of myofibrillar proteins during muscle atrophy. Electroporation of a dominant negative p97 (DNp97), but not the WT, into mouse muscle reduced fiber atrophy caused by denervation and food deprivation. DNp97 (acting as a substrate-trap) became associated with specific myofibrillar proteins and its cofactors, Ufd1 and p47, and caused accumulation of ubiquitinated components of thin and thick filaments, which suggests a role for p97 in extracting ubiquitinated proteins from myofibrils during atrophy. DNp97 expression in myotubes reduced overall proteolysis by proteasomes and lysosomes and blocked the accelerated proteolysis induced by FoxO3, which is essential for atrophy. Expression of p97, Ufd1 and p47 increases following denervation, at times when myofibrils are rapidly degraded. Surprisingly, p97 inhibition, though toxic to most cells, caused rapid growth of myotubes (without enhancing protein synthesis) and hypertrophy of adult muscles. Thus, p97 restrains post-natal muscle growth, and during atrophy, is essential for the accelerated degradation of most muscle proteins.

## 1–09
## Inhibition of RANK/RANKL pathway greatly improves SERCA activity and force production in atrophic and dystrophic skeletal muscles

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**Background and aim:** Receptor-activator of nuclear factor-κB (RANK), its ligand RANKL and the soluble decoy receptor osteoprotegerin (OPG) are members of the tumor necrosis factor (TNF) superfamily that control osteoclast differentiation, activation and survival and consequently bone remodelling and osteoporosis. Although there is a clear association between osteoporosis and skeletal muscle atrophy/dysfunction, the existence and functional relevance of a particular biological pathway that regulates synchronously bone and skeletal muscle physiopathology is still unknown. The aim of this study was to test whether RANK/RANKL pathway is involved in muscle dysfunction following different muscle wasting conditions.
**Methods**: In vivo: contractile properties of EDL muscles were obtained following 14 days of denervation in mice specifically deficient in RANK skeletal muscle or in dystrophic mdx mice treated daily for 10 days with OPG [0.3 mg/kg], the natural inhibitor of RANKL. In vitro: C2C12 myotubes were stimulated with RANKL [100 μg/ml] for 15, 30, 60 or 120 min. The activity, ubiquitination and sumoylation of sarco/endoplasmic reticulum Ca2+ ATPase (SERCA) were determined in C2C12 myotubes and denervated and dystrophic muscles.
**Results**: RANK muscle deletion did not prevent atrophy but greatly reduced the losses of force production and SERCA activity in denervated EDL muscles. Force production of EDL muscles also increased by 140 % in OPG-treated relative to PBS-treated mdx mice while the treatment with OPG prevented the loss of SERCA1 protein content in dystrophic mice. Conversely, the stimulation of C2C12 myotubes with RANKL induced a rapid and massive ubiquitination and sumoylation of the fast-twitch skeletal muscle SERCA1 but not the slow-twitch skeletal muscle SERCA2.
**Conclusion**: Together, these data indicate that the deletion or blockage of RANK/RANKL pathway has inotropic effects and increases SERCA activity and expression in fast-twitch skeletal muscles; this opens promising new avenues for several atrophic and cachectic conditions in which fast-twitch glycolytic fibers are more vulnerable than slow-twitch oxidative fibers.

## 1–10
## Exercise capacity in patients with and without anemia and iron deficiency with preserved ejection fraction (HFpEF) compared to reduced ejection fraction (HFrEF). Results from the Studies Investigating Co-morbidities Aggravating Heart Failure (SICA-HF)

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**Background:** Anaemia and iron deficiency (ID) are important factors for muscle function and exercise capacity in patients with chronic heart failure (HF). Their interaction in HF remains to be defined.
**Methods:** A total of 174 patients with chronic HF were enrolled (mean ± SD, age: 65±10 years, 22 % female, NYHA class 2.3±0.6, body mass index 29.4±5.3 kg/m$^2$). After matching for age, 86 patients were found to have a left ventricular ejection fraction (LVEF) ≥40 % (HF with preserved ejection fraction, HFpEF) and 88 to have an LVEF <40 % (HF with reduced ejection fraction, HFrEF). Anaemia was defined according to World Health Organization criteria (Haemoglobin [Hb] <13 g/dL in men and <12 g/dL in women, ID was defined as ferritin <100 μg/L or ferritin <300 μg/L with transferrin saturation (TSAT) <20 %. Exercise capacity was assessed by spiroergometry (peak$VO_2$), hand grip strength was measured using a hand grip dynamometer.
**Results:** A total of 28 patients (33 %) with HFpEF and 22 patients (25 %) with HFrEF presented with anaemia ($p$ = 0.71). ID was present in 40 patients (45 %) with HFpEF and 48 patients (55 %) with HFrEF ($p$ =0.97). Using data from all patients, peak $VO_2$ was 19.5±4.7 in HFpEF vs. 16.5±4.7 mL/kg/min in HFrEF ($p$ =0.0004), hand grip strength was 37.6±11.8 vs. 38.1±12.7 kg, respectively ($p$ =0.81). Differentiating all patients according to iron status, patients with ID had peak $VO_2$ 17.1±5.1 vs. patients without ID 19.1±4.6 mL/kg/min ($p$ =0.02). Likewise, hand grip strength was significantly reduced in patients with ID (35.6±12.1 vs. no ID 40.2±11.9 kg, $p$ =0.02). Regarding presence or absence of ID, these findings could only be confirmed in patients with HFrEF (both $p$ <0.01), but not in patients with HFpEF (both $p$ > 0.7). Peak$VO_2$ in all patients with anaemia was 15.5±4.5 vs. 19.1±4.7 mL/kg/min in patients without anaemia ($p$ <0.001). Hand grip strength was 33.2±10.4 in patients with anaemia as compared to 39.8±12.5 kg in patients without anaemia ($p$ = 0.002). In contrast to ID, peak $VO_2$ was significantly different

in patients with HFpEF and HFrEF when anaemia was present as compared to patients without anaemia (both $p<0.02$). In terms of hand grip strength, anaemic patients with HFrEF presented with lower values than non anaemic patients ($p=0.01$). A trend was detected in patients with HFpEF in this regard ($p=0.07$).

**Conclusion:** Both anaemia and ID predict reduced exercise capacity and hand grip strength in patients with HF. The effect of anaemia is stronger than that of ID alone, and altogether the effects are more pronounced in patients with HFrEF but appear to be present to a lesser extent also in patients with HFpEF.

## 1–11
## Body composition in patients with heart failure and preserved ejection fraction and its effect on muscle strength and exercise capacity

Results are from SICA-HF: Studies Investigating Co-morbidities Aggravating Heart Failure

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**Purpose:** Heart failure (HF) is associated with changes in body composition that include obesity, muscle wasting, or cachexia. These may have significant effects on quality of life. The purpose of this study was to compare parameters of body composition between patients with HF with preserved vs. those with reduced ejection fraction (HFpEF vs. HFrEF).

**Methods:** A total of 204 outpatients with stable HF ($n=173$, mean ± SD, age: 65.4±9.4, 21 % female, New York Heart Association [NYHA] class 2.2±0.6, body mass index [BMI] 30.3±5.5 kg/m$^2$) and controls (31=X, age 61.6±10.8, 45 % female) participating in the Studies Investigating Co-morbidities Aggravating Heart Failure (SICA-HF) were enrolled. Eighty-five patients (42 %) presented with HFpEF, defined as clinical signs and symptoms of HF with left ventricular ejection fraction ≥40 %. Patients with HFpEF were further subgrouped by echocardiographic parameters into three groups (E/e' ≥15 [group A: $n=15$], ≥8 E/e' <15 [group B: $n=52$], and E/e' <8 [group C: $n=18$]). Body composition was analysed using Dual-energy X-ray absorptiometry.

**Results:** No major differences were detected between patients with HFrEF and HFpEF with regards to fat and lean mass. Patients with HFpEF had higher values of fat and lean mass than controls. Patients with the most severe diastolic dysfunction (HFpEF group A) showed significantly lower lean mass in different body compartments than patients in HFpEF group C (appendicular lean mass 20.5±6.9 vs. 26.6 ±4.6 kg, $p=0.005$, total 51.8±9.4 vs. 58.9±9.1 kg, $p=0.03$). Hand grip strength in both arms was significantly lower in HFpEF group A than in group C (right arm: 28.6±10.2 vs. 45.7±9.1 kg, $p<0.0001$, left arm: 26.0±9.9 vs. 40.5±9.6 kg, $p=0.0008$). Intermediate values were detected in group B. Spiroergometry showed a statistically significant difference in peak oxygen consumption between patients with HFrEF and HFpEF (16.6±4.6 vs. 19.1±4.9 mL/min/kg, $p=0.002$) and decreasing values of peak oxygen consumption with increasing degree of diastolic dysfunction (ANOVA $p=0.0002$).

**Conclusion:** Appendicular and total lean mass in patients with HFpEF is reduced in concert with increasing degree of diastolic dysfunction. Functional parameters like hand grip strength and peak oxygen consumption follow this pattern. Insight into the pathophysiological pathways may have therapeutic implications.

## 1.12
## Body composition profiles in relation to physical performance in patients with COPD

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**Background and aims:** The past decade an increasing number of 'metabolic' syndromes have been proposed to characterize abnormal body composition, reflecting complex interactions between (epi)genetics, lifestyle and disease triggers on muscle, bone and adipose tissue as typically reflected in COPD heterogeneity. Aim of this study was to characterize metabolic profiles in relation to physical performance of COPD patients based on commonly used criteria for abnormal body composition.

**Methods:** Body composition was assessed by Dual-energy X-ray Absorptiometry in 505 COPD patients (age: 63± 8.9 years; FEV1: 51±18.9 %). Low muscle mass was defined according to cut-offs for sarcopenia (skeletal muscle mass-index (SMI) <7.23 kg/m2 (M) and <5.67 kg/m2 (F)), and gender- and age-group specific fat free mass-index values (<10th and <25th percentile values for healthy subjects). Abdominal obesity was defined as android/gynoid %fat mass <1.0 (M) and <0.8 (F). BMI and osteoporosis were classified by the WHO criteria. Physical performance was assessed by

quadriceps muscle strength, 6 min walking distance, and cycle ergometry.

**Results:** The prevalence of low muscle mass ranged from 34 (10th percentile)-87 % (SMI). Nearly all underweight patients ($n$ = 74) had low muscle mass (81–100 %), 60 % osteoporosis, and generally decreased physical performance relative to other BMI categories. In normal weight patients ($n$ = 191), 74 % had a muscle mass <25th percentile which was also reflected in significantly lower physical performance. According to SMI 97 % had low muscle mass and 35 % had osteoporosis. Overweight and obese patients with low SMI (88 % and 54 %), were characterized by significantly lower physical performance; 19 % and 15 % had osteoporosis. Abdominal obesity was not only present in 95 % overweight and all obese patients, but also in 77 % of normal weight patients.

**Conclusion:** Body composition assessment is a prerequisite for personalized metabolic intervention strategies in COPD; there is a need for harmonization of definitions and reference values of abnormal body composition.

## 1–13
## Impact of peripheral tissue endothelial dysfunction on exercise capacity in patients with chronic heart failure

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**Background:** The impact of endothelial dysfunction [ED] on atherogenesis and increased cardiovascular risk is clearly established and has important implications for blood supply to the muscle and development of muscle wasting. Current assessment methods of ED are, however, complicated by invasive protocols or laborious Doppler ultrasound procedures and are often more strenuous for the elderly fragile patients in cachexia research. The aim of the study was to examine ED in patients with chronic heart failure (HF) by a novel non-invasive and easily applicable method. We aimed to assess the relationship of ED to exercise capacity and clinical status in chronic HF patients with and without cachexia.

**Methods:** We studied 81 patients with chronic HF [age 64± 11 years, 24 % female, body mass index [BMI] 28.6±5.5 kg/m$^2$, New York Heart Association [NYHA] class (I/II/III/IV, 4/41/24/3), left ventricular ejection fraction [LVEF] 36±11 %, $pVO_2$ 16.6±5.0 ml/min/kg, 6MWT 408.6±149.2 m (all mean ± SD)]. Endothelial dysfunction was assessed by non-invasive arterial tonometry [EndoPAT] using the reactive hyperaemia index [RHI]. RHI is defined as a ratio between the post- and pre-occlusion arterial tonometry signal of the index finger corrected for baseline vascular tone and for the signal of the non-occluded contra lateral arm. Exercise capacity was assessed by symptome limited treadmill spiroergometric exercise test (modified Bruce protocol) and 6 min walk test (6MWT). For comparison, we studied 21 healthy controls [CON] of similar age.

**Results:** Compared to controls (RHI 2.15±0.61), endothelial function was 16 % lower in chronic HF (RHI 1.82±0.41, $p$ = 0.004) and decreased stepwise with advancing disease severity (NYHA I + II/III + IV 1.89±0.37/1.63±0.33, ANOVA $p$ <0.005). RHI was more reduced in chronic HF patients with ischaemic aetiology than in non-ischaemic chronic HF (ischaemic chronic HF vs non-ischaemic chronic HF, 1.64±0.3 vs 1.96 ±0.5; $p$ <0.05) and more reduced in cachectic patients (RHI 1.72±0.36) compared to non cachectic patients (RHI 1.86±0.43, $p$ <0.05). In linear regression analyses lower RHI was associated with lower $pVO_2$ ($r$ =+0.30, $p$ <0.05) as well as lower 6 min walking distance ($r$ =+0.40, $p$ =0.01). 6MWT distance was reduced in chronic HF vs CON (408±149 m vs 556±100 m, $p$ < 0.001). In multivariable analysis the association between RHI and 6MWT distance was independent of age and LVEF($r$ =+0.50, $p$ <0.05).

**Conclusions:** Endothelial dysfunction as assessed by EndoPAT is predictive of reduced functional status and impaired exercise capacity in patients with chronic HF. Endothelial dysfunction may impact on development of poor muscle perfusion, particularly during exercise, and contribute to skeletal muscle wasting. Assessment of endothelial function by this novel non-invasive method is a simple and easily applicable method for the use in ambulatory and clinical settings, and can be applied in patients with and without cachexia.

## 1–14
## Insulin resistance in HFrEF differs from HFpEF – specific characteristics of glucose metabolism according to HF type

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**Background:** Insulin resistance (IR) is a common metabolic characteristic in the pathophysiology of chronic heart failure

(CHF) that relates to symptomatic status and to mortality. Differences in the pathophysiological profile of IR in patients with preserved left ventricular ejection fraction (EF) (HFpEF) and patients with reduced EF (HFrEF) are less well characterised.
The aim of this study was to evaluate characteristics of IR in HFrEF vs. diastolic HFpEF in comparison to healthy controls.
**Methods:** 20 HFrEF patients (age 65.2±10.3, BMI 25.9±4.4 kg/m$^2$, EF 30±10 %, peak $VO_2$ 18.4±4.1 ml/kg/min, E/E' 15.6±9.6, all mean ± SD) and 22 HFpEF patients (age 69.1±5.9, BMI 28.4±4.7, EF 62±8 %, peak $VO_2$ 19.5±5.1 ml/kg/min, E/E' 11.5±4.1) as well as 21 healthy controls (age 70.3±4.8, BMI 25.4±2.9 kg/m$^2$) were studied. Patients were all non-diabetic and were in stable ambulatory condition and on standard medical therapy for HF. HOmeostasis Model Assessment (HOMA) index was calculated as a measure of IR at fasting state (IR = fasting glucose × fasting insulin/22.5). Short insulin test (SIST) was used to assess insulin resistance within the physiologic range of insulin-glucose interaction. For SIST, a bolus injection of insulin (0.1 IE/kg body weight) was administered under standardised conditions and glucose profiles were analysed from minute sampling for 15 min.
**Results:** HOMA was elevated in patients compared to healthy controls, indicating IR in HFpEF and worse IR in HFrEF despite lower BMI in contrast to controls (HOMA 2.6±1.6 vs. 3.0±2.3 vs. controls: 1.5±1.2, respectively, ANOVA, $p<0.01$). Both patient groups showed higher fasting glucose levels compared to controls (HFpEF 107.4±10.5 vs HFrEF 102.4±8.8 vs. controls: 96.8±10.0, all mg/dl, $p<0.01$). The SIST showed a more sever IR over the physiologic range of insulin-glucose interaction in patients with HFrEF but not in HFpEF. Glucose levels remained highest in HFrEF patients after Insulin bolus (75.5±15.1 vs. HFpEF 66.2±13.3 vs. controls 56.7±15.0 mg/dl, $p<0.001$). Moreover, HFrEF patients had the lowest glucose response after insulin bolus (28±12 %, $p=0.0043$) while the glucose profile decreased similarly in patients with HFpEF (38±12 %) and in controls (40±14 %).
**Conclusions:** Sings of insulin resistance can be observed in non diabetic HF patients with both HFpEF as well as HFrEF. However, distinct differences in the insulin sensitivity characteristics between these types of heart failure become apparent by more advanced testing. Patients with HFrEF showed more severe insulin resistance.

**1–15**

## Statin myalgia does not reduce muscle strength, mass or protein turnover, but is associated with impaired dynamic muscle function and insulin resistance in older male volunteers

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**Background and aims:** We investigated whether statin myalgia was associated with impaired isometric strength and work capacity in older volunteers compared to age and sex matched controls, and whether any group differences were allied to dissimilarities in muscle mass, protein turnover and insulin sensitivity.
**Methods:** Nine healthy males with no history of statin use (control 70.4±0.7 years) and 9 males with statin myalgia (71.5±0.9 years; 4 fold greater muscle soreness, $p<0.001$) participated in this ethically approved study. Body composition was measured using DEXA, and isometric strength and work output during 30 maximal isokinetic contractions (90o/s) was measured in the knee extensors. Muscle protein synthesis (MPS) and leg protein breakdown (LPB) were determined during a two-stage euglyceamic insulin clamp; enabling fasted (insulin 5 mU.l−1 for 120 min) and fed (insulin 40 mU.l−1 and 6 g.hr−1 infusion of mixed amino acids for 120 min) state responses to be examined. Whole body glucose disposal was determined during the fed-state clamp, and muscle biopsies were obtained at baseline and after 120 and 240 min.
**Results:** Isometric strength and peak isokinetic work were no different between groups; however time to peak work was delayed in the statin group, such that work output over the initial 3 contractions was 19 % lower ($p<0.05$). LPB and MPS in the fasted and fed state were no different between groups. In keeping with this, lean body mass was no different between groups. Statin users had markedly greater muscle creatine kinase (CK) mRNA expression (89 fold, $p<0.001$) and reduced glucose disposal (control 2.9±0.46 vs. statin 1.7±0.23 mg.kg−1.min−1, $p<0.001$).
**Conclusions:** Lean body mass, protein turnover and strength in statin related myalgia were no different to control. Statin myalgia was associated with impaired dynamic muscle function and insulin resistance, possibly occurring secondary to muscle soreness and/or metabolic dysfunction (evidenced by elevated muscle CK and insulin resistance).

**1–16**

**Skeletal muscle wasting and cardiac structural changes in cirrhotic patients with preserved left ventricular ejection fraction**

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**Background and aims:** Muscle wasting is a systemic and generalized phenomenon in cachexia. Animal models of cachexia showed that cardiac atrophy [ie, left ventricular (LV) mass reduction] occurs concurrently with skeletal muscle wasting, but is unproven in humans.
Substantial degrees of cachexia have been shown in cirrhotic patients. We evaluated a population of patients with cirrhosis with computed-tomography (CT) based assessment of skeletal muscle and fat as well as - echocardiography-based measures of LV and left atrium (LA) dimension and LV mass index. This exploratory study was intended to reveal variations in skeletal muscle wasting and cardiac mass and structure in cirrhotic patients.
**Methods:** Clinical charts as well as echocardiography reports of 79 patients with cirrhosis listed for liver transplant with different underlying etiologies [54 male (68.3 %)] were investigated. We also measured adipose tissue, skeletal muscle, and skeletal muscle index by measuring CT-defined lumbar cross-sectional areas of the tissues. Skeletal muscle wasting was defined using previously published sex-specific cutoffs.
**Results:** All patients showed preserved LV ejection fraction, but in 39 (49.4 %) and 42 (53.2 %) patients, diastolic dysfunction and left atrium enlargement (LAE) were found respectively. Skeletal muscle wasting was present in 35 (44.3 %) patients. Diastolic dysfunction and LAE were not associated with skeletal muscle wasting. Left atrial enlargement was associated with increased visceral and total adipose tissue in females ($p<0.05$).
Skeletal muscle wasting ($p<0.01$), reduced LV mass index ($p<0.05$) and increased LA dimension ($p<0.05$) were associated with presence of ascites and hepatic encephalopathy (HE) and these effects were very prominent in men.
**Conclusion**: Skeletal muscle wasting is strongly associated with presence of ascites and HE in the patients suffer from end stage liver failure. Increased LA dimension and decreased LV mass index in severe features of liver failure seem to be an initial cachexia-induced alteration of cardiac structure.

**1–17**

**Echocardiographic and hemodynamic parameters and their relation to exercise capacity in patients with and without cardiac cachexia**

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**Introduction**: We aimed to assess left and right heart function and peripheral blood flow in relation to exercise capacity in patients with and without cardiac cachexia.
**Methods**: We prospectively investigated 128 patients with left ventricular ejection fraction ≤40 %, which were subgrouped as follows: New York Heart Association (NYHA) class II ($n=59$), NYHA class III without cachexia ($n=46$), and NYHA class III with cachexia ($n=23$). All patients underwent echocardiography, venous occlusion plethysmography, treadmill test and 6-minute walk test (6MWT). Low exercise capacity was defined as 6MWT distance below the lower quartile in all patients.
**Results**: Treadmill test was completed by 76.3 % of NYHA II vs. 58.7 % of non-cachectic NYHA III vs. only 30.4 % of cachectic patients ($p=0.001$) due to premature fatigue of cachectic patients. Cachectic patients had lowest exercise capacity (peak oxygen consumption: 18.3 [15.3–21.2] vs. 14.1[12.4–14.8] vs. 11.3 [8.5–13.2] mL/min/kg, respectively; 6MWT distance: 455 [403–538] vs. 388 [283–438] vs. 280 [211–451] m, respectively; both $p<0.001$).
When comparing NYHA class II vs. NYHA class III vs. cachectic patients we found a stepwise decrease in systolic *right* ventricular (RV) function (tricuspid annular plane systolic excursion 19 [15–23] vs. 16 [13–19] vs. 14 [13–15] mm, respectively; $p<0.001$) and an increase in right atrial pressure (RAP; >10 mmHg 6.8 vs. 33.3 vs. 61.9 %, respectively; $p<0.001$). Systolic and diastolic function of the *left* ventricle (LV) and peripheral blood flow were similar in non-cachectic vs. cachectic patients in NYHA class III. In multivariate logistic regression, reduced RV function (odds ratio [OR] 0.89, 95 % confidence interval [CI] 0.91–1.00, $p=0.049$), elevated RAP

(OR 1.2, 95 % CI 1.0–1.3, $p=0.02$), and diastolic LV dysfunction (OR 1.1, 95 % CI 1.0–1.1, $p=0.02$) were independent predictors of low exercise capacity.

**Conclusion**: Patients with cardiac cachexia displayed a higher degree of right heart failure and a lower exercise capacity compared to non-cachectic patients of similar NYHA class. Right heart failure and diastolic LV dysfunction were independent predictors of low exercise capacity in patients with and without cardiac cachexia.

## 1–18
## Sleep apnea syndrome: Body composition and exercise capacity

Results are from SICA-HF: Studies Investigating Co-morbidities Aggravating Heart Failure

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**Background:** Sleep apnea syndrome (SAS) is an increasingly recognized co-morbidity in patients with heart failure. The aim of this study was to analyse exercise capacity and the effects of body composition in patients with SAS with particular focus on obesity and muscle wasting.

**Methods:** A total of 86 out-patients with stable chronic heart failure (mean ± SD, age 66.7±10.5, 15 % female, body mass index [BMI] 29.2±5.8 kg/m$^2$, LVEF 35.9±12.8, NYHA 2.3± 0.7) and 13 controls (mean ± SD, age 68.4±12.7, 46 % female, BMI 24.6±3.4 kg/m$^2$) were enrolled in the Studies Investigating Co-morbidities Aggravating Heart Failure at the Charité Medical School, Campus Virchow-Klinikum, Berlin, Germany. Body composition was assessed using Dual-energy X-ray absorptiometry. A polygraphy was performed as screening test for detecting patients with SAS. Muscle wasting was defined as the appendicular muscle mass 2 standard deviations below the mean of healthy reference group of adults aged 18–40 years. We divided each of the patients and the controls groups according to Apnea/Hypopnea Index (AHI) into three subgroups (patients: [group A: AHI <5, $n=21$; group B: AHI 5–20, $n=28$; group C: AHI ≥20, $n=37$], controls: [group $A_1$: $n=7$, group $B_1$: $n=4$, group $C_1$:2]).

**Results:** Sleep apnea with an AHI value ≥20 was present in 37 patients (43.1 %) and was associated with lower LVEF (AHI ≥20/<20: 30 %/40 %, $p=0.0005$), higher values of NYHA (AHI≥20/<20: 2.3/2.2, $p=0.66$). Muscle wasting was present in 15 patients (17.4 %). Muscle wasting was not associated with increasing values of AHI, neither in patients with heart failure nor in controls (chi square $p=0.53$). Simple regression analysis showed that increasing AHIs were associated with increased values of BMI ($p=0.02$, $R=0.25$), hip- ($p=0.02$, $R=0.24$) and waist ($p=0.04$, $R=0.22$) circumferences. Values of 6- minutes-walk were negatively correlated with AHI values ($p=0.02$, $R=-0.25$).

**Conclusion:** This preliminary analysis shows that values of AHIs as indicators of sleep apnea syndrome are not associated with muscle wasting. Functional parameters like 6-minute-walk are inversely proportional to AHI. Further and larger studies are recommended for better understanding of possible changes in the body composition of patients with sleep apnea syndrome.

## 1–19
## Comparison of body composition assessment in patients with heart failure

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**Background:** Fat-free mass (FFM) is increasingly recognized as determining factor for functional status in patients with chronic heart failure (HF). It is therefore a promising target for physiologic and pharmacologic interventions to improve functional status and potentially outcome. In clinical and ambulatory settings, however, assessment of fat free mass is rarely performed and applicable methods are poorly established. The aim of this study is to compare two methods for measurement of FFM in patients with chronic HF.

**Methods:** A total of 100 patients with stable ambulatory chronic HF were enrolled [mean age ± SD 69±8 years, 22%female, NYHA (I + II/III + IV) 64/36, BMI 26.3± 3.2 Kg/m$^2$]. Additionally we measured 20 healthy persons of similar age and BMI [age 66±9 years, 55%female, BMI 25.0±2.7 Kg/m$^2$]. Body composition was measured with dual-energy-X-ray absorptiometry (DEXA), which is widely reported as reference method. Tissue distribution for fat tissue, fat free (lean) tissue and bone tissue was assessed for total body and separately for arms, legs, and appendicular mass (arms plus legs). In comparison to this reference method, FFM was assessed by Bioelectrical impedance analysis (BIA). Presence of peripheral oedema was meticulously recorded and taken into account.

**Results:** In chronic HF patients FFM measured by BIA ($FFM_{BIA}$ ± SD 53.7±11.1Kg) was strongly correlated to FFM measured by DEXA of total and regional FFM ($FFM_{totalDEXA}$ $r=0.93$, $FFM_{armDEXA}$ $r=0.84$, $FFM_{legDEXA}$ $r=0.83$ $FFM_{appendicularDEXA}$ $r=0.86$, all $p<0.0001$). In controls we found the same correlation between $FFM_{BIA}$ and FFM measured by DEXA of total and regional FFM ($FFM_{totalDEXA}$ $r=0.94$, $FFM_{armDEXA}$ $r=0.92$, $FFM_{legDEXA}$ $r=0.91$ $FFM_{appendicularDEXA}$ $r=0.93$, all $p<0.0001$). A total of 52 patients with CHF had oedema, but $FFM_{BIA}$ correlated with $FFM_{DEXA}$ in patients with and without oedema ($FFM_{DEXA\ total}$ in patients with oedema $r=0.9$ and $FFM_{DEXAtotal}$ in patients without oedema 0.7, both $p<0.0001$). Comparison of both methods using Bland-Altman plots confirmed a good concordance between both methods in both patients (SD 11.4) and controls (SD 9.7) although an increasing difference between methods in very high FFM values was observed.

**Conclusion:** BIA assessment provides reasonable good measure of fat free mass in comparison to the reference method (DEXA) in clinical stable heart failure patients regardless of the presence or absence of oedema. BIA is, in comparison to DEXA, much more suitable for fast and routine clinical application due to mobility, simple and affordable technical application without use of radiation. More consequent application of BIA seems a suitable tool to assess and monitor fat free mass.

**1–20**

## Handgrip strength as a robust marker of muscle wasting and functional status after acute stroke

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**Background:** Muscle wasting is a common observation in patients with ischemic stroke. The main clinical manifestations of wasting such as loss of muscle mass and loss of muscle strength should be detected early in order to prevent further decline. Hand grip strength is a simple isometric method for assessment of muscle strength of the upper extremities; is easy to apply at bed side and independent of physical performance. We studied hand grip strength as a metrical marker of muscle wasting in patients with ischemic stroke.

**Methods:** 101 patients with acute ischemic or haemorrhagic stroke (age 70±11y, BMI 26.7±5.7 kg/m$^2$, all mean ± SD) admitted to hospitalized early rehabilitation centre were studied. Base line (BL) physical examinations performed at admission (23±18 days after acute stroke, all mean ± SD) followed by follow up (FU) examinations (27±5 days) included functional assessment scores: Barthel Index [BI], modified Rankin Score [mRS], and Rivermead Motor assessment [RMA], hand grip testing, body composition analysis by bioelectrical impedance analysis (BIA) and blood samples collected after over night fasting.

**Results:** Hand grip strength of the weaker/paretic arm improved at discharge from the rehabilitation centre in 61 patients by 5.7±4.5 kg (+46 %), and hand grip strength of the stronger arm improved in 50 patients by 4.1±2.5 kg (11 %). Improvement of hand grip strength of the both arms was related to lean mass (BIA) at discharge (weaker arm $r=0.305$, $p<0.05$ vs. stronger arm $r=0.6$, $p<0.0001$). Hand grip strength of the weaker hand was associated with Barthel Index (admission: $r=0.430$, $p<0.0001$; discharge $r=0.330$, $p<0.01$), modified Rankin scale (admission $r=0.488$, discharge $r=0.435$, both $p<0.0001$) and Rivermead Motor Assessment score (admission $r=0.284$, $p=0.0337$, discharge $r=0.357$, $p=0.0045$).

**Conclusion:** Hand grip strength could be a simple and robust metrical marker for stroke-induced muscle wasting and to monitor rehabilitation progress in stroke patients.

**1–21**

## Association between muscle strength of head lifting, dysphagia, and malnutrition in Japanese elderly: possibility of sarcopenic dysphagia

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**Background and aims:** Sarcopenia in swallowing muscles may cause sarcopenic dysphagia in the elderly. In a systematic review, head lifting exercise was revealed to eliminate dysphagic symptoms, partly due to improvement of swallowing muscle mass and strength. The purpose is to assess the association between muscle strength of head lifting, dysphagia, and malnutrition in Japanese elderly.

Method: A cross-sectional study was performed in 386 elderly aged 65 years and older with dysphagia or suspected dysphagia. Muscle strength of head lifting was assessed by manual muscle testing. Severity of dysphagia was evaluated by Dysphagia Severity Scale (DSS). Nutrition status was assessed by Mini Nutritional Assessment Short Form (MNA-SF). Associations between muscle strength of head lifting, DSS, and MNA-SF were examined using Spearman rank correlation coefficient and logistic regression.

**Results:** There were 129 men, 257 women. Mean age was 83 years. The median Barthel Index score was 30 (5, 65) points. A total of 189 elderly (49 %) were able to lift their head by themselves. Based on DSS, 79 were normal swallowing function, 138 were dysphagia without aspiration, and 169 were dysphagia with aspiration. MNA-SF revealed that 175 elderly were malnourished, 171 were at risk for malnutrition, and 40 had a normal nutritional status. There were significant correlations found between muscle strength of head lifting, age ($r=-0.256$), DSS ($r=0.458$), MNA-SF ($r=0.331$), and Barthel Index ($r=0.54$). DSS, MNA-SF, and Barthel Index were independently associated with muscle strength of head lifting, after adjust for age and sex.
**Conclusion:** Muscle strength of head lifting is associated with dysphagia, and malnutrition. Malnutrition is common in Japanese elderly with dysphagia or suspected dysphagia. Sarcopenia in swallowing muscles might be responsible for the association between muscle strength of head lifting, dysphagia, and malnutrition, suggesting the presence of sarcopenic dysphagia in Japanese elderly.

## 1–22
## Assessment of oral cavity function and its clinical significance: Is ROAG valuable for sarcopenia screening?

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**Background and aims:** Oral cavity dysfunction is one of the key causes of malnutrition leading to sarcopenia. But sarcopenia itself may weaken the muscles involved in oral cavity function, and lead to more severe dysfunction of oral cavity and physical performance. The purpose of this study is to evaluate the relationship between oral cavity function and sarcopenia of skeletal muscle.
**Methods:** The observational prospective study (ID; UMIN000010925) involved 65 consecutively enrolled hospitalized elderly patients from Kumamoto Rehabilitation Hospital. Revised Oral Assessment Guide (ROAG) as assessment of oral cavity function, hand grip strength (HG), arm and calf circumference (AC and CC), body mass index (BMI) and MNA-SF, serum albmin level (Alb), Functional Independence Measure (FIM) and Fujishima's swallowing grade were investigated on admission. For statistical analyses Spearman rank correlation and multiple regression analyses were used.
**Results:** 65 patients (35 male) with mean age of 80.9 years were included. According to ROAG (score from 8 to 24), 23.1 % of subjects were at risk of moderate to severe oral cavity dysfunction (score >12) and 61.4 % were of slight dysfunction (score from 9 to 12). There were significant correlations between AC, CC, HG, BMI, FIM, Fujishima's swallowing grade and ROAG. Especially, correlation between AC and ROAG was quite strong ($R=-6.87$, $p<0.01$). In multiple regression analysis, ROAG and Alb was significantly associated with AC ($AC=27.86+ROAG\times(-0.8)+Alb\times2.6$, $R=0.753$, $p<0.001$).
**Conclusions:** Oral cavity dysfunction prevails among hospitalized elderly patients. Muscle mass, especially arm circumference, was strongly and negatively correlated to oral cavity function. As such, ROAG can be a clinically valuable tool for screening both for oral cavity function and sarcopenia.

## 1–23
## Higher prevalence of sarcopenia in older adults with type 2 diabetes

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**Aims**: Our aim was to clarify the association between type 2 diabetes and the risk of sarcopenia in older adults. **Methods**: In this study, 414 adults aged 65 years or older (144 patients with type 2 diabetes and 270 control subjects) were included. Body composition was measured by dual-energy X-ray absorptiometry. Sarcopenia was defined as the appendicular skeletal muscle mass/height2 (ASM/Ht2) or appendicular skeletal muscle mass/weight (ASM/Wt) of <2 SD below the sex-specific normal mean of the young reference group, or < lower 20th percentile of total body skeletal muscle mass/weight (TSM/Wt) from control subjects.
**Results**: Older men with type 2 diabetes showed significantly lower appendicular skeletal muscle mass than those without diabetes (19.5±3.5 kg vs. 21.0±2.8 kg, $P<0.001$). The prevalence of sarcopenia was consistently higher in older men with diabetes than those without diabetes defined by ASM/Ht2 (57.6 % vs. 41.5 %, $P=0.040$), ASM/Wt (23.7 % vs. 12.3 %, $P=0.046$), and TSM/Wt (49.2 % vs. 20.0 %, $P<0.001$). In older women with diabetes, the prevalence of sarcopenia was higher than those without diabetes by ASM/Wt (25.9 % vs. 15.0 %, $P=0.044$), and TSM/Wt (32.9 % vs. 20.0 %, $P=0.030$), but not by ASM/Ht2 (7.1 % vs. 8.6 %, $P=0.685$). The risk of

sarcopenia was about two to four fold higher in older adults with type 2 diabetes even after adjusting for age, body mass index, current smoking, and other risk factors.
**Conclusions**: In Korean older adults, type 2 diabetes is associated with sarcopenia.

**1–24**
**Sarcopenia in Ukrainian women of different age**

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The aim of this study is evaluating of body composition and frequency of sarcopenia in women depending on age.
**Materials and methods:** We have examined 8637 women aged 20–89 years (mean age – 56.7±0.14 years; mean height – 162.5±0.07 cm; mean weight – 73.5±0.16 kg). The patients were divided into two groups depending on age: 20–24 ($n$ = 143), 25–29 ($n$ = 209), 30–34 ($n$ = 271), 35–39 ($n$ = 326), 40–44 ($n$ = 419), 45–49 ($n$ = 794), 50–54 ($n$ = 1292), 55–59 ($n$ = 1534), 60–64 ($n$ = 1193), 65–69 ($n$ = 943), 70–74 ($n$ = 877), 75–79 ($n$ = 384), 80–84 ($n$ = 204) and 85–89 years ($n$ = 48). Lean and fat masses and total body, lumbar spine, femoral neck bone, forearm bone mineral density (BMD) were measured by DXA using a densitometer Prodigy, GE.
**Results:** We have found the significantly differences of fat and lean masses in women with age:- fat mass: 20–24 years – 18630.12 g; 25–29 years – 18630.12 g; 30–34 years – 19201.00 g; 35–39 years – 21528.15 г.; 40–44 years – 24611.77 g, 45–49 years – 2750.54 g; 50–54 years – 27501.54 g; 55–59 years – 29909.92 g; 60–64 years – 31600.27 g; 65–69 years – 33508.25 g; 70–74 years – 33155.54 g; 75–79 years – 32284.86 g, 80–84 years – 30595.53 g; 85–89 years – 30303.68 g; $F$ = 83.19; $p$ < 0.0000001; - lean mass: 20–24 years – 37271.57 g; 25–29 years – 37954.09 g; 30–34 years - 39019.72 g; 35–39 years – 39928.62 g; 40–44 years – 40929.67 g, 45–49 years – 41407.19 g; 50–54 years – 41936.27 g; 55–59 years – 42564.79 g; 60–64 years – 42519.73 g; 65–69 years – 41758.95 g; 70–74 years – 41233.77 g; 75–79 years – 41105.52 g, 80–84 years – 40308.00 g; 85–89 years – 38454.61 g; $F$ = 29.15; $p$ < 0.0000001. Frequency of sarcopenia in women aged 65 years and older was 7 % (women aged 65–69 years ($n$ = 943)–7.6 % ($n$ = 72), 70–74 years ($n$ = 877)–6.1 % ($n$ = 54), 75–79 years ($n$ = 384)–6.3 % ($n$ = 24), 80–84 years ($n$ = 204)–6.9 % ($n$ = 14), 85–59 years ($n$ = 48)–10.4 % ($n$ = 5).
**Conclusion:** Fat and lean masses were significantly decreased with age. The maximal accumulation of fat and lean masses was in women aged 50–59 years. Frequency of sarcopenia in women aged 65 years and older was 7 %.

**1–25**
**Prevalence of sarcopenia in community-dwelling Japanese older adults**

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**Background and aims:** Sarcopenia, the age-dependent loss of skeletal muscle mass, is highly prevalent among older adults in many countries. However, the prevalence of sarcopenia in healthy Japanese community-dwelling older adults is not well characterized. The aim of this study was to evaluate the prevalence of sarcopenia and to examine the association of sarcopenia with falls and fear of falling in community-dwelling Japanese older adults.
**Methods:** Healthy men (568) and women (1,314) aged 65–89 years participated in this research. For all participants, three measurements were taken: skeletal muscle mass measurement using bioelectrical impedance, 10 m at a usual walking speed, and handgrip strength. Sarcopenia was defined as the presence of both poor muscle function (low physical performance or low muscle strength) and low muscle mass.
**Results:** The prevalence of sarcopenia, determined using the European Working Group on Sarcopenia in Older People (EWGSOP)-suggested algorithm, in men and women aged 65–89 years was 21.8 % and 22.1 %, respectively. The prevalence of sarcopenia increased age-dependently, especially after 75 years in both genders. In the young old, the prevalence of sarcopenia was higher in women than in men; however, in those over 85 years, the prevalence of sarcopenia was lower in women than in men ($p$ <0.05). In addition, fall incidents and fear of falling were more prevalent in sarcopenic older adults than in non-sarcopenic older adults ($p$ <0.05).
**Conclusions:** These results suggested that sarcopenia is highly prevalent in community-dwelling Japanese older adults and is related to falls and fear of falling.

**2–01**
**Abnormal liver metabolism and pro-inflammatory responses are modulators of cardiac cachexia in a rat model of heart failure**

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**Background and aims:** Congestive heart failure (CHF) often accompanies cachexia, namely body wasting, which is a potential target of therapeutic intervention in cases of CHF.

However, its pathophysiology is poorly understood. To gain insight into the mechanism of cardiac cachexia, we have analyzed the change of hepatic metabolism since the liver plays a central role in the systemic regulation of catabolism and anabolism.

**Methods**: We examined energy metabolism in the liver of Dahl salt-sensitive rats, which show a transition from hypertension to CHF. We analyzed markers of cachexia in Dahl salt-sensitive rats which show marked hypertension with preserved systolic function at 11 weeks and CHF at 17–19 weeks of age. We also analyzed the change in hepatic metabolism associated with CHF using quantitative RT-PCR, Western blottings, metabolome analyses, and analysis of radio isotopes.

**Results**: In CHF rats, a failure to grow was observed and blood hepatic protein levels were decreased associated with increased blood pro-inflammatory cytokine levels, indicating that Dahl rats serve as a model of cardiac cachexia. Food intake was reduced, and blood sugar and insulin levels were decreased. Despite the apparent fasting condition, blood fatty acid levels were decreased and triglycerides levels were increased. In CHF rats, liver incorporated more glucose, the gene expression related to gluconeogenesis was decreased, the gene expression related to lipogenesis was increased, and the triglyceride content of the liver was increased. The paradoxical production of triglycerides synthesis in fasting rats was associated with a pro-inflammatory response in liver.

**Conclusions**: The cachexia was associated with abnormal hepatic metabolism that might work as a maladaptive response during the progression of heart failure when the body is losing weight and peripheral tissues need more substrates to maintain tissue homeostasis.

## 2–02
## Growth differentiating factor-15 (GDF-15) induces anorexia and cachexia in mice: a novel pathway for cachexia

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**Background**: Cachexia is associated with increased inflammatory markers and decreased survival in cancer. Also, elevated GDF-15 has been associated with poor prognosis in several cancer types and it is known to induce anorexia but its role and mechanisms of action in cachexia is not well-understood.

**Methods**: Human GDF-15 (hGDF-15) or vehicle were administered subcutaneously to male and female adult mice. Outcome measures included body weight, body composition by NMR, food intake, locomotor activity, and energy expenditure (EE) assessed by a comprehensive laboratory animal monitoring system (CLAMS) and muscle strength by handgrip dynamometry. Upon completion of the experiments, animals were sacrificed and hindleg muscles and mesenteric, epidydimal, perirrenal and inguinal fat pads were dissected.

**Results**: hGDF-15 administration when compared to placebo induced a significant decrease in body weight, food intake, total body fat and lean body mass. Analyses of CLAMS data showed that hGDF-15-receiving animals showed a decrease in locomotor activity, probably part of a sick behavior syndrome, and a decrease in energy expenditure when compared to placebo. Quadriceps, gastrocnemius and tibialis anterioris muscles and all fat pads were significantly decreased by hGDF-15 administration. This decrease in muscle mass induced by hGDF-15 was associated with a significant decrease in muscle strength.

**Conclusions**: GDF-15 induces significant weight, muscle and fat loss and weakness and decreased locomotor activity in mice. These changes are associated with decreases in food intake and an appropriate reduction in energy expenditure. This suggests that anorexia is at least in part responsible for the effects of GDF-15 in this setting. However, other contributing mechanisms may play a role by affecting directly target organs and are currently under investigation. This pathway may be a promising therapeutic target for cachexia, anorexia and other wasting disorders.

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## 2–03
## The characterization of the fertilized chicken egg proteome by accurate mass, high resolution mass spectrometry: therapeutic implications for the treatment of sarcopenia and cachexia

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**Background and Aims:** Many have argued that eggs are perhaps the most nutritionally dense substance known to man. We have established that fertilized chicken egg yolk contains significant levels of follistatin. The protein, follistatin, is a powerful antagonist of the myostatin-mediated inhibition of myogenesis. Accurate mass, high-resolution mass spectrometry has been applied to characterize the proteome of fertilized chicken eggs to gain further insight

into other proteins that may promote myogenesis thereby reversing sarcopenia and cachexia.
**Methods:** Dehydrated fertilized chicken egg yolk protein was solubilized. The disulfide bonds were reduced and alkylated and the resulting protein mixture was then digested with trypsin. This tryptic digest was separated on a nanoLC column, ionized using nanoelectrospray ionization with 1.2 kV and then analyzed using a Q Exactive bench-top Orbitrap mass spectrometer (Thermo Scientific). Tryptic peptide fragmentation spectra were searched against a Gallus gallus database using MASCOT software (Matrix Science).
**Results:** Inflammatory markers such as IL-6 and C-reactive protein (CRP) increase the risk of muscle strength loss. We have used pathway analysis software (Ingenuity Systems) to ascribe function to 268 Gallus gallus proteins that we identified using mass spectrometry with a 1.4 % false discovery rate. Pathway analysis of the fertilized egg yolk proteome revealed proteins associated with myogenesis along with anti-inflammatory functions. Hen egg lysozyme along with RGD peptides are examples of molecular entities with known anti-inflammatory properties that we identified in fertilized egg yolk powder using mass spectrometry which may help to reduce the risk of muscle loss.
**Conclusions:** Proteomic profiling studies of fertilized chicken egg yolk have identified proteins relevant to myogenesis along with anti-inflammatory properties. These results suggest that fertilized egg yolk may have therapeutic value in the treatment of sarcopenia and cachexia.
**References:**
1. Schaap LA, Pluijm SM, Deeg DJ, Visser M. Am J Med. 2006 Jun;119(6):526.e9–17.

## 2–04
## Increased expression of protein catabolism and ActRIIB pathway in a non-human primate (NHP) radiation-induced cachexia model

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**Background and Aims:** Radiation therapy has been associated with anorexia and weight loss, thus severely affecting patients' quality of life. Animal models of radiation-induced cachexia that recapitulate the clinical findings from human disease and enable testing of therapeutic drugs are urgently needed. Protein catabolism pathway and ActRIIB pathway have been shown to play important roles in cachexia. We aim to develop a non-human primate (NHP) radiation-induced cachexia model and study the molecular changes contributing to the disease.
**Methods:** We have developed NHP radiation models to assess the efficacy of radiation medical countermeasures. NHP were exposed to high dose radiation, and then were treated with either test or control articles. All animals received supportive care. Clinical parameters including complete blood counts, body weights and core body temperatures were recorded. Cageside observations were graded and recorded. Skeletal muscle tissues were collected at the time of euthanasia. Total RNA were extracted and realtime PCR were performed to quantify gene expression levels.
**Results:** We have observed severe cases of cachexia after radiation exposure. Animals may lose as much as 25 % body weight prior to euthanasia. On the molecular level, multiple components of the protein catabolism pathway such as ubiquitin, Atrogin-1 and MuRF1 were significantly increased in cachectic animals. These results are in agreement with the ubiquitin-proteasome system's role in the breakdown of the bulk of muscle proteins and the accelerated muscle proteolysis. To investigate whether the ActRIIB pathway was elevated in our NHP model, we measured the mRNA levels of ActRIIB and myostatin. We found that levels of ActRIIB were significantly increased while myostatin levels were decreased in the cachectic animals.
**Conclusion:** In summary, we have developed a NHP model of radiation-induced cachexia and our studies suggest the important roles of protein catabolism and ActRIIB pathways in radiation-induced cachexia.

## 2–05
## Cardiac cachexia is associated with cholestatic liver dysfunction and reduced liver perfusion

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**Introduction**: The aim of this study was to assess the prevalence of liver function test (LFT) abnormalities and their relation to hepatic blood flow in patients with heart failure (HF) with and without cachexia.
**Methods**: We measured serum LFTs in 177 chronic HF patients (91 in New York Heart Association [NYHA] class I/II and 86 in NYHA III) enrolled as part of the Studies

Investigating Co-morbidities Aggravating Heart Failure (SICA-HF). Blood flows in the hepatic artery (HA) and portal vein (PV) were assessed by Doppler sonography in a subgroup of 54 patients.

**Results**: Cachectic patients had an increased prevalence of elevated cholestatic LFTs compared to non-cachectic NYHA class III and class II patients (60.0 vs. 20.3 vs. 6.6 %, respectively; $p<0.001$) whilst transaminases were in normal range in most patients (Table 1). Cachectic patients ($n=12$) compared to non-cachectic ($n=42$) had lower peak systolic, mean systolic and total flow in the HA (total HA flow: 57.6 [28.1–93.6] vs. 95.3 [68.0–122.5] mL/min, $p=0.008$). Flow in the PV was similar in both groups (309.6 [216.6–362.3] vs. 242.4 [194.2–378.7] mL/min; $p=0.5$) with higher pulsatility index in the cachectic group (0.42 [0.29–0.63] vs. 0.30 [0.23–0.41]; $p=0.003$), reflective of higher systemic and liver congestion.

Cholestatic LFTs were associated with both reduced HA flow (alkaline phosphatase [AP]: $r=-0.31$, gamma-glutamyl transferase [GGT]: $r=-0.37$, total bilirubin [TB]: $r=-0.35$ and direct bilirubin [DB]: $r=0.36$; all $p\leq0.04$) and dilation of inferior vena cava (AP: $r=0.43$, GGT: $r=0.32$, TB: $r=0.47$, DB: $r=0.55$; $p\leq0.04$) and liver veins (AP: $r=0.49$, GGT: $r=0.45$; $p\leq0.001$).

**Conclusion**: Cholestatic liver dysfunction but not elevation of transaminases is very frequent in patients with cardiac cachexia and is associated not only with venous congestion but also with reduced arterial perfusion of the liver.

**Table 1** LFTs in patients with chronic HF according to functional status and presence of cachexia

| | Non-cachectic NYHA II ($n=91$) | Non-cachectic NYHA III ($n=66$) | Cachectic NYHA III ($n=20$) | $p$-value |
|---|---|---|---|---|
| AP (U/L) | 61.0 [50.0–75.0] | 74.0 [54.0–96.0] | 107.0 [79.3–156.0] | <0.001 |
| GGT (U/L) | 35.0 [24.0–55.0] | 64.0 [38.0–133.5] | 95.0 [41.3–256.8] | <0.001 |
| Total bilirubin (mg/dL) | 0.50 [0.40–0.70] | 0.60 [0.40–0.83] | 0.80 [0.60–0.45] | <0.001 |
| Direct bilirubin (mg/dL) | 0.15 [0.11–0.23] | 0.20 [0.15–0.30] | 0.42 [0.23–0.86] | <0.001 |
| Indirect bilirubin (mg/dL) | 0.39 [0.33–0.51] | 0.41 [0.32–0.55] | 0.47 [0.39–0.63] | 0.1 |
| Alanine aminotransferase (U/L) | 22.0 [16.0–31.0] | 24.0 [17.0–33.3] | 19.0 [13.5–24.0] | 0.2 |
| Aspartate aminotransferase (U/L) | 25.0 [21.0–34.0] | 28.0 [21.0–34.3] | 28.5 [17.3–30.8] | 0.7 |

**2-06**

## Adipose tissue lipolytic activity in advanced heart failure patients with or without cardiac cachexia

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**Objective**: Fat mass loss in heart failure associated with cachexia is associated with a poor prognosis. We evaluated in heart failure patients with (CXC) or without cachexia (nCXC) compared to age-matched healthy controls (CTR) the mechanisms involved in altered adipose tissue metabolism.

**Design and method:** Sixteen advanced heart failure patients (6 CXC, mean age 74 years; 10 nCXC, 69 y) and 5 CTR (70 y) underwent clinical, hormonal, biochemical evaluation, and a subcutaneous bioptic sampling. Bioptic samples were processed to obtain freshly isolated mature adipocytes whose volume was evaluated and basal free fatty acid (FFA) release measured after 2 h of medium incubation. A portion of the bioptic sample was frozen and hormone sensitive lipase (HSL) activity measured. Isolated adipocytes were seeded and stimulated (dose response curves) with isoproterenol, recombinant natriuretic peptides (ANP and BNP) and fatty acids release measured.

**Results:** CXC showed lower nutritional parameters (prealbumin, albumin, total cholesterol, triglycerides) vs nCXC. Both CXC and nCXC had higher BNP, plasma renin activity, aldosterone levels vs CTR, whereas plasma norepinephrine was increased in CXC vs nCXC. Adipocyte volume was similar in nCXC and CTR (1426”b325 and 1578”b241fL, respectively) and significantly lower in CXC (857”b156 fL, $p<0.05$). Plasma FFA were higher in CXC (+121 %; +402 %) as compared to nCXC and CTR, respectively. High HSL activity was observed in CXC vs nCXC and CTR. Basal release of FFA from cultured adipocytes showed a significant increase in palmitic, oleic and arachidonic acids in CXC vs nCXC and CTR. In contrast,

linolenic acid release was lower in CXC vs nCXC and higher vs CTR.

**Conclusions:** Cardiac cachexia is associated with an activation of lipolytic pathway expressed by higher HSL activity and plasma FFA. A diversified pattern of FFA released from isolated mature adipocytes could suggest differences in triglycerides hydrolysis of those stored in adipocyte

## 2–07

## Incidence of weight loss, pre-cachexia and cachexia in a court of cancer outpatient in public hospital in Brazil

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One most important cancer feature is an involuntary weight loss known as cachexia. Pre-cachexia is weight loss less than 5 % of body weight and cachexia is patient who has more than 5 % loss of stable body weight increasing patient mortality. Depending on the tumor type, weight loss occurs in 30–80 % of cancer patients and is severe (with loss of >10 % of initial body weight) in 15 %. A cross-sectional study involving outpatients treated by the Public Hospital Guarapuava–Paraná–Brazil, was conducted in 2012 to investigate % Weight Loss, pre-cachexia and cachexia incidence preceding diagnosis (PD) or after chemotherapy treatment (AT). 143 (55,1±12, 7 years) patients, were 34.3 % ($n$=49) male and 65.7 % (n-94) female. Time treatment was 151±110 days. There were 20.3 % of deaths ($n$=29), 3 patients with pre-cachexia and 13 patients with cachexia and lifetime was 148±83 days. Gastrointestinal ($n$=32) with WLPD 12.0±11,0 % and pre-cachexia-PD 3.1 % and cachexia-PD 78.1 %, WLAT was 13.0±11.7 % and pre-cachexia-AT 12.5 % and cachexia-AT 68.8 %. Head and neck ($n$=5) with WLPD 8.1±7.6 % and pre-cachexia-PD 20 % and cachexia-PD 60 %, WLAT was 8.8±9.3 % and pre-cachexia-AT 13.3 % and cachexia-AT 60 %. Lung ($n$=13) with WLPD 7.5±12.9 % and pre-cachexia-PD 15.4 % and cachexia-PD 61.5 %, WLAT was 10.7±15.8 % and pre-cachexia-AT 7.7 % and cachexia-AT 69.2 %. Pancreatic ($n$=4) with WLPD 18.7±12.6 and pre-cachexia-PD 25.0 % and cachexia-PD 75.0 %, WLAT was 20.9±12.8 % and pre-cachexia-AT 25.0 % and cachexia-AT 75.0 %. Ovarian ($n$=8) with WLPD 4.6±9.7 and pre-cachexia-PD 12.5 % and cachexia-PD 62.5 %, WLAT was 6.8±14.0 % and pre-cachexia-AT 37.5 % and cachexia-AT 37.5 %. Other tumors ($n$=13) with WLPD 1.2±9.5 and pre-cachexia-PD 15.4 % and cachexia-PD 38.5 %, WLAT was 2.8±13.6 % and pre-cachexia-AT 30.8 % and cachexia-AT 23.1 %. Breast ($n$=58) with WLPD +1.4±7.6 (gain) and pre-cachexia-PD 12.1 % and cachexia-PD 6.9 %, WLAT was +3.7±11.5 %* (gain) and pre-cachexia-AT 17.2 % and cachexia-AT 15.5 %. All cancer had weight loss and cachexia was present and ranged in preceding diagnosis to after treatment except in breast cancer. $p$<0,05 *WLPD x WLAT. Thanks to support: Fundação Araucária-PR/SESA-PR/MS-Decit/CNPq.

## 2–08

## Serum phosphorus is independently associated with catabolic/anabolic balance in chronic heart failure

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**Background:** Epidemiological studies have found association of higher normal serum phosphorus (sP) with adverse clinical outcome in patients with normal kidney function. In more advanced stages of heart failure (HF) sP level increase independently of GFR. Most body phosphorus resides in bones and cells from which it may be released during hypoxia or catabolism. In HF oedema-free body weight trajectories reflect global catabolic/anabolic balance (CAB). From HF onset different patterns are observed: only loss, loss and regain or only gain. The impact of metabolic status on sP has never been examined.

**Aim:** We wanted to test hypothesis that CAB as reflected by weight trajectory was associated with sP.

**Material & methods:** In 1029 patients with HF (12 % female, NYHA – 2.7±0.9, LVEF – 25±7 %, 67 % - ischaemic) weight changes were analysed. We recorded maximal weight within 1 year before HF onset (Wmax), nadir weight (Wmin) and weight at index date (Windex) where we

measured sP. We calculated weight loss (Wmin-Wmax), weight gain (Windex-Wmin), and CAB as a sum of the two. We divided CAB into quintiles and using logistic regression analysis we computed odds for s $P$ >1.25 mmol/L taking quintile 4 with neutral CAB as reference.

**Results:** Independent of kidney function dominance of catabolism was associated with higher risk of sP above the level linked to elevated cardiovascular risk. Dominance of anabolism did not alter the odds for higher sP.

**Conclusion:** In HF higher serum phosphorus is independently associated with catabolic profile of weight change 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).

## 2–09
## Weight trajectory during treatment of heart failure is related to body mass before the onset of the syndrome

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**Background:** Oedema-free weight changes are typical in heart failure (HF). Initial weight loss induced by dominance of catabolic drive may be lessened, inhibited or even reversed following introduction and uptitration of recommended therapy. As a result different weight patterns are observed. Resting energy expenditure is positively correlated to body mass while anorexia limiting energy intake is not. This may result in higher dynamic of weight loss and smaller od weight gain in bigger patients.

**Aim:** We intended to test the hypothesis that bigger HF patient loses more and gains less weight.

**Material & methods:** In 1029 patients with HF (12 % female, NYHA – 2.7±0.9, LVEF – 25±7 %, 67 % - ischaemic) weight changes were analysed. We recorded maximal weight within 1 year before HF onset (Wmax), nadir of weight (Wmin) and weight at index date (Windex). We calculated weight loss (Wmin-Wmax) expressed as percentage of Wmax and weight gain (Windex-Wmin) expressed as percentage of Wmin. Weight loss and weight gain was calculated in consecutive quintiles of BMI and their change analysed using ANOVA.

**Results** Weight loss was more pronounced at higher initial BMI and decreased in consecutive quintiles ($p$ < 0.001 ANOVA). Weight gain was smallest in initially biggest patients and gradually increased in smaller patient.

**Conclusion** In HF weight loss and weight gain is dependent on pre HF body size 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).

## 2–10
## Splanchnic congestion: a typical finding in patients with cardiac cachexia

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**Background:** Splanchnic congestion as a consequence of right heart failure (HF) may cause hepatic and intestinal dysfunction leading to cachexia. We therefore analysed indices of splanchnic congestion and right heart function in patients with and without cachexia.

**Methods:** We prospectively studied 20 cachectic and 157 non-cachectic ambulatory HF patients. Patients underwent echocardiography, abdominal sonography of the liver and the gut. Cholestatic liver function tests (LFTs) were measured in serum.

**Results**: Congestive right HF was present in 88.9 % of cachectic patients compared to 54.3 % of non-cachectic patients ($p$ =0.005). Cachectic patients, compared to non-cachectic, had higher prevalence of dilated inferior vena cava (IVC; 73.3 vs. 40.5 %, $p$ =0.02) and elevated cholestatic LFTs (60.0 vs. 12.3 %; $p$ <0.001) indicating congestive liver dysfunction. Cachectic patients also showed increased bowel wall thickness in the ascending colon (1.8 [1.2–1.5] vs. 1.3 [1.1–1.5] mm), descending colon (2.1 [1.9–2.3] vs. 1.3 [1.1–1,7] mm), sigmoid (2.3

[1.7–2.7] vs. 1.5 [1.3–2.0] mm; all $p \leq 0.02$) and in trend in the terminal ileum (1.3 [1.0–1.6] vs. 1.1 [0.9–1.3] mm; $p = 0.08$) suggestive of bowel wall edema. Interestingly, indices of both hepatic and intestinal congestion were higher in cachectic patients even when compared with non-cachectic subjects in the same (NYHA III) functional class. Dilated IVC, elevated cholestatic LFTs as well as wall thickness in the ascending and descending colon, and the sigmoid were associated with reduced systolic right ventricular (RV) function and elevated right atrial pressure (all $p \leq 0.03$) indicating relation between splanchnic congestion and backward right heart failure. In multivariable regression analysis, the presence of cachexia was associated with lower RV function, higher RAP, dilated IVC, elevated cholestatic LFTs and thickened intestinal walls independently of LVEF or NYHA class (Table 1).

**Conclusion:** Patients with cardiac cachexia presented with signs of splanchnic congestion related to backward right HF. Splanchnic congestion as well as right HF were associated with the presence of cachexia independently of the functional status or systolic left ventricular function.

**Table 1** Logistic regression analysis with the presence of cachexia serving as the dependent variable

| | Adjusted for NYHA class | | Adjusted for LVEF | |
|---|---|---|---|---|
| | Odds ratio (95 % CI) | p | Odds ratio (95 % CI) | p |
| TAPSE (per 1 mm increase) | 0,8 (0,7–0,9) | 0,007 | 0,8 (0,7–0,9) | 0,001 |
| RAP (per 1 mmHg increase) | 1,1 (1,0–1,3) | 0,05 | 1,2 (1,1–1,4) | 0,003 |
| IVC diameter (per 1 mm increase) | 1,1 (1,0–1,2) | 0,2 | 1,1 (1,0–1,3) | 0,03 |
| AP (per 1 U/L increase) | 1,021 (1,008–1,034) | 0,002 | 1,030 (1,016–1,044) | <0,001 |
| GGT (per 1 U/L increase) | 1,005 (1,000–1,010) | 0,04 | 1,009 (1,004–1,013) | <0,001 |
| Total bilirubin (per 0,1 mg/dl increase) | 1,1 (1,0–1,3) | 0,03 | 1,2 (1,1–1,3) | 0,001 |
| Direct bilirubin (per 0,1 mg/dl increase) | 1,6 (1,2–2,0) | <0,001 | 1,9 (1,4–2,5) | <0,001 |
| Terminal ileum (per 1 mm increase) | 3,3 (0,6–16,9) | 0,2 | 6,4 (1,1–38,5) | 0,04 |
| Ascending colon (per 1 mm increase) | 3,9 (1,1–14, 2) | 0,04 | 5,4 (1,3–21,7) | 0,02 |
| Transverse colon (per 1 mm increase) | 2,6 (0,6–11,0) | 0,2 | 5,0 (1,0–26,4) | 0,06 |
| Descending colon (per 1 mm increase) | 3,8 (1,2–12,1) | 0,02 | 4,3 (1,5–12,5) | 0,006 |
| Sigmoid (per 1 mm increase) | 2,9 (1,1–7,5) | 0,03 | 3,9 (1,5–10,6) | 0,007 |

*TAPSE* tricuspid annular plane systolic excursion, *RAP* right atrial pressure, *IVC* inferior vena cava, *AP* alkaline phosphatase, *GGT* gamma-glutamyl transferase, *CI* confidence interval

**2–11**

## The role of reactive oxygen species and xanthine oxidase in the development and progression of cancer-induced cachexia

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**Background:** Cancer cachexia is a profound wasting condition, driven by systemic inflammation and oxidative stress. The study aimed to establish if there was a change in gene expression and activity of pro- and antioxidants, and components of the ubiquitin proteolytic pathway (UPP) in skeletal muscle during cachexia development. It was hypothesised XDH levels increase in cachexia, increasing xanthine oxidase activity and decreasing antioxidant activity, leading to oxidative stress, in turn driving increased breakdown of muscle via the UPP.

**Methods**: Nude mice were randomized to Cachexia (MAC16) or Cancer Control (MAC13), then to time-points of 0, 12 or 28 days. Analysis of antioxidant, pro-oxidant and UPP component gene expression, and enzyme activity of pro- and antioxidants were completed on gastrocnemius muscle.

**Results:** Compared to the control, there was an increase in MnSOD expression in both Cachexia groups. The D28

Cachexia group also had increased expression compared to D28 Cancer. There was no change in activity of Total SOD in any group. Compared to control, significantly higher expression of GPx1 was observed in D28 Cachexia. D12 Cachexia group exhibited lower expression of GPx1 than Cancer D12, but increased at D28 Cachexia compared to D28 Cancer. GPx activity increased in D12 Cancer compared to control. There were no significant differences in XDH/XO expression or activity between groups. Expression of UPP components increased in the D28 cachexia group compared to Day 0 and D28 Cancer Controls, and UbiquitinB was increased at D12 Cachexia compared to Control.

**Conclusion:** The increased expression of antioxidant precursor genes was not accompanied by corresponding change in enzyme activity, suggesting that while there may be a drive for increased antioxidant capacity to counteract oxidative stress, post-transcriptional conditions prevented this from being achieved. Results also indicate Ubiquitin tagging of muscle proteins may be active during early cachexia, driving initial wastage.

## 2.12
## Identification of serum peptides associated with low lean mass using novel population based proteomics

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**Background and Aims:** The biological pathways that mediate age-related sarcopenia are unclear; there are few biomarkers for the disorder. We utilized a unique population-based approach to identify serum peptides and proteins associated with low lean mass (LLM) in older men.

**Methods:** 1923 men >65 years (mean 73) were randomly chosen from the Osteoporotic Fracture in Men Study after excluding relevant comorbidities/medications. LLM was defined as DXA appendicular lean mass/height2 <7.26 kg/ m2 (prevalence 19 %). A novel high throughput liquid chromatography–ion mobility separation-mass spectrometry (LC-IMS-MS) platform was used to quantify serum peptides. ANCOVA/linear regression models adjusted for age and total body fat mass were used to compare peptide abundance between LLM and non-LLM groups. We adjusted for multiple comparisons using the Storey method. To better understand the biology of identified proteins, they were assessed using PANTHER—a comprehensive, curated database of protein families and functions.

**Results:** Among 3099 identified peptides analyzed, 117 were differentially abundant in LLM (false discovery rate <0.1 and fold differential >10 %). They were mapped to 54 unique proteins. The top up-regulated proteins in LLM included sex hormone binding globulin, complement C4-A, inter-alpha-trypsin inhibitor heavy chain H4, heparin cofactor 2. The top down-regulated proteins included IGF-binding protein 3, pigment epithelium-derived factor, IGF-binding protein complex acid labile subunit, gelsolin, retinol-binding protein 4. The major protein classes associated with LLM included defense/immunity, transfer/carrier, protease and hydrolase. These proteins involve various important biological processes, such as metabolic, immune system process and response to stimuli.

**Conclusions:** Using novel, in depth proteomic methods in a large population of older men, we identified multiple serum peptides/proteins associated with low lean mass, including a variety previously linked to muscle biology and thus with biological plausibility. These methods and our results provide an opportunity to better understand the etiology, and to develop biomarkers, for sarcopenia.

## 2–13
## Fat-free and fat mass can be measured with D2O dilution and Gas Chromatography-Tandem Mass Spectrometry

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We describe three easy and sensitive methods using GC/MS and GC/MS/MS to measure D2O in body water to estimate fat mass (FM) and fat-free mass (FFM). Data were compared to a 4 compartmental (4C) model. In 6 subjects, 30 ml D2O was consumed and urine/plasma samples were collected before and 8 h after. Samples were reacted with 1 M NaOH and U-13C-acetone in an autosampler vial to allow deuterium exchange with acetone hydrogens. Headspace injections were made of acetone-saturated air onto a 30 m DB-1MS column. Product fragment ions monitored included 45 and 46 using single ion monitoring (SIM:GC/MS; Method1), the 61>45 and 62>46 transition using multiple reaction monitoring (MRM; GC/MS/MS; Method2) and Method 2 with the Neutral Loss, 62>45, transition (Method3). MRM methods were optimized for collision energy and collision-induced disassociation argon gas pressure. Body water was determined by standard equation. DXA scans were performed to calculate body mass, body volume and bone mineral content and D2O-

dilution for body water estimates were fitted to a 4C model to calculate FM and FFM. Limits of quantification for deuterium enrichment were determined to be 0.005 % for all methods. The coefficient of variation was 0.60 %, 0.46 %, and 2.10 % at 0.0005 % enrichment for Method 1, 2 and 3. Correlation between 4C and DXA or D2O-determined FFM using methods 1, 2, and 3 were 0.90vs0.92, 0.92vs0.86, and 0.93vs0.86. We have developed an easy and sensitive method to determine D2O enrichment in body water to enable measurement of FM and FFM. In addition, this method can be utilized in a 4C model to more accurately determine body composition.

## 2–14
## Comparable upregulation of Whole body Protein turnover but differences in Arginine and Nitric Oxide metabolism among the chronic wasting diseases Chronic Obstructive Pulmonary Disease (COPD), Non-Small Cell Lung cancer (NSCLC), and Cystic Fibrosis (CF)

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**Background**: COPD, NSCLC and CF are chronic inflammatory wasting diseases with pathology originating in the lung. As inflammation can reduce arginine (ARG) availability by stimulating nitric oxide (NO) and arginase enzyme activity, it could contribute to a cascade of metabolic events leading to muscle wasting via increased protein breakdown. Although local changes in ARG concentrations were observed, it remains unclear whether whole body (Wb) ARG and protein metabolism, and NO synthesis are comparably affected in COPD, NSCLC and CF, and if nutritional status plays a role.
**Methods**: In patients with COPD ($n=23$), advanced NSCLC ($n=13$), CF ($n=15$), and age-matched healthy controls, Wb Protein breakdown/synthesis, ARG and citrulline (CIT) production were assessed by stable isotope technique. De novo ARG production (from CIT) and NO synthesis were calculated, and muscle wasting assessed by DXA. Plasma isotope enrichments were measured by LC/MS/MS, and statistics done by unpaired $t$-test.
**Results**: Wb ARG clearance, Protein breakdown and synthesis were higher in COPD, NSCLC and CF than in healthy controls ($p<0.05$), but plasma ARG concentration was unchanged. Reduced values for Wb NO synthesis were found in COPD and NSCLC, and for de novo ARG and CIT production only in COPD ($p<0.05$). Stratification by nutritional status revealed increased de novo ARG production, Wb CIT and NO synthesis in CF with muscle loss only ($p<0.05$).
**Conclusions**: The impaired Wb NO synthesis in older adults with NSCLC and COPD indicates that total ARG production from de novo ARG production is not sufficient despite elevated protein breakdown, independent of nutritional status. The younger CF patients were able to preserve Wb NO synthesis by increasing ARG availability via de novo ARG production and protein breakdown, and even more in those with muscle loss. This suggests that the underlying disease influences Wb ARG and NO but not Protein metabolism.
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## 3–01
## PGC-1α counteracts cancer-induced muscle wasting in female mice

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**Background and Aims:** PGC-1α overexpression in the skeletal muscle produces changes typically associated with endurance exercise including: mitochondrial biogenesis, fiber type switch (fast to slow) and resistance to fasting or denervation-induced atrophy. Aim of this study was to investigate if PGC-1α hyperexpression prevents muscle wasting in experimental cancer cachexia.
**Methods:** C57/BL6 male and female wild-type or PGC-1α transgenic mice were divided into two groups: controls (C) and tumor-bearers (TB). TB received subcutaneously 1*106 Lewis Lung Carcinoma cells. All the animals were sacrificed 28 days after.
**Results:** In males, tumor mass was significantly higher in transgenic than in wild-type mice. Gastrocnemius weight was significantly reduced in both C and TB transgenic mice vs. wild-type animals. Tibialis mass equally reduced in both wild-type and transgenic TB; in the same muscle, fiber cross sectional area (CSA) was only slightly improved in transgenic vs. wild-type TB. In females, tumor mass was comparable in transgenic and wild-type mice. Gastrocnemius weight was equally reduced in both wild-type and transgenic TB. Tibialis mass was reduced only in TB wild-type mice while was

unchanged in TB transgenic mice with respect to C. Consistently, CSA decrease occurring in wild-type TB was prevented in transgenic TB.

**Conclusions:** These results suggest that the enhanced oxidative capacity induced by PGC-1α overexpression in the skeletal muscle improves cancer-induced muscle wasting in female but apparently not in male mice. Such discrepancy could be partially explained by the different tumor mass. Further studies are needed to clarify the effective role played by PGC-1α overexpression in the prevention of cancer-induced muscle wasting.

### 3–02
### Are there a relation between patients undergoing chemotherapy, anemia and cachexia?

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Weight Loss (WL) and anemia is common in cancer patients undergoing chemotherapy. WL is classified: pre-cachexia and cachexia when is less than 5 % of body weight and more than 5 % loss of stable body weight correspondingly. Cancer patients have WL and anemia preceding or developing during treatment often decreasing physical/non-physical functioning, increasing treatment toxicity resulting in higher patient mortality. Outpatient cancer who underwent nutritional consultation (2012) in a public hospital at Guarapuava-Paraná-Brazil was investigate %WL, pre-cachexia, cachexia, anemia incidence Preceding Treatment (PT) or After chemotherapy Treatment (AT 151±116 days). Anemia was classified a level below of hemoglobin (Hb) 13,3 g/dl in man and 11,7 g/dl in woman. PT: Pre-cachexia was present in 17 patients (11.8 %) and had WL 2.6±1.4 % and HbPT 12.6±2.1 g/dL being 5 patients (29.5 %) anemic. Cachexia was present in 59 patients (41.3 %) and had WL 14.8±7.4 % and HbPT 12.6±2.0 g/dL being 28 patients (47.5 %) anemic. AT: Pre-cachexia was present in 24 patients (16.8 %) and had WL 2.9±1.2 %, HbAF 12.9±1.4 g/dl being 9 patients (37.5 %) anemic. Cachexia was present in 59 patients (41.3 %) and had WL 18.2±8.0 % and HbAF 11.1±1.8 g/dl# being 40 patients (67.8 %) anemic. All patients ($n$=143): HbPT was 13.0±2.0 g/dl and 30.8 % ($n$=44) was anemic, HbAT was 12.0±1.7 g/dl* and 50.3 % ($n$=72) was anemic. Gastrointestinal ($n$=32): HbPT 12.7±1.6 g/dl, HbAT 11.3±1.9 g/dl*, 53.3 % and 80.0 % was amenic respectively. Head/neck ($n$=5): HbPT 12.8±1.7 g/dl, HbAT 11.5±2.1 g/dl*, 37.5 % and 62.5 % was amenic respectively. Breast ($n$=58): HbPT 13.4±1.9 g/dl, HbAT 12.4±1.4 g/dl*, 10.3 % and 31.0 % was amenic respectively. Ovarian ($n$=8): HbPT 13.5±1.5 g/dl, HbAT 12.4±1.8 g/dl*, 12.5 % and 50.0 % was amenic respectively. Pancreatic ($n$=4): HbPT 9.6±0.9 g/dl, HbAT 10.1±2.0 g/dl, 100 % and 75.0 % was amenic respectively. Lung ($n$=13): HbPT 12.2±2.0 g/dl, HbAT 12.3±1.2 g/dl, 61.5 % and 38.5 % was amenic respectively. Others ($n$=13): HbPT 13.2±2.4 g/dl, HbAT 11.8±1.1 g/dl*, 38.5 % and 76,9 % was amenic respectively. Patients underwent chemotherapy there have been decreased in Hb level and have occurred statistical significance ($p$<0.05) in *HbPTxHbAT and #HbPTxHbAT between cachetic patients. Thanks to support: Fundação Araucária-PR/SESA-PR/MS-Decit/CNPq

### 3–03
### Staging cachectic cancer patients using the cachexia score (CASCO)

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**Background and aims:** Cachexia has been defined but the definition does not consider the problem of staging it. Stratification of patients is important when considering therapy. The object of the cachexia score (CASCO) is to fulfill the existing gap in the classification of cachectic cancer patients.

**Methods**: CASCO takes into consideration five main different factors: body weight and lean body mass loss; anorexia; inflammatory, immunological, and metabolic disturbances; physical performance; and quality of life. The scoring scale goes from 0 to 100: mild cachexia (less than 25), moderate (more than 26 and less than 50), severe (more than 51 and less than 75), and terminal phase (more than 76 and up to 100). The score also takes into consideration the condition known as pre-cachexia (without lean body mass loss and the sum of the other 4 factors more than 25).
**Results**: The analysis of all these components allow the classification of the cachexia degree in cancer patients recruited in the University Hospital (University of Cagliari, Cagliari, Italy). 169 cancer patients (digestive tract, lung, gynecologic, head and neck, prostate, lymphoma, neuroendocrine and urinary) were included. The utilization of CASCO resulted in the following percentages: 36 % no cachectic; 8 % pre-cachectic; 8 % mild; 32 % moderate; 15 % severe and 1 % terminal phase.
**Conclusions**: CASCO is certainly a tool for the (a) identification of pre-cachectic patients, and (b) classification and staging of the syndrome according to body weight loss and composition, inflammation/metabolic disturbances/ immunosupression, physical performance, anorexia, and quality of life. CASCO could be also a useful tool for the treatment and nutritional recommendations of cachectic cancer patients and will therefore allow for a more adequate therapy.

### 3–04
### Prediction of weight loss in metastatic colorectal cancer

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**Background**: Colorectal cancer is less frequently associated with cachexia as compared to other tumor types. Predictors of colorectal cancer cachexia are poorly defined.
**Methods**: We analyzed patients treated for metastatic pancreatic cancer at M.D. Anderson, dividing patients into those with and without 5 % weight loss over the first 2 months of therapy. To ensure a homogenous group, we limited our sample to patients with intact primary tumors. We compared weight loss to clinical features and a panel of 56 cytokines drawn at baseline.
**Results**: 59 patients were available for analysis; the >5 % weight loss group constituted 17 patients. On univariate analysis, IL-8 (median 57.09 vs. 26.98 ng/ml, $p = .045$) and LDH (874 vs 603 IU/L, $p = .022$) correlated with weight loss. Median CEA was 260 ng/ml in the weight loss group and 38.75 ng/ml in the non-weight loss group, but was highly skewed in the population (median 75.2, mean 565). Surprisingly, IL-6 did not predict weight loss. Likewise, neutrophil/lymphocyte ratio (4.04 vs 3.91) and choice of initial therapy (FOLFOX vs FOLFIRI) was not different between the groups. On multivariate analysis, IL-8 remained a significant predictor of weight change. ROC analysis for IL-8, IL-12, LDH, and NLR showed AUCs of 0.61, 0.61, 0.57, and 0.58, respectively. Only IL-8 and IL-12 predicted for overall survival (HR 1.101 and 1.34 for each 10 unit change).
**Conclusions**: In this very well characterized cohort of 59 patients with newly diagnosed, primary-tumor intact mCRC patients, 29 % lost 5 % of body weight at the first restaging. Serum IL-8 appears to offer an ability to predict weight loss.

### 3–05
### A novel platform for mediator and therapeutic discovery in cancer cachexia

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To make rapid progress towards truly effective therapies for cancer cachexia requires a transformative approach to the discovery and validation of the driving mediators of this complex syndrome. We have developed a novel series of cellular assays using primary human myocytes, adipocytes, and hepatocytes that represent the major target tissues of cancer cachexia. Critically, these in vitro assays faithfully recapitulate the cellular phenotypes induced by patient plasma and a range of human cancer cell lines that generate the phenotype in vivo. We have integrated these cellular assays with cutting-edge genomic technologies (transcriptomic, proteomic, and metabolomic profiling) for high-resolution readouts of the cachectic cellular state. This integrated platform tracks mediator activity across a broad range of proteomic, genetic, and small molecule perturbations, providing orthogonal approaches for unbiased mediator discovery and validation. It also provides a platform for testing therapeutic candidates, and can provide important insights into mechanism of action at the molecular level. In a proof of concept study that combined small-molecule screening by gene-expression signature and quantitative proteomic analysis, we identified MEK-ERK as a key pathways in human muscle response to cachectic stimuli in vitro. We then used inhibitors of this pathway to block

and reverse the cachectic phenotype to cachexia-inducing cancer cell lines. The confirmation of the MEK-ERK pathway in the cachectic response is reassuring, given the role this pathway plays in muscle development and hypertrophy. Taken together, this platform represents a novel and unbiased approach for the discovery of the driving mediator(s) of cancer cachexia, their mechanism of action, and new targeted therapeutics.

### 3–06
### Distinct age-related muscle wasting pathways are activated in samples of human cancer cachexia patients

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Cancer cachexia is a multifactorial syndrome characterized by an ongoing loss of skeletal muscle mass. Currently there is no treatment for this condition. Cachexia induces loss of lean muscle that cannot be reversed nutritionally, and it affects the majority of patients with advanced cancer and is associated with a reduction in survival, treatment tolerance, response to therapy and quality of life. We analyzed muscle samples from cancer non-cachectic patients, cancer-cachectic patients and healthy controls according to distinct clinical criteria (as described by Hatakeyama et al1), to identify new disease biomarkers. A set of genetic and biochemical markers of pathways known to play a role in muscle degradation was chosen. In contrast to pre-clinical in vivo models of cancer-induced cachexia, we have not identified pathways exclusively activated in the cancer cachexia patients; rather the activation is a question of degree. One of the most activated pathways is the TGFbeta-signaling pathway, which is one of the best studied pathways perturbed in muscle wasting and ageing. Despite general activation of this pathway in muscle of all investigated groups, there is a trend toward even higher activation in patients with cachexia. The current data indicates that in the muscle of patients in the age group 61–68 years there is a "basal" activation of age related muscle wasting and additional activation of these pathway(s) mediates the cancer cachectic phenotype. (1) Submitted abstract: "Comparison of histology phenotypes and clinical classification of cancer cachexia": S. Hatakeyama

### 3–07
### Investigating the coexistence of comorbidity and cachexia in patients with lung cancer

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**Background:** Cachexia is common in patients with lung cancer, may preclude aggressive treatment and is a predictor of poor survival. Many patients have co-existing chronic diseases, which may also be associated with cachexia and reduced survival.

**Aim:** To.determine the prevalence of cachexia and comorbidity in patients with lung cancer and evaluate their relationship to survival duration.

**Methods:** A retrospective review of electronic case notes for all patients presenting to the Edinburgh Cancer Centre with lung cancer between January and March 2008. Cachexia was defined as weight loss (WL) >5 % and comorbidity was scored using the Charlson Comorbidity Index (CCI).

**Results:** Records for 140 consecutive patients were accessed (non-small-cell lung cancer [NSCLC] $n=79$, small cell $n=14$, others $n=47$). At presentation only 80/140 patients had WL recorded. Of these, 37/80 had cachexia (4/12 Stage 1, 3/7 Stage II, 5/10 Stage III and 21/42 Stage IV NSCLC). Cachexia was associated with poor survival (median 219 versus 391 days, $p<0.024$), as was poor performance status. Older age was not. Comorbidity was common, with a mean CCI of 4. Those with a CCI of 4 or more had much shorter survival than those scoring 3 or less (median 167 versus 442 days, $p<0.01$). There was no difference in co-morbidity scores between those with and without cachexia.

**Conclusion:** Cachexia and co-morbidity are both prevalent and associated with reduced survival in lung cancer. When considering the potential for rehabilitation, the nature of the interaction between cachexia and co-morbidity is worthy of evaluation in large prospective data sets.

### 3–08
### Clinical significance of Ghrelin and Peptide tyrosine tyrosine expression in patients with cancer-related anorexia/cachexia syndrome

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**Background:** Cachexia has been recognized for a long time as an adverse effect of cancer. It occurs in 50–80 % of cancer patients and is associated with severe muscle wasting, ongoing catabolism, low performance status, metastatic disease refractory to anti-cancer therapy and reduced survival. Various hormones and cytokines play crucial role in the pathophysiology of cancer-related anorexia/cachexia syndrome (CACS). Recent evidence suggests that a complicate interaction between multiple hypothalamic effector pathways and afferent hormonal signals different systemic origin, such as ghrelin and peptide tyrosine tyrosine (PYY) from gastrointestinal tract, insulin from pancreas, leptin and adiponectin from adipocytes, is important in functions of energy intake and expenditure.
**Materials and Method:** Forty-one subjects (14 CACS, 14 non-CACS and 13 healthy control) were enrolled this study. We analyzed fasting serum level of regulator of food intake (ghrelin, PYY and Leptin) and inflammatory cytokine (IL-6) and other laboratory index. We also investigated Body Composition and Quality of Life(QOL) and correlation between 3groups.
**Results:** The experimental results of serum levels of ghrelin, PYY and leptin indicated there were no significant differences between 3groups. There were significant differences in results of IL-6 (CACS vs Non-CACS; $P<0.05$, CACS vs control; $P<0.001$) and CRP (CACS vs Non-CACS; $P<0.05$, CACS vs control; $P<0.001$, Non-CACS vs control; $P<0.05$). We confirm the inverse correlation between markers of chronic inflammation (i.e., IL-6 and CRP) and appetite, quality of life, and muscle mass. Of note, we found a significant correlation between ghrelin levels and body composition parameters: in particular, an inverse correlation between ghrelin and lean body mass and fat mass (total and abdominal fat) have been observed in cachectic cancer patients.
**Conclusion:** Our preliminary results confirm the correlation of inflammatory mediators with the main CACS markers (anorexia, lean body mass and quality of life). The role of ghrelin as a marker of body composition in cachectic cancer patients is interesting but warrant further investigation in a larger sample size.

### 3–09
### Cancer anorexia: a new mechanism by which serotonin signalling induces hypothalamic resistance of the neuropeptide Y system

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**Background:** Appetite is frequently affected in cancer patients, leading to anorexia and consequently insufficient food intake. In cancer-induced anorexia, the hypothalamic neuropeptide Y system (NPY) seems to fail to respond adequately to changes in energy balance. Here, we investigate the role of serotonin in tumour-induced hypothalamic resistance in cancer cachexia.
**Methods:** Two tumour cachectic mouse models with different food intake behaviours were used: a C26-colon adenocarcinoma model with compensatory eating behaviour and a Lewis lung carcinoma model with decreased food intake. C26 cells were subcutaneously inoculated in 6 weeks old male CDF1 mice. Lewis lung cells were injected intramuscularly in 6w old C57/bl6 male mice. The hypothalamus was used for transcriptomics using Affymetrix chips. Hypothalamic neuronal cells mHypoA-2/12 (serotonin unresponsive cells) and mHypoE-46 (serotonin sensitive cells) were used to study the effect of serotonin on messenger NPY expression and NPY excretion.
**Results:** In C26 tumour-bearing (TB) mice, food intake increased, while in mice bearing the Lewis lung carcinoma food intake decreased. The progression of cachexia, as

reflected in a decrease in skeletal muscle weight and carcass weight (approximately 20 % decrease) was similar between the two different models. In both models, hypothalamic gene expression of orexigenic neuropeptides NPY and AgRP was significantly higher compared to controls, suggesting that these changes do not directly reflect food intake status. Serotonin signalling pathway showed to be decreased in C26 TB mice, while in Lewis lung TB mice a tendency for increased serotonin signalling was shown. Brain serotonin levels were correlated to food intake in both models. In vitro, serotonin repressed neuronal hypothalamic NPY excretion, while not affecting messenger NPY expression, suggesting that serotonin signalling can interfere with NPY synthesis, transport or excretion.

**Conclusions:** Serotonin signalling is important in food intake behaviour in cancer cachexia, probably by mediating its inhibitory effect on food intake via affecting the neuropeptide Y system. Serotonin regulation might therefore be a therapeutic target to prevent the development of cancer-induced eating disorders.

### 3–10
### Repression of p38 pathway and cachexia underlies ZIP4 regulation of post-surgical survival of pancreatic cancer

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**Background and aims:** Cachexia and muscle wasting are hallmarks of pancreatic cancer, and chemo- and radiotherapies provide little benefit in improving patient survival or quality of life, highlighting the importance of understanding the mechanism of cancer cachexia and developing new adjuvant therapies. Here, we describe a novel surgical xenograft mouse model and a new signaling pathway through which a cancer-promoting zinc transporter ZIP4 regulates pancreatic cancer cachexia and muscle wasting.

**Methods**: Human pancreatic cancer cells were used to create orthotopic xenografts in nude mice, distal pancreatectomy was performed to remove the pancreatic tumors, and sham surgery was performed in the control group. ZIP4 expression in cancer cells was knocked down by shRNA. Conditioned medium of cancer cells with or without ZIP4 knockdown was used to treat C2C12 myotubes. Muscle wasting was evaluated by measuring body, fat and muscle mass, tyrosine release, fiber cross sectional area, p38 MAPK activation and atrogin1/MAFbx levels.

**Results**: Our data demonstrate that surgical removal of tumors with blocked ZIP4 levels significantly improved survival and reduced loss of body weight and muscle wasting in comparison to high ZIP4 tumors. Mechanistically, we demonstrated that reduced ZIP4 levels in pancreatic cancer cells limits muscle wasting due to attenuated p38 activation and subsequent Atrogin1/MAFbx upregulation possibly through reducing the secretion of immunoregulatory molecules.

**Conclusions**: Activation of the p38 MAPK/Atrogin1/MAFbx pathway appears a critical component of ZIP4-mediated pancreatic cancer cachexia and tumor growth. The mouse model we developed provides a unique resource to test future adjuvant therapies in combination with surgery to develop more effective treatments for pancreatic cancer.

### 3–11
### Comparative study of the detection threshold (DT) and recognition threshold (RT) of the basic four flavours in patients with breast cancer

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**Background and aims:** Derangements on the taste sense following chemotherapy have not been thoroughly studied. Alterations on the perception of flavours in an American subset of patients with infiltrative breast cancer (IBC) have been reported; however, there is a lack of information on the quantitative/qualitative

changes on the taste sense in Mexican patients with IBC after systemic chemotherapy (SC). Therefore the aim was to compare the DT and RT in healthy women and in patients with IBC before and after SC.

**Methods**: Thirty healthy women (H group), matched by age and hormonal status to 30 patients with IBC (C group) were compared to C before SC and after the first cycle of SC (anthracyclines/taxanes). The DT and RT thresholds were measured on two occasions on the H group, and before SC and at day 12 after the first cycle of SC on the C group. The differences among data were detected by the Mann–Whitney's *U* test.

**Results**: The DT were different among the H and C group before SC on the sweet, salty and bitter tastes ($p<0.001$, $p=.015$, $p=.037$; respectively). Differences on the DT before and after SC were found on group C on the bitter and acid tastes ($p=.046$ and $p=.012$, respectively). Furthermore, the RT for the salty and bitter tastes on the H group were different when compared to the RT of women on the C group before SC ($p=0.003$ and $p=0.007$ respectively). Lastly, the RT for the acid flavour before SC was different from the RT after SC on group C ($p=.002$).

**Conclusions**: The DT and RT are different among the H and C groups. Both thresholds are, indeed, affected in IBC patients even before receiving SC. The difference between the DT for the acid and bitter flavours before and after SC may be attributed to metabolites produced by protein degradation and/or chemotherapy (drugs with metallic and/or bitter taste), among other reasons. The RT for the acid taste before and after SC are different.

### 3–12
### Systemically accelerated autophagy causal in cancer cachexia?

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Development of cancer cachexia involves loss of cellular proteins and reduced muscle mass. The tumor induced signalling substances that cause a systemically loss of proteins is not completely understood. Also, the cellular mechanism(s) for the loss of proteins is poorly defined. Two major systems exist for the degradation of intracellular proteins; the proteasomes and lysosomes. Lysosomal hydrolysis depends on isolation of cellular constituents into a double membrane vesicle that fuses with the lysosomes and the isolated material is degraded in the process known as autophagy. All cells have a basal level of protein turnover via autophagy and this level can be induced under starving or stressful conditions. We hypothesized that increased protein turnover in cancer cachexia could be due to cancer cell derived signalling substances that raise the level of autophagy in normal cells. Serum from in total 344 cancer patients and 185 healthy controls were screened for autophagy inducing activities using a novel bioassay. We find that 20–30 % of the samples from cancer patients possess an autophagy inducing activity. By analysing a subset of the lung cancer patients ($n=46$) we find a positive correlation between autophagy inducing activity and self-reported weight loss ($p=0.031$). To identify cancer cell derived signalling substances with autophagy inducing activities we analysed conditioned media from cultures of the ovarian carcinoma cell line TOV21G. This cancer cell line induced cachexia at low tumor burden in mice. Conditioned media from the cells increased autophagy and a multi-plex ELISA of 27 pro-inflammatory cytokines identified high levels of IL-6. Recombinant IL-6 was found to induce autophagy both in the reporter cells and in differentiated myotubes. An IL-6R neutralizing antibody, Tocilizumab, blocked autophagy induced by recombinant IL-6 and interfered with the autophagy induced by the TOV21G conditioned media. These results demonstrate that an increased autophagy in normal cells in response to cancer derived signalling substances could contribute to the development of cancer cachexia. IL-6 was identified as a cytokine that could mediate autophagy activation and this could be affected by Tocilizumab. The results suggest that at least some aspects of cancer cachexia could be counteracted by compounds that interfere with cytokine signalling and/or by modulators of autophagy.

### 3–13
### Characterisation of a patient population presenting to an interdisciplinary cancer cachexia clinic in regional Victoria, Australia

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**Background**: Cancer cachexia is a condition of tissue wasting affecting 50 % of cancer patients. Cachexia is associated with a significant decrease in response to

anti-neoplastic therapy, quality of life, and survival. The prevalence and clinical course of cachexia has not been characterised in an Australian population, but the condition is recognised as under-diagnosed. The study proposed to characterise the patient population captured by an interdisciplinary cachexia clinic in regional Victoria, and assess the success of the service model.

**Methods:** A retrospective longitudinal study was conducted from records of cachectic patients that attended an interdisciplinary cachexia clinic from 2008 to April 2013 ($n$ =142). The study assessed survival intervals and treatment response. Patients with ≤2 attendance dates were further assessed to establish follow-up intervals, cachexia progression, patient performance in standardised muscle function tests, and anthropometric measures were evaluated as measures of for relevance as prognostic risk markers.

**Results:** During the period January 2008-February 2013, 354/364 appointments were attended (97 %). Patients attended an average 2.5±2 appointments, with 43 % attending one appointment only, and 25 % of patients first attending within 60 days of death. 62 % of patients were male, 38 % female. 33 % of patients had a primary diagnosis of lung cancer, with gastrointestinal 32 %, uriogenital 17 %, head & neck 7 %, and multiple primary, unknown primary, or other cancer types 11 %. 49 % of patients attending 2+ appointments displayed <2 kg weight gain at any interval, with 59 % of patients showing improved handgrip strength, and 53 % improved Sit-To-Stand functional muscle strength scores between any two appearances.

**Conclusion:** The cohort of patients attending the cachexia clinic is comparable to those previously reported in the literature. Whilst patients are experiencing some benefit from the clinic, there is a high attrition rate, and a more nuanced analysis is required to establish whether outcomes are improving as the service model evolves.

### 3–14
### Preserved muscle oxidative metabolic phenotype in clinical cancer cachexia

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**Background and aims:** Cancer cachexia augments mortality and has a negative impact on quality of life. Muscle oxidative phenotype (Oxphen), including mitochondrial oxidative capacity and slow-twitch oxidative type I fibre proportion, has been implicated in muscle mass maintenance. Indeed, studies using animal cancer cachexia models indicate that loss of muscle Oxphen, induced by systemic inflammation, contributes to cancer-induced muscle wasting. However, this has never been validated in clinical cancer cachexia. Therefore, we hypothesized that muscle Oxphen loss, associated with systemic inflammation, is present in clinical lung cancer cachexia.

**Methods:** Patients with newly diagnosed stage III/IV non-small cell lung cancer ($N$=26) and healthy control subjects ($N$ =22) were enrolled in the study. Patients were subdivided into pre-cachexia ($N$=10) and cancer cachexia ($N$=16), defined according to the recent international cancer cachexia consensus (Fearon et al., Lancet 2011). Muscle biopsies were obtained from the vastus lateralis. Muscle Oxphen was determined by muscle fiber type distribution (immunohistochemistry), enzyme activities (spectrophotometry), and protein expression levels of mitochondrial complexes (Western blot) as well as transcript levels of (regulatory) oxidative genes (QPCR). Also, muscle fiber size (immunohistochemistry) and circulating pro-inflammatory cytokines (multiplex analysis) were assessed.

**Results:** Surprisingly and in contrast to the animal models, no differences in any of the muscle Oxphen parameters were found between lung cancer patients and controls, despite significantly elevated plasma inflammatory markers IL-6 (268 %) and soluble TNF-receptor-1 (160 %) in the cancer cachexia subgroup and to a lesser degree in the pre-cachectic group. Moreover, muscle fiber size of all fiber types (oxidative type I and glycolytic type II) was decreased in cachectic patients ($P$< 0.05).

**Conclusion:** despite evident systemic inflammation, muscle Oxphen is preserved and therefore not an important trigger of muscle wasting in clinical lung cancer cachexia.

### 3–15
### Anthropometric follow-up in patients with acute leukemia

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**Background and aims:** Cancer is characterized by weight loss associated to a decrease in adipose tissue and muscle mass. Anthropometric assessment ensures a good practice by detecting a decreased intake or increased energy requirement that has not been met, so needing nutritional support. Acute

leukemia is a life-threatening, fast-evolving disease requiring close supervision. Body changes along leukemia treatment have not been thoroughly described. Therefore, our aim was to assess the anthropometric changes in patients with acute lymphocytic and myelocitic acute leukemia after starting the first cycle of chemotherapy.

**Methods:** Patients were recruited upon diagnosis and were assessed on days 0 (diagnosis) and 7, 14, 21 and 28 days after chemotherapy infusion. Assessment included the measurement of height upon diagnosis, of weight upon diagnosis and weekly thereof, and measurement of the mid-arm circumference and triceps skinfold upon diagnosis and day 28. Body mass index (BMI), ideal body weight according to body frame, percentage of ideal body weight (%IBW), mid-arm muscle circumference (MAMC) and mid-arm muscle area (MAMA) were estimated. Percentiles were used as reference values for MAMC and MAMA. Data were compared by Student's *t* test, and differences were significant when a $p$ value was $<0.05$.

**Results:** Twenty-six patients with acute leukemia were included (14 men, age 27.8+9.5 years, and 12 women, age 35.4+16.5 years). BMI decreased –although nonsignificantly- both in men and women (Day 0: 29.2+8 Kg/m2 and 25.4+4,4 Kg/m2, vs. Day 28: 26.4+8.3 Kg/m2 and 24.7+5.2 Kg/m2, respectively), as well as %IBW (Day 0: 123.2+14 % and 111.29+14.9 %, vs. Day 28: 109.3+3 % and 98.4+16.2 %, respectively). No difference on MAMC at day 28 was found. However, MAMA was significantly reduced in men after follow-up.

**Conclusion:** Male patients with leukemia show a loss of muscle mass.

### 3–16
### Fat loss in cancer cachexia – Exploration of lipolytic factors as potential targets for prevention and effective therapeutic treatment

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Cancer cachexia develops in up to 80 % of patients with advanced cancer disease and is associated with severe loss of muscle and fat mass, poor quality of life, poor response to therapy, and shortened survival time. At present, there is no effective treatment or robust and clinically applicable biomarkers available to predict the syndrome, and the understanding of the molecular mechanisms involved is limited. Based on the current knowledge of demographic and clinical factors, no one is able to predict, for any given cohort of patients, who will develop cancer cachexia and who will not. Recent studies indicate that disturbance in lipid metabolism (lipolysis) is an early event in cachexia and may contribute to both fat- and muscle wasting. The current project aims to explore the relevance of blood levels of glycerol, free fatty acids and the lipolysis-inducing factor Zinc-alpha2 glycoprotein-1 (ZAG-1), interleukin-6 (IL-6), adiponectin and tumor necrosis facotor alpha (TNF-alpha), as potential biomarkers of ongoing lipolysis in early cachexia. Further, these findings will be correlated with levels of lipolytic activity (triglyceride lipase and hormone-sensitive lipase activity) in adipose tissue and skeletal muscle in cachectic (weight-losing) and non-cachectic (weight-stable) cancer patients. Cancer cachexia mediated by increased lipolysis may be due to inflammation and/or endocrine signaling from the tumor. The project aims to explore whether such signaling is reflected in the gene expression profile in blood, as a potential predictor of early stage cachexia. This project will contribute to increased understanding of the molecular mechanisms involved in the development of cancer cachexia and aims at uncovering potential useful therapeutic targets.

### 3–17
### Cancer cachexia: a possible link between hydration of skeletal muscle, mitochondrial creatine kinase activity and muscle loss

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**Background:** Dehydration of skeletal muscle plays a role in muscle dystrophy and impaired muscle performance.

Aquaporin 4 (AQ4), a waterchannel expressed at the sarcolemma of fast-twitch skeletal muscle fibers, is important to maintain hydration in skeletal muscle putatively via regulation of muscle mitochondrial creatine kinase (CKMT2) activity. Here, we investigate the role of AQ4 and CKMT in tumour-induced cachexia.

**Methods:** Male CD2F1 mice, aged 5–6 weeks, were randomly divided into a control (C) or a tumour-bearing group (TB). At day 20 after tumour inoculation, the experiment was ended and muscle mass of hind limb muscles was determined. The mGastrocnemius was used for pooled transcriptomics using Affymetrix chips. Expression of genes that were highly up or downregulated were confirmed with real time PCR. Whole muscle homogenate creatine kinase activity was analysed by enzyme coupled colorimetry in the mTibialis. Hydration status of muscle was determined as water content in the mPlantaris, by weighing the muscle before and after freeze drying.

**Results:** Skeletal muscle weight loss was approximately 20 % in TB mice. Gene expression of AQ4(−8) and CKMT2(−2) were both downregulated in the mGastrocnemius of TB mice compared to controls. Furthermore, mTibialis total creatine kinase activity was lower in TB mice. Moreover, muscle water content of mPlantaris showed a tendency to be lower in TB mice. In a similar second experiment it was confirmed that for the larger muscles, muscle water content were significantly lower in TB mice vs control mice (mTibialis: 73.6±0.5 % vs 72.5±0.6 % and mGastrocnemicus: 74.7±1.2 % vs 74.1± 0.4 %).

**Conclusions:** In this cancer cachectic model, muscle dehydration seems to be present and to coincide with a downregulation of both AQ4 and CKMT2, resulting in decreased overall creatine kinase activity. Future experiments with AQ4 inhibitors on ex vivo muscles of non-TB mice might reveal if activity of this gene directly relates to creatine kinase activity and muscle performance.

### 3–18
### Major abdominal cancer surgery and appetite loss: is parenteral nutrition deleterious?

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**Background:** Major abdominal cancer surgery needs subsequent nutritional support. Parenteral nutrition is used in most of these patients at least in the early postoperative period in order to prevent undernutrition and surgical complications. However, appetite loss following parenteral nutrition was often described but biological mechanisms are unknown.

**Objective:** To assess ghrelin before and after major abdominal cancer surgery, in patients with or without total parental nutrition during early postoperative period. Blood samples were collected each morning at 06 h00 and 08 h00, before the procedure and at day 1, 3, 5 and 7 after surgery.

**Patients and methods:** 85 patients undergoing major abdominal cancer surgery were included consecutively into the study : 41 patients were allowed to an early enteral diet as intestinal motility/permeability were preserved (ED group); 44 patients needed total parenteral nutrition before recovering an enteral diet (TPN group). The following parameters were assessed: Albumin, prealbumin, IGF-1, free T3for nutritional status and total and acylated ghrelin for appetite regulation.

**Results:** When compared to baseline all patients presented a significant decrease in hormonal and protein metabolism parameters in day 1 and 3 following surgery: IGF-1 (140± 12 vs 93±9 vs 85±8 ng/ml); prealbumin (0.20±0.01 vs 0.12± 0.01 vs 0.12±0.01). No differences were noticed between ED and TPN group. Overall, total ghrelin decreased in early postoperative period (day 1, 3 and 5) and increased by day 7. This total ghrelin dynamic profile characterized ED group but was blunted in TPN group, especially for 08 h00 (Figure 1). No significant differences were noticed between groups for acylated ghrelin.

**Conclusion:** Major abdominal cancer surgery exacerbate undernutrition, regardless of post operative diet. In addition, total parental nutrition leads to prolonged appetite loss, confirmed by blunted levels of ghrelin, as orexigenic signal.

### 3–19
### Predictive value of sarcopenia and skeletal muscle density on prognosis in aggressive lymphoma patients treated with rituximab containing therapy

**Michael P. Chu[1], Jessica Lieffers[2], Andrew R. Belch[1], Neil Chua[1], Amelie Fontaine[1], Randeep Sangha[1], A. Robert Turner[1], Sunita Ghosh[1], Michael B. Sawyer[1]**
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**Background and Aims:** Sarcopenia (Sarc) is an adverse feature for carcinomas. A study showed sarc predicts poor prognosis in elderly diffuse large B-cell lymphoma (DLBCL) patients (pts). Studies suggest low skeletal muscle density (SMD) is a better predictor than sarc. SMD can be measured by CT scans using muscle attenuation in Hounsfield Units (HU).

**Methods**: We studied pts from 2004 to 2009 ($n$=224) who received rituximab-based chemo. Demographics and

progression free (PFS) and overall survival (OS) were collected. Skeletal muscle area and SMD were calculated using Slice-o-Matic with pts' baseline CT scans using prior HU tissue values at L3 vertebrae levels. Sarc was defined by prior carcinoma and elderly DLBCL studies.
**Results:** Median age was 62 years (range 21–88) with 124 M/100 F. Most received R-CHOP. Median PFS and OS were 55 and 56 mths, respectively. Sarc pts did not have differences in PFS or OS. PFS hazard ratio (HR) of 0.70 suggests sarc was protective ($p=0.19$). In elderly DLBCL pts (>70 years) sarc was protective for PFS and OS with HRs of 0.24 and 0.45 ($p=0.002$ and 0.05). An insignificant PFS improvement was seen below and above the median SMD with 61 vs 53 mths. OS was better in those above the median SMD at 65 vs 51 mths, HR 2.02 ($p=0.006$). A SMD cut-off point was at 26.63 HU where PFS was worse in those that had lower SMD with 53 vs 56 mths, HR 1.74 ($p=0.03$). OS was poorer with SMD less than this cut-off 52 vs 59 mths HR 1.92 ($p=0.01$). SMD was not significant in analysis with IPI and gender.
**Conclusions:** Contrary to a prior study, we showed sarc may be protective in elderly. SMD is more prognostic than sarc. These findings suggest muscle mass and quality play roles in DLBCL, but R-IPI scores better predict outcomes.

### 3–20
### Skeletal muscle density predicts overall survival in indolent lymphoma treated with rituximab based chemotherapy

**Michael P. Chu[1], Jessica Lieffers[2], Andrew R. Belch[1], Neil Chua[1], Amelie Fontaine[1], Randeep Sangha[1], A. Robert Turner[1], Sunita Ghosh[1], Michael B. Sawyer[1]**
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**Background and Aims:** Sarcopenia (Sarc) is an adverse risk factor for carcinomas. Low skeletal muscle density (SMD) has been identified as a risk factor for poor prognosis. SMD can be approximated using CT scans and measuring muscle attenuation in Hounsfield Units (HU). An average muscle SMD of <30 HU is considered to be poorly functioning muscle.
**Methods:** Follicular lymphoma (FL) patients (pts) $n=145$ from 2004 to 2009 who had rituximab-based chemo at our center were reviewed. Baseline demographics, FL International Prognostic Index-1 score [FLIPI-1], chemo, progression free (PFS) and overall survival (OS) were collected. Skeletal muscle area (SMA) and SMD were calculated using Slice-o-Matic with pts' pre-treatment CT scan using previously described HU tissue ranges at L3 vertebrae. Sarcopenia was defined by prior carcinoma and elderly DLBCL studies.
**Results:** Median age was 59 years (range 29–83),79 M/66 F. Most pts received R-CVP chemo. Median PFS and OS were 44.7 and 56.8 mths, respectively. Sarc pts failed to have differences in PFS or OS. PFS hazard ratio (HR) of 1.26 showed a trend for poor outcomes in sarc pts ($p=0.17$). Pts with SMD below the median had a PFS 41 vs 50 mths above it (HR 1.91; $p=0.01$) respectively. Differences in OS below and above the median SMD with 53 vs 63 mths (HR 2.61; $p=0.01$). A SMD cut-off was identified at 36.61 HU. PFS for those with lower than this SMD was worse at 39.3 vs 55.3 mths (HR 2.76; $p=0.0005$). OS was 52 vs 65 mths (HR 4.67; $p=0.0001$) at levels below and above the SMD cut-off, respectively. Multivariate analysis found SMD was adverse risk factor (HR=4.08; $p=0.004$) independent of FLIPI-1 or gender.
**Conclusions:** In FL, SMD is a strong prognostic marker independent of FLIPI-1. SMD can be used as an additional tool to stratify FL patients.

### 3–21
### Prognostic impact of cancer cachexia on patients with limited-stage small-cell lung cancer

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**Background:** Cancer cachexia (CC) is frequently observed in lung cancer and was reported to be associated with poor prognosis. However, there is little information of prognostic impact of CC on limited-stage small-cell lung cancer (LD-SCLC) receiving chemoradiation. The aim of this study is to reveal the association between CC and overall survival of LD-SCLC.
**Methods:** We retrospectively reviewed 55 patients who had LD-SCLC and was treated with concurrent chemoradiation therapy between September 2002 and August 2011 at the Shizuoka Cancer Center in Japan. The diagnosis of CC was made according to the consensus criteria (Fearon K et al., Lancet Oncol. 2011) at baseline, 3 months after diagnosis, and 6 months after diagnosis. The patients were also classified by Glasgow Prognostic Score (GPS).
**Results:** The median age and PS were 65 years (range, 34–76) and 0 (0–1). Forty-two patients were male. At baseline, 14 patients (25.5 %) had experienced weight-loss during the last 6 months. Means (SD) of BMI, serum albumin, and C-reactive protein were 22.5 (3.2) kg/m^2, 4.2 (0.4) g/dL, 0.81 (1.2) mg/dL. At baseline, only 15 patients were assessable for the consensus criteria because of the missing value of weight.

A total of 8/15 patients (53.3 %) at baseline, 18/55 patients (32.7 %) at 3 months after diagnosis, and 16/55 patients (29.1 %) at 6 months after diagnosis were diagnosed as having CC. A total of 12 patients (21.8 %), 10 (18.2 %), and 12 (21.8 %) had GPS of ≥1, respectively. The diagnosis of CC at baseline was not associated with survival (Log-rank $p = 0.3$). The GPS at baseline was strongly associated with survival (Log-rank $p = 0.006$).

**Conclusion:** CC was frequently observed in patients with LD-SCLC receiving concurrent chemoradiotherapy and the GPS at baseline was strongly associated with prognosis.

### 3–22
### Prognostic impact of cancer cachexia in patients with advanced non-small-cell lung cancer

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**Background:** Cancer cachexia (CC) occurs commonly in patients with non-small-cell lung cancer (NSCLC). We evaluated the association between CC and survival in NSCLC patients receiving chemotherapy.

**Methods:** We retrospectively reviewed the clinical data of NSCLC patients administered first-line chemotherapy between January 2010 and August 2011. CC was defined as a body weight loss of >5 %, or body weight loss of >2 % in patients with a body mass index of <20 kg/m2, as assessed before the start (T1), and 3 (T2) and 6 (T3) months after the start of chemotherapy.

**Results:** A total of 134 patients were enrolled; 121 had an ECOG-PS of 0–1, and 43 had epidermal growth factor receptor (EGFR) mutations. CC was observed in 26, 53, and 36 patients at T1, T2, and T3, respectively. The overall survival was significantly longer in patients without CC at all the time points ($P < 0.05$). Cox's proportional hazard ratios for CC at T1 and T3 were 3.2 and 2.1, adjusted for PS and EGFR gene status, and 1.7 at T2, adjusted for PS, EGFR gene status, and best response.

**Conclusion:** CC, which frequently developed in patients with NSCLC during chemotherapy, was strongly associated with a poor prognosis.

### 3–23
### Comparison of histology phenotypes and clinical classification of cancer cachexia

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Cancer cachexia has been defined as a multifactorial syndrome characterized by an ongoing loss of skeletal muscle mass that cannot be fully reversed by conventional nutritional support and leads to progressive functional impairment. Cachexia affects the majority of patients with advanced cancer and is associated with a reduction in treatment tolerance, response to therapy, quality of life and duration of survival.

The aim of this study is to compare skeletal muscle phenotypes to a range of current diagnostic criteria for cancer cachexia. Four diagnostic criteria were used to classify patients as cachectic: >5 % weight loss (>5%WL), >10 % WL (>10%WL), low muscularity (LM), and LM plus >2 % WL (LM + >2%WL). At the time of surgery, 41 patients with resectable upper gastrointestinal or pancreatic cancer underwent open biopsy of the rectus abdominis muscle. A set of immunohistochemistry analysis was performed to evaluate muscle fiber and other biomarkers such as inflammation pathway, vascularity and satellite cells.

Patients who were cachectic by LM + >2%WL had mean muscle fiber size that was 26 % smaller compared to Not LM + >2 % WL (4,045 and 2,979 μm2, $p = 0.001$). No difference in fiber diameter was observed when patients were classified by WL alone. Regardless of classification, there was no difference in fiber number or proportion of myosin heavy chain isoforms. Analyses of other biomarkers are continuing. In conclusion, muscle fiber size is significantly reduced only when the diagnostic criteria for cachexia include both a measure of low muscularity and weight loss. Such correlation was not observed when cachexia was diagnosed based on weight-loss or low muscularity alone, and this finding suggests that current diagnostic criteria identify groups of patients with different skeletal muscle phenotypes. Identification of a more homogeneous patient cohort for

musculo-centric intervention trials may require use of such combined criteria.

### 3–24
### Variation of lean body mass predicts treatment toxicity in platinum - based chemotherapy in non small cell lung and head and neck cancer patients

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**Background and aims:** Cancer patients are notably variable in their proportions of lean (LBM) and adipose tissues. We hypothesize that for chemotherapy drugs metabolized in LBM, relatively muscular individuals may have a lower drug dose/LBM and individuals with muscle depletion (sarcopenia) may have high drug dose/LBM, with corresponding variations in treatment toxicity.
**Methods**: Consecutive patients with head and neck ($n=71$) and non-small cell lung cancer ($n=76$) receiving cisplatinum chemotherapy were evaluated. Skeletal muscle cross-sectional areas at standard vertebral landmarks were measured by computerized tomography. Toxicity (i.e. febrile neutropenia, thrombocytopenia, and nausea/vomiting) was assessed by CTCAE criteria and resulted in dose reductions and incomplete treatment. The chemotherapy dose: skeletal muscle surface area ratio (DSMA, mg/cm2) was calculated. Characteristics for patients at the median DSMA ±0.99 SD were compared with those with DSMA ≥1 SD above and below the median DSMA.
**Results**: We considered $n=26$ (6♀, 20♂) with DSMA ≥1 SD below median, $n=23$ (14♀, 9♂) with DSMA ≥1 SD above median, and $n=98$ (24♀, 74♂) with median DSMA. Dose-limiting toxicity occurred in 65.2 % of high DSMA, 38.8 % of median DSMA ($p=0.06$), and 38.5 % of the low DSMA patients ($p=0.02$). Incomplete treatment occurred in 69.6 % of high DSMA, half of median DSMA ($p=0.092$) and 34.6 % of low DSMA ($p=0.01$) patients. Sarcopenia was present in 34.8 % of patients with high DSMA, 18.4 % with medium DSMA ($p=0.086$) and 11.5 % with low DSMA ($p=0.05$). Of 14 female patients with high DSMA, 71.4 % had a dose-limiting toxicity, vs. 33.3 % of the low DSMA ($p=0.1$) and 20.8 % of the median DSMA ($p=0.001$) patients. Also, 71.4 % of the female high DSMA, 33.3 % of low DSMA ($p=0.1$) and 37.5 % of median DSMA ($p=0.04$) patients experienced incomplete treatment.
**Conclusions**: Patients with less skeletal muscle mass, particularly female patients, are significantly more likely to experience increased toxicity and an incomplete treatment.

### 3–25
### Formoterol in the treatment of experimental cancer cachexia: effects on heart function

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**Background and aims:** Formoterol is a highly potent β2–adrenoceptor–selective agonist, which is a muscle growth promoter in many animal species, resulting in skeletal muscle hypertrophy. Previous studies carried out in our laboratory have shown that formoterol treatment in tumour–bearing animals resulted in an amelioration of muscle loss through different mechanisms that include muscle apoptosis and proteolysis.
**Methods**: The study presented involved rats bearing the Yoshida AH-130 ascites tumour model –which induces a high degree of cachexia— treated with the beta-2 agonist formoterol (0.3 mg/kg BW).
**Results**: Administration of formoterol to cachectic tumour-bearing rats resulted in a significant reduction of muscle weight loss. The treatment also increased lean body mass and body water. The treatment, however, did not influence heart weight, which was very decreased as a result of tumour burden. Untreated tumour-bearing rats showed important changes in parameters related with heart function heart rate, LV ejection fraction, fractional shortening, LV diameter and volume (diastolic) and LV stroke volume, LV mass and posterior wall thickness (PWT) (both systolic and diastolic). Administration of formoterol affected heart rate, LV diameter and volume, LV stroke volume and LV mass.
**Conclusions**: The results suggest that formoterol treatment, in addition to reducing muscle wasting, serves to improve heart function in animals affected by cancer cachexia.

### 3–26
### The Animal CAchexia SCOre (ACASCO): a tool for evaluating the cancer cachexia degree in different experimental models

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**Background and aims:** The aim of the Animal CAchexia SCOre (ACASCO) is to overcome the problem of cachexia staging in experimental animals. The score considers five main different factors involved in the patophysiology of cachexia and classifies cachexia in 4°: mild, moderate, severe and terminal phase.

**Methods**: Three different experimental models have been used for the evaluation of the cachexia degree: Yoshida AH-130 ascites hepatoma (rat), Lewis lung carcinoma (mouse) and C26 colon adenocarcinoma (mouse). The parameters analyzed were the following: 1) BWC component: body and muscle weights; 2) IMD component: plasma levels of IL-6, serum amyloid A, albumin, glucose, and triglycerides. 3) ANO component: food intake; 4) PHP component: grip strength test and total physical activity; 5) QoL component: signs of distress, the intruder-resident paradigm and forced swim tests.

**Results**: The analysis of all these components allows the classification of the cachexia degree in these tumour models: • Yoshida AH-130 ascites hepatoma: The resulting scores of cachexia at days 2, 4, 6, 8, 10 and 11 were respectively: no cachexia (7), pre-cachexia (27), moderate (48), moderate (48), severe (68) and terminal phase (78). • Lewis lung carcinoma: The resulting score of cachexia at days 4, 6, 10, 14, 18 and 20 were respectively: no cachexia (14), no cachexia (21), moderate (48), severe (61), severe (65) and terminal phase (78).• C26 colon adenocarcinoma: The resulting score of cachexia at days 6, 8, 10, 12, 14, 15, 20 and 24 were respectively: no cachexia (6), no cachexia (14), pre-cachexia (35), pre-cachexia (30), severe (63), terminal phase (79), terminal phase (75), terminal phase (77).

**Conclusions**: The present score facilitates cachexia staging in the above-mentioned tumour models. ACASCO could be a useful tool for the evaluation of cachexia in experimental tumours allowing for a more appropriate measurement of the degree of cancer wasting.

### 4–01
### Ghrelin prevents cancer-related muscle wasting through GHSR-independent regulation of myostatin and p38

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**Background and Aims:** Cachexia and muscle atrophy are common and often lethal consequences of cancer and, paradoxically, of chemotherapy administration. The novel hormone ghrelin has been proposed as a treatment for cachexia. However its mechanisms of action are not well-understood. We characterized the molecular pathways involved in muscle atrophy induced by tumor implantation and by cisplatin administration. We also determined the effects of ghrelin in these settings and the mechanisms mediating these effects in muscle.

**Methods**: Two different models of cachexia were set up in c57bl male adult mice: a) cisplatin induced cachexia and anorexia, and b) Lewis lung cell carcinoma-induced cachexia. Also, C2C12 myotubes were treated with cisplatin or ghrelin.

**Results**: We show here how multiple pathways interact and are involved in the development of cachexia; either induced by a tumor or, paradoxically, by the chemotherapeutic agent cisplatin. Activation of p38/C/EBPβ, myostatin, and inflammatory cytokines (in tumor-bearing animals) and a decrease in akt and Myogenin/myoD ultimately leads to increased proteolysis, and decreased muscle mass and strength. Ghrelin prevents muscle atrophy by decreasing inflammation, and by downregulating the p38/C/EBPβ/myostatin pathway and increasing akt phosphorylation and activating myogenin and myoD. These changes appear, at least in part, to target muscle cells directly and to be GHSR1a-independent. Ghrelin also decrease mortality in tumor bearing animals, highlighting the clinical relevance of these findings.

**Conclusions**: In summary, we characterize the effect of ghrelin in different models of cancer-related cachexia and show how multiple pathways are modulated leading to the prevention of muscle atrophy, weakness and ultimately death. Work funded by the U.S. Dept of Veterans Affairs (I01-BX000507 and I01 CX000174 to

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### 4–02
### Ghrelin promotes skeletal muscle regeneration

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**Background and aims:** Acylated and unacylated ghrelin (AG and UnAG, respectively) are peptide hormones released by the stomach during fasting. AG, but not UnAG, stimulates growth hormone release, appetite, and positive energy balance through binding to its receptor GHSR1a. An increasing body of evidence indicates that ghrelin peptides act on the skeletal muscle. AG and UnAG promote differentiation of C2C12 myoblasts and prevent skeletal muscle atrophy by acting directly on the skeletal muscle. We have recently shown that the pro-differentiative and anti-atrophic activities of AG and UnAG are mediated by the activation of signaling pathways involving p38 and mTORC2-mediated activation of Akt without the involvement of GHSR1a. Based on these findings, we aimed to explore the role of ghrelin peptides on skeletal muscle regeneration.
**Methods:** We exploited Myh6/Ghrl transgenic mice, characterized by constitutively high UnAG circulating levels, and investigated if UnAG affected skeletal muscle regeneration after cardiotoxin-induced injury. **Results:** Skeletal muscle regeneration was enhanced in Myh6/Ghrl mice, with new-formed myofibers characterized by increased area and number of central nuclei. Evaluating the quiescent pool of satellite cells (SCs) in uninjured adult mice, surprisingly we found that muscles of Myh6/Ghrl mice have a greater number of quiescent Pax7 + ve SCs compared to WT mice, suggesting that a UnAG-rich environment may enhance SCs proliferation and/or self-renewal. Finally, we observed that the expression of Ghrl gene, barely detectable in the skeletal muscle, is strongly promoted after cardiotoxin-induced regeneration.
**Conclusions**: These findings, together with the anti-atrophic activity of AG and UnAG, the reported increase of plasmatic AG in cachexia, and the observation that Ghrl gene is up-regulated in the skeletal muscles of cachectic mice, suggest that ghrelin peptides may play a pivotal role in the stress-induced adaptive response of the skeletal muscle and that they may also act distally as novel myokines.

### 4–03
### Acylated and Unacylated Ghrelin protect the skeletal muscle from atrophy by acting directly on the muscle fiber, independently on GHSR1a-mediated orexigenic and anabolic activities

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**Background:** Ghrelin is an acylated peptide hormone stimulating GH release and appetite through its GHSR1 receptor expressed in the hypothalamus and pituitary. Conversely, the unacylated ghrelin peptide, which is also released in plasma, does not bind GHSR1a nor induces appetite or GH release. Nevertheless acylated and unacylated ghrelin share high affinity binding sites, distinct from GHSR1a, and inhibit apoptosis and trigger muscle differentiation, respectively in cardiomyocytes and skeletal myoblasts. Ghrelin administration to cachectic patients counteracts muscle wasting through a yet unidentified mechanism, thereby suggesting that the observed up-regulation of circulating ghrelin in cachectic patients is likely an adaptive response to muscle atrophy. Thus we set to investigate whether unacylated and acylated ghrelin act directly in the skeletal muscle to prevent skeletal muscle atrophy.
**Methods:** We investigated acylated and unacylated ghrelin anti-atrophic activity in transgenic mice featuring high plasmatic level of unacylated ghrelin and upon administration of acylated and unacylated ghrelin in either wt or GHSR1 KO mice. In addition, the molecular mechanisms of ghrelin anti-atrophic activity have been investigated in C2C12 myotubes.
**Results:** Acylated and unacylated ghrelin i) protect the skeletal muscle from atrophy induced by either fasting or denervation without stimulating muscle hypertrophy and GHSR1a-mediated activation of the GH/IGF-1 axis; ii) rapidly stimulate anti-atrophic signaling in the skeletal muscle of wt and GHSR1 KO mice, thus protecting it from atrophy; iii) prevent atrophy through

activation of cAMP, PI3Kβ, mTORC2, and p38 pathways, thus preventing atrogenes-mediated protein degradation, while it does not involve mTORC1-mediated activation of proteins synthesis. Finally, ghrelin expression is up-regulated in the skeletal muscle of cachectic mice, suggesting that the skeletal muscle may be central for ghrelin anti-cachectic activity
**Conclusions:** In here we unveil a novel pathway by which both acylated and unacylated ghrelin contribute to counteract muscle wasting, independently on the GHSR1-mediated orexigenic and anabolic activities.

### 4–04
### Orexigenic and hemodynamic effects of small molecule ghrelin receptor modulators

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**Background and aims:** Modulation of the ghrelin receptor (GHSR-R1) has the potential to be an effective treatment for cachexia and anorexia of aging. The growth hormone secretion effects of ghrelin and ghrelin agonists have been well-documented in the literature, but perhaps less appreciated are the orexigenic and associated hemodynamic effects of ghrelin receptor modulators.
**Methods:** Small molecule ghrelin receptor modulators were synthesized and profiled in in vitro assays to determine their functional potency and selectivity. Molecules with suitable pharmacokinetic profiles were investigated in a telemetered rat model that simultaneously measures feeding and hemodynamic effects.
**Results:** Functional agonist and antagonists of the ghrelin receptor show increases in feeding in rats. Agonist compounds demonstrate a greater degree of feeding induction, but also display the greatest increases in blood pressure parameters. A ghrelin receptor antagonist shows a delayed and less robust feeding response with little measured hemodynamic effects.
**Conclusions:** A rat model was developed to simultaneously measure orexigenic and hemodynamic effects of small molecule ghrelin receptor modulators. Initial profiling studies with tool ghrelin receptor functional agonists and antagonist demonstrate varying degrees of orexigenic and hemodynamic effects that help define a therapeutic window.

### 4–05
### Small molecule inhibitors of myostatin/activin signaling prevent muscle loss in the mouse hindlimb unloading model

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**Background and Aims:** Myostatin (GDF8) and Activin A, members of the transforming growth factor beta (TGF beta) family of ligands, are known negative regulators of skeletal muscle growth and have been implicated in a variety of muscle wasting conditions, including cancer cachexia. Members of the TGF beta family of ligands bind to different combinations of paired cell surface receptor kinases that transmit signals directly to receptor-specific SMAD transcription factors. The overlapping and combinatorial use of a limited number of receptors by this large family of secreted ligands presents unique challenges for developing selective small molecule inhibitors of these signaling pathways.
**Methods:** A combination of cell-based assays, using either GDF8 or TGF beta to induce SMAD phosphorylation, were used to direct a medicinal chemistry effort to develop compounds with selectivity for GDF8/Activin A signaling relative to TGF beta.
**Results:** We describe here, potent inhibitors of GDF8/Activin A signaling with selectivities over TGF beta ranging from 10 to 47-fold. These inhibitors, represented by several distinct chemical scaffold series, are active in a variety of cell-based assays and demonstrate the ability to modulate GDF8/Activin A signaling in vivo. A representative of one of these scaffold series, R462, is orally bioavailable, has favorable pharmacokinetic properties and demonstrated efficacy in the murine hindlimb unloading model of disuse atrophy when dosed orally for 14 days.
**Conclusions:** These data show that GDF8/Activin A signaling can be selectively targeted with small molecule inhibitors and this approach holds promise for addressing numerous conditions associated with muscle loss.

**4–06**

**Efficacy of REGN1033, a fully human anti-myostatin antagonist antibody, in rodent muscle function**

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**Background and aims:** Loss of skeletal muscle mass and strength plays a significant pathological role in the progression of a wide variety of disorders associated with aging and catabolic conditions. Neutralization of myostatin (GDF8) activity results in skeletal muscle hypertrophy and prevents atrophy in adult skeletal muscle. We tested the efficacy of REGN1033, currently in clinical development, in increasing muscle mass, force production, physical performance outcomes in aged mice, and preventing the loss of muscle mass in multiple atrophy settings. The pharmacodynamics effects of REGN1033 were further compared to the soluble decoy receptor-body ActRIIB-hFc including a skin wound healing model.
**Methods:** REGN1033 was tested in mice with weekly or bi-weekly subcutaneous injections ranging from 2.5 to 30 mg/kg. Muscle mass, force, and endurance exercise were assessed in mice treated for 21–28 days. Muscle mass was analyzed after 14 days of immobilization or dexamethasone treatment, and after recovery from 7 days of hindlimb suspension (HLS). Skin wound size was analyzed over 14 days in response to treatment. ActRIIB-Fc ligand specificity was determined by immunoprecipitation of sera from injected mice, and confirmed by signaling studies in human myoblasts.
**Results:** REGN1033 increased fiber size, muscle mass, and force production in young mice by approximately 20 %, and improved physical performance outcomes in combination with treadmill exercise in two-year-old mice. REGN1033 prevented the loss of muscle mass induced by immobilization or dexamethasone treatment by 80 %, and increased the gain of muscle mass after recovery from 7 days of HLS by 15 % compared to isotype controls. ActRIIB-hFc administration in mice elicits greater efficacy than REGN1033, but significantly impaired wound healing whereas no effects were seen with REGN1033.
**Conclusions:** Specific antagonism of GDF8 with REGN1033 enhanced muscle mass and function, and had beneficial effects in multiple models of atrophy without the adverse effects associated with broadly inhibiting TGFβ ligands.

**4–07**

**Anabolic effect of A Novel Long-Acting SARM in Rat ORX model**

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Androgen plays an essential role in male physiology such as maintenance of muscle and bone mass, prostate growth, spermatogenesis. Steroidal androgens, such as Nandolone, oxandrolone and fluoxymesterone have beneficial effects on muscle and bone but are not widely used due to safety concerns. Selective androgen receptor modulators (SARMs), which bind to androgen receptor (AR) and display tissue-selective activation, will be a preferable alternative. We have discovered different types of SARM with novel non-steroidal scaffold. They bind to AR with high affinity, and showed strong transcriptional activity in HEK293 cells. Among them, TEI-E0001 is classified as long-acting SARM which has excellent oral bioavailability and longer half-life in the blood at least than known SARMs including those under clinical trials. Anabolic activity and tissue selectivity of TEI-E0001 were evaluated in castrated (ORX) rats by assessing the effects on the levator ani muscle (LA) and prostate. ORX rats were orally administrated TEI-E0001 once, and then tissues were dissected and weighed 2 weeks later. Nandororone decanoate (ND) was used as a reference (once subcutaneous injection in oil). In ORX rats, LA and prostate weight was decreased, and serum luteinizing hormone (LH) level was elevated. TEI-E0001 treatment recovered LA weight to the level of that in sham-operated animals, while the influence on the prostate and serum LH were minimal. ND also increased LA weight without affecting prostate weight much. However, serum LH level was substantially suppressed. We conclude that TEI-E0001 is a novel long-acting SARM. Its application to several disease conditions is expected by potent anabolic activity on muscle and bone, reduced androgenic side effects and hormonal perturbation, and improved dosing regimen (oral and lower frequency).

## 4–08
## Acotiamide hydrochloride (Z-338), a novel drug for functional dyspepsia, restrores feeding inhibition and delayed gastric emptying induced by restraint stress in rats

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**Background & Aims:** Acotiamide hydrochloride (Acofide®), a novel upper gastrointestinal motility modulator, has been approved for the indication of meal-related symptoms (postprandial fullness and early satiety) of functional dyspepsia in Japan. The clinical effect of acotiamide has been explained by the enhancement of gastrointestinal motility. The mechanism underlying gastrointestinal prokinetic effect has been proposed to be based on its inhibitory effect on acetylchol inesterase activity. The aim of this study is to elucidate novel aspects of putative effects of acotiamide on FD treatment. As stress is considered a major contributing factor in the development of FD, effects of acotiamide on stress-related symptoms in rat were examined.
**Methods:** For stress-induced feeding inhibition, rats were restrained in a wire cage to apply restraint stress after administration of the test drugs and then the amount of food consumption was measured. For stress-induced delayed gastric emptying, a solid test meal was ingested within 10 min and the test drugs were administered. The rats were restrained in a wire cage to apply restraint stress and the gastric emptying ratio was calculated. Gene expression analysis in rats was performed using DNA microarray or real time RT-PCR.
**Results**: Acotiamide significantly improved both feeding inhibition and delayed gastric emptying in restraint stress-induced model, but did not affect both normal feeding and gastric emptying in intact rats. In addition, gene expression of neuromedin U increased by restraint stress in hypothalamus was suppressed by administration of acotiamide, while acotiamide had no effect on delayed gastric emptying induced by intracerebroventricular administration of neuromedin U, suggesting that the suppressive effect of acotiamide on the gene expression might be important to restore delayed gastric emptying induced by restraint stress.
**Conclusion**: These results suggest that acotiamide might play an important role in regulation of stress response, which may be relevant for symptom improvement in FD.

## 4–09
## Rikkunshito, Japanese herbal medicine reduces urocortin 1-induced anorexia and Fos expression in the central nerve nuclei of autonomic nerves

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**Background:** Cancer anorexia-cachexia syndrome is characterized by decreased food intake, weight loss, muscle tissue wasting and psychological stress. In the pathophysiogy of cancer related cachexia xia corticotropin-releasing factor (CRF) seems to be involved. Rikkunshito iRKT j, a Japanese traditional medicine is used clinically to treat patients with dyspepsia symptoms and anorexia. Recently, RKT was shown to improve the anorexia in tumor bearing rats. We also demonstrated that intracerebroventricularly-injected (ICV) urocortin 1 (UCN)-induced anorexia was restored with RKT through an increase of ghrelin. Aim: To investigate the involvement of central nervous system in the restoration of anorexia by RKT using the expression of c-fos as a marker of neuronal activation in brain regulating appetite and visceral function.
**Methods**: Adult male rats were injected ICV with UCN (300 pmol/rat) or PBS, and brain was processed for expression of c-fos by in situ hybridization and Fos by immunohistochemistry respectively. To elucidate effects of RKT, amount of food intake and plasma ghrelin levels were measured, RKT (1000 mg/kg) was orally administered 2 h before ICV UCN.
**Results**: RKT significantly restored the decrease of food intake, and plasma ghrelin levels after ICV UCN. UCN induces c-fos mRNA and Fos protein expression in paraventricular nucleus of hypothalamus (PVN), locus coeruleus, solitary tract nucleus (NTS), and ventrolateral nucleus of medulla oblongata (VLM). Fos expression in NTS was markedly suppressed by coadministration of RKT. UCN-induced Fos expression in VLM was also suppressed by RKT, but not significant ($P=0.08$). RKT also suppressed UCN-induced c-fos expression in PVN.
**Conclusions**: These data indicate that a) RKT inhibits the activity of the neuron of NTS suggesting that RKT inhibits vagal input from the periphery to central nerves. b) Activity of PVN is also suppressed possibly resulting in the decrease of the sympathetic outflow from the central nerve to the periphery. RKT may restore disturbed appetite and ghrelin secretion through the restoration of balance of central autonomic nervous system.

**4–10**

**Effects of oral administration of Rikkunshito on cisplatin-induced anorexia in rats**

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Cisplatin-induced anorexia is known as side effects. Here we studied the changes in feeding and plasma ghrelin secretion in cisplatin-induced anorexic rats after oral administration of Rikkunshito (RKT), a traditional herbal medicine. Adult male rats were divided into 4 groups: water + saline (WS), water + cisplatin (WC), RKT + saline (RS), and RKT + cisplatin (RC) groups. Water or RKT was intragastrically administered daily, and saline or cisplatin was intraperitoneally administered at day 0. Body weight and cumulative food intake in WC dramatically decreased compared to WS, but not changed in RS and RC. Three days after the start of the experiment, trunk blood sample was taken, followed by measuring plasma active/desasyl ghrelin. Plasma desasyl ghrelin was comparable in all among the groups, whereas plasma active ghrelin dramatically increased only in RC. RKT does not cause any change in plasma active ghrelin in RS, which indicating that RKT acts only in pathological condition, but not in physiological state. These results suggest that RKT may attenuate anorexia induced by cisplatin via active ghrelin.

**4–11**

**The metabolic modulator trimetazidine enhances myoblast differentiation and potentiates skeletal muscle regeneration**

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Trimetazidine (TMZ) is a modulator of cell metabolism, which optimizes energy production. This drug acts by blocking fatty acid β-oxidation and by shifting ATP production towards glucose oxidation. We investigated the metabolic effect of TMZ on skeletal muscle cells and its role during myogenic differentiation and during muscle regeneration. In the present study we analysed the effect of TMZ on skeletal muscle metabolism. We have recently published that TMZ has a protective effect against atrophy in vitro this supporting a possible reappraisal of TMZ in the treatment of cachexia. Cachexia is also associated with loss of regenerative potential, which we, here, analysed upon TMZ treatment. We incubated C2C12 and satellite myoblasts with TMZ during differentiation and we administered TMZ to mice during regeneration following muscle injury. Our results show that TMZ significantly stimulates glucose and glycogen consumption in C2C12 myotubes. We also found that it, transcriptionally, down-regulates PDK. TMZ also induces hypoglycemia in mice and increases myoblast fusion in differentiating cells. In order to study the effectiveness of TMZ on muscleα, suggesting that treated animals are using more glucose than the untreated ones. Moreover, we interestingly found that the administration of TMZ potentiates myogenic differentiation in both C2C12 and satellite cells. In fact, TMZ up-regulates MyoD, Myogenin, MyHC and PGC-1 regeneration in vivo, we administered TMZ to mice following focal injury on Tibialis anterior (TA). TA analysis after the injury shows that TMZ enhances the expression of Pax7 and desmin, used as markers of satellite cell activation. In addition, TMZ potentiates the expression of MyoD, Myogenin as well as of neonatal MyHC expressed by nascent regenerating myofibers. Our finding strongly suggests that TMZ stimulates myoblast differentiation and muscle regeneration following injury; this makes this drug appealing for its possible use in the treatment of several skeletal muscle pathologies.

**4–12**

**Trimetazidine counteracts stress-induced atrophy in C2C12 myotubes and improves muscle function in mice bearing the C26 tumor**

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The metabolic modulator Trimetazidine (TMZ) blocks fatty acid β-oxidation and shifts ATP production towards glucose oxidation, resulting in improved cell energy metabolism. TMZ is commonly used to treat angina pectoris and has been

found to enhance both the efficiency of myocardium metabolism and patient exercise capacity. The results show that TMZ significantly prevents myotube reduction in size caused by both treatments. In addition TMZ also markedly increases MyHC expression. Such an effect is associated with: a) increased levels of phosphorylated S6-kinase, suggestive of enhanced protein synthesis, and b) activation of the PI3K-AKT-mTORC2 pathway, and reduction of muscle-specific ubiquitin ligase mRNA levels, likely inhibiting proteasome-dependent degradation. Finally, TMZ also induces autophagy in untreated myotubes. TMZ effects on skeletal muscle cells were investigated in the present study, with particular reference to its potential protective effect against atrophy-inducing stimuli. C2C12 myotube cultures were exposed to serum deprivation or to the proinflammatory cytokine TNF. In order to study the effectiveness of TMZ also in vivo, the drug was administered to mice bearing the C26 colon-carcinoma, a well-characterized model of cancer cachexia. Treatment of tumor hosts with TMZ does not modify food intake, body weight and muscle mass. By contrast, muscle fiber cross-sectional area and voluntary muscle grip strength are improved by TMZ; the latter also correlates with TMZ-induced hypoglicemia, suggesting that treated animals are effectively using more glucose than the untreated ones. On the whole, these results suggest that TMZ positively interferes with skeletal muscle cell response to stress both in vitro and in vivo, supporting a possible reappraisal of TMZ in the treatment of diseases characterized by muscle atrophy, among which cancer cachexia.

## 5–01

## Efficacy and safety results from a phase II study of anamorelin HCl, a ghrelin receptor agonist, in NSCLC patients

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**Background**: Anamorelin HCl is in development for treating non-small cell lung cancer (NSCLC)-associated anorexia-cachexia. Anamorelin is an orally active ghrelin receptor agonist with appetite-enhancing and anabolic activity.
**Methods**: An international, randomized, double-blind, 12-week Phase II trial (NCT00622193) enrolled 226 patients ($N$=76 for 50 mg anamorelin; $N$=73 for 100 mg anamorelin; $N$=77 for placebo). Patients with Stage IIIB/IV NSCLC, ECOG ≤1, and scheduled treatment with carboplatin/paclitaxel (± bevacizumab) were eligible. Co-primary endpoints were body weight and handgrip strength (HGS). Safety endpoints included adverse event (AE) profile and overall survival; secondary efficacy endpoints included IGFBP-3 and Quality of Life (MDASI).
**Results**: A beneficial effect on weight was observed as early as 1 week after anamorelin treatment. Over 12 weeks, the 100 mg anamorelin group gained an average 0.14 kg from baseline, compared to mean losses of 0.3 kg and 1.32 kg for the 50 mg and placebo groups, respectively (mean treatment difference between 100 mg anamorelin and placebo was 1.47 kg; $p$=0.0005). For HGS, mean treatment difference between 100 mg anamorelin and placebo was 0.58 kg, but was not statistically significant. Anamorelin was safe and well-tolerated, and AEs of anorexia, nausea, and fatigue were reported in fewer anamorelin than placebo-treated patients. There was no statistically significant effect on long-term overall survival in the anamorelin groups compared with placebo. Anamorelin also increased IGFBP-3, a marker of drug activity ($p$<0.0001 for both treatments vs placebo). MDASI scores improved with 100 mg anamorelin treatment (mean ± SE change from baseline at Week 12 for total score was -8.6±4.58 for 100 mg anamorelin vs −1.5±3.29 for placebo), but were not statistically significant from placebo.
**Conclusions**: In this study, anamorelin significantly increased body weight, improved HGS and QoL, and had an overall favorable safety/tolerability profile. These data support further investigation of anamorelin for treating NSCLC anorexia-cachexia.

## 5–02

## 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/m2). 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.

### 5–03
### Individually dose-optimized Phase I-II study with natural ghrelin in advanced cancer patients with cachexia

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**Background:** Natural ghrelin, a peptide growth hormone secretagogue has a therapeutic potential in cachexia. We designed a dose-finding trial of subcutaneous natural ghrelin to improve nutritional Intake (NI) in advanded cancer patients.

**Methods**: Advanced cancer patients with cachexia management (symptom management, physiotherapy, nutritional and psychosocial support) started with ghrelin at 32 μg/kg, followed by 50 % dose increases. Patients self-injected ghrelin twice daily for 4 days followed by a wash-out period. Pharmacokinetics was measured. After reaching the primary endpoint: Minimal Dose for Maximal Nutritional Intake(MD-MANI) defined as ≥10% NI increase [2 days food diaries, weighing food at home] reaching a plateau or maximum tolerated dose (MTD), a maintenance period followed, where patients injected 10 doses of ghrelin per week. Safety parameters, NI, and cachexia outcomes (symptoms, narratives, muscle mass and strength) were measured over 6 weeks.

**Results:** 10 patients with metastatic solid tumors were included and 6 received ghrelin. Median survival was 59 days (28–412 days). 5 patients reached the primary endpoint and 3 patients reached the end-of study visit. MD-MANI was reached on dose level 3 (72 μg/kg) in 3 patients, dose level 2 & 4 in 1 patient each. Final pharmacokinetic results are pending. On request, two patients were approved to receive ghrelin on compassionate use. Subjective tolerability was high. An episode of hypothermia occurred as related, one atrial fibrillation and a secondary malignancy as unrelated adverse events. Of the 6 patients reaching MD-MANI muscle mass was stable in 2 patients, increased in 1 patient, muscle strength was stable in 3 patients. Selected narratives were: "I feel fresher and I eat more", "This is the first therapy which improves my well-being".

**Conclusion:** Ghrelin was safe in patients with cancer cachexia without dose-limiting toxicity and well tolerated. There was a positive effect on nutritional intake and patient narratives.

### 5–04
### Phase II study of OHR/AVR118 in anorexia-cachexia

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OHR/AVR118, a peptide drug to manage symptoms of anorexia-cachexia modulates cytokine action. The study objectives were to determine the effect of OHR/AVR118 on appetite, early satiety and nutritional intake in patients with advanced cancer. Secondary endpoints included changes in performance status, lean muscle mass and quality of Life (QOL). Eligible adult patients received 4.0 ml of OHR/AVR118 subcutaneous daily injections. Patients underwent bi-monthly evaluations during the 28 day initial treatment (phase A). Evaluations included Karnofsky performance status, Edmonton Symptoms Assessment Scale (ESAS), Patient Generated Subjective Global Assessment (PG-SGA), Simmonds Functional Assessment, Dyspepsia Symptom Severity Index, Weight, Lean Body Mass, skin fold thickness and grip strength.

Eighteen patients, 3 with stage III and 15 with stage IV cancers completed the treatment protocol; six pancreatic cancer, five lung cancer, two prostate cancer patients, and one each with colon, stomach, esophageal, liver cancer and multiple myeloma. At completion of treatment, patients achieved stabilization of mean body weight, body fat and muscle mass with a significant increase in appetite ($p = .001$). Moreover, PG-SGA (Patient Generated Subjective Global Assessment) scores demonstrated improvement ($p = .025$), indicating improved nutritional status. No statistically significant differences from baseline (indicated by the paired $t$ test) were observed in body fat content, arm circumference, triceps fold measurement, nausea or vomiting.
Patients had the option to continue receiving study drug after the initial 28 day treatment period; 11 of 18 patients (61 %) elected to do so, being treated with the drug for a total of 42 to 153 days. Sustained body weight stabilization was maintained on prolonged therapy with the drug in this sub-group of patients. These results were seen despite the fact that 7 of the 18 patients received concomitant chemotherapy, and 1 received radiotherapy during the trial treatment period with OHR/AVR118. The drug was well tolerated by the patients in the study.

## 5–05
## Siltuximab reverses muscle wasting in patients with multicentric Castleman's disease

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**Background and Aims:** Multicentric Castleman's disease (MCD) is characterized by fevers, night sweats, fatigue, anorexia, and wasting. IL-6 plays pivotal roles in MCD and muscle wasting. Siltuximab (siltux), a chimeric IgG1κ Mab binds IL-6. We studied prospectively collected CT scans to assess siltux's effects on muscle mass (MM).
**Methods**: Patients (Pts) ($n = 37$) were treated with siltux in a phase I study q wk, 2 week or 3 week at doses from 2.8 to 11 mg/kg, and 34/37 pts had CTs suitable for analysis. Median age was 49 (range 18–76) with 18 M/16 F. Median pt wt was 78 kg (range 40–170). CTs were landmarked at the L3 vertebra. L3 images were analyzed using Slice-O-Matic® with Hounsfield units set at different levels for each tissue.
**Results:** During siltux txt 38 % of pts gained >1 kg of MM, 2.3±1.1 (mean ± SD or range), 47 % of pts had stable MM ± 1 kg, 0.2±0.5 and 15 % of pts lost >1 kg, loss 3.1±1.7. For all pts ($n = 34$) MM gain was 0.5 kg (−5.5 to.4.4); and fat mass (FM) gain was 3.1 kg (−13.8 to 35.8). By Cheson criteria MM change was 0.9 kg in PR pts ($n = 11$), 0.5 kg in SD pts ($n = 22$), and −3.7 kg in a PD pt. FM change was 8.5 kg for PR pts, 2.85 for SD pts and −0.6 for a PD pt. At phase II dose 11 mg/kg q 3 week ($n = 17$), gain in MM was 0.6 kg (−5.5 to 4.4). Interestingly FM increased first followed by a delay and then MM gains occurred. Pts responding to siltux gained MM up to time of censoring.
**Conclusions**: In addition to durable MCD objective responses (median response duration not reached at 29 mths follow-up), siltux reversed MM wasting in 38 % of pts, stabilized MM in 47 % and in only 15 % did MM decrease.

## 5–06
## Food aversion and ingestion in patients with breast cancer undergoing systemic chemotherapy

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**Background:** Cancer patients frequently develop food aversion -even before receiving systemic chemotherapy (SC)- a treatment know to further increase it. A decreased food ingestion ensues food aversion, and adds up to the toxic effect of SC on the digestive tract (mucositis, nausea, vomit and/or diarrhea).
**Aim:** To describe the most common food aversions on patients with infiltrative breast cáncer (IBC) and to compare food ingestion before and 12 days after the infusion of SC.
**Methods**: Food ingestion was assessed in 30 patients before and 12 days after receiving the first cycle of SC (anthracyclines and/or taxanes). A questionnaire including seven items aimed to assess the number and type of food aversions was administered. Categoric variables were expressed as frequencies and proportions, and continuous variables were referred using measures of central tendency and dispersion. In order to calculate the differences in ingestion, the Student's $t$ test for related samples was used.

**Results:** The median basal typical ingestion was 2144 Kcal, and 1202 kcal 12 days after SC infusion ($p<0.001$). The most common food aversion was to red meat and fat before ($N=11$, 36.7 %) and at day 12 after SC infusion ($N=18$, 60 %).
**Conclusions:** Patients with IBC receiving chemotherapy experience a decreased food ingestion 12 days after starting SC. Our findings with respect to food aversion are in agreement to what has been published: red meat and fat aversion are the most common food aversions. The decreased food ingestion in patients due to aversion or other causes is an important factor contributing to the development of malnutrition in this set of patients.

## 5–07
## An assessment of curcumin's impact on muscle mass and mediators of inflammation

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**Background**: Patients with pancreatic cancer are at risk for muscle loss. STAT3 and NF-kB are inflammatory mediators that may contribute to this process. Pharmacological interference of these pathways may reduce the development of sarcopenia.
**Methods**: We re-analyzed a phase II study evaluating curcumin (8 gm/day) in patients with metastatic pancreatic cancer. We compared muscle mass change with baseline and 8-day PBMC measurement of NFkB and pSTAT3.
**Results**: 15 patients were available for assessment (9 M/7 F); median age 67 with median 2 prior cancer treatments. 8/15 pts were sarcopenic at baseline (7 M/1 F). Baseline CA 19–9, pSTAT3, and NFkB levels were similar between the sarcopenic and non-sarcopenic groups ($t$-test $p$ =NS). 12 patients were reimaged after a median of 65 days. All but one patient lost muscle over this period, and one additional pt met the definition of sarcopenia by this time. Baseline sarcopenic and non-sarcopenic patients lost 10 % and 7 % of muscle mass, respectively. Eight patients had both follow-up imaging and 8 days assessments of NFkB and pSTAT-3. Patients with below median pSTAT3 decrease lost less muscle than those in the upper half (3.7 % vs 9.2 %, NS). Conversely, patients with greater than median NFkB decline lost muscle similar to patients in the lower half (5.57 % vs 7.36 %, NS). One patient had a radiologic response with a 66 % decrease in pSTAT3 d8 but a rise in NF-kB and muscle mass loss of −10 %.
**Conclusion**: We saw continued muscle mass loss at 60 days in most patients despite treatment with curcumin. The low bioavailability of curcumin and the aggressive nature of PC may each partly explain the absence of stability. Despite small numbers, we observed an unexpected trend towards more muscle loss in patients whose pSTAT3 decreased with curcumin

## 5–08
## The effects of pharmacological, nutraceutical and exercise-based interventions on vascular and muscle protein anabolic responses to feeding in older aged men

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Perfusion of the skeletal muscle microvasculature after feeding is thought to be central for delivery of amino acid substrates and consequently, maintenance of skeletal muscle mass. In older age fed-state protein turnover responses can be compromised ("anabolic-resistance"), possibly contributing to sarcopenia. We therefore, explored links between leg blood flow (LBF), muscle microvascular blood volume [flow] (MBV) and muscle protein metabolism under both postabsorptive and fed (I.V glamin (~10 g EAA), glucose ~7.5 mmol•l−1) conditions in 30 older men (71.9±0.9 y). We assessed these links in response to (10 men per group): (i) acute pharmacological vasodilation (unilateral methacholine infusions to create "non-intervention vs. intervention" legs), (ii) an acute putative nutraceutical vasoactive supplement (350 mg cocoa flavanols), or (iii) resistance exercise training ((RET); 20 weeks, fully-supervised). LBF was measured by Doppler ultrasound, MBV by contrast-enhanced ultrasound (CEUS) and muscle protein turnover using stable isotope methodologies. While LBF was unaltered in response to feeding without intervention, pharmacological vasodilation resulted in ~100 % increases in LBF (0.32±0.04 vs. 0.74±0.08 l•min −1, $P<0.001$). Nutraceutical supplementation (0.46±0.03 vs. 0.54±0.04 l•min−1, $P<0.01$) and RET (0.37±0.03 vs. 0.45± 0.04 l•min−1, $P<0.05$) also resulted in significant increases in LBF in response to feeding. These results were mirrored for MBV where pharmacological (+56.2±15.5 %, $P<0.01$), nutraceutical (+20.6±6.9 %, $P<0.01$) and RET (+55.6± 11.5 %, $P<0.01$) interventions all enhanced fed-state MBV versus non-intervention conditions. Despite these

intervention(s) dramatically altering blood flow profiles, fed-state increases in muscle protein synthesis were similar between all-conditions. Furthermore, although fed-state muscle protein breakdown was suppressed only following RET (87.9±12.2 to 63.6±6.9 Phe•100 ml leg−1•min−1, $P < 0.05$) this was insufficient to improve net protein balance versus non-intervention conditions. Therefore, we conclude that enhancing LBF and MBV is unable to modulate fed-state anabolism in older age. Nonetheless, the positive effects of both RET and flavanol supplements on circulation are notable and likely afford other metabolic benefits.

**5–09**

**Impact of a two-drug combination regimen for cancer-related cachexia on nutritional, anabolic/metabolic, physical activity, anti-inflammatory and quality of life variables**

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**Background and aims:** Cancer progression is characterized by loss of lean body mass (LBM), inflammatory status, metabolic derangements and poor quality of life (QL) which result in cancer-related anorexia/cachexia syndrome (CACS). The aim of the present study was to test the safety and efficacy of a combination treatment (including nutraceuticals, i.e. quercetin, alpha lipoic acid and curcumin) with carnitine+celecoxib for the treatment of CACS. Primary efficacy endpoints were: increase of LBM, resting energy expenditure (REE) and improvement of QL, particularly fatigue. The following were assessed as secondary endpoints: physical performance (tested by grip strength and 6-min walk test, 6MWT), appetite, chronic inflammatory variables (IL-6 and CRP), Performance Status (PS) and Glasgow prognostic score (GPS).
**Patients and methods:** Outpatients with advanced cancer at different sites with CACS (i.e. loss of body weight >5 % of the pre-illness (or ideal) weight in the last 3 months) received L-carnitine 4 g/day plus Celecoxib 300 mg/day plus nutraceuticals/antioxidants, i.e., quercetin 300 mg/day, lipoic acid 300 mg/day, carbocysteine 2.7 g/day, curcumin 2 g/day (i.e.400 mg/day of active curcuminoids extract (Meriva, Indena, Milan, Italy). Treatment duration was 4 months. Results: From June 2011 to October 2012, 80 patients with advanced cancer (all stage IV) at different sites were enrolled: 70 completed the treatment and were evaluable (mean age 65 ±9.6, range 32–82 years). Ten patients did not complete the treatment for death due to disease progression. Results showed a significant increase of LBM and a significant improvement of QL (by EORTC-QLQ-C30), and particularly fatigue (by MFSI-SF). Moreover, an improvement of physical performance assessed by 6MWT as well as a decrease of inflammatory parameters (IL-6 and CRP), ECOG PS and GPS was observed. The treatment was very well tolerated (no grade 3–4 toxicities occurred) and no patient discontinued the treatment due to severe adverse events.
**Conclusions:** The results of the present study showed that a combined treatment with anti-inflammatory, anabolic/metabolic agents plus antioxidants was able to improve the main nutritional, metabolic and physical activity variables as well as QL of cachectic cancer patients with an optimal safety and cost-benefit profile, so that it may be suggested in the clinical practice as treatment for CACS.

**6–01**

**Dietary fat and fatty acid profile are associated with indices of skeletal muscle mass in women which, may be relevant for the development of sarcopenia**

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**Background and aims:** Age-related skeletal loss of muscle mass results in the reduction in metabolically active tissue, and has been related to the onset of obesity and sarcopenia. While the causes of muscle loss are poorly understood, dietary fat has been postulated to have a role through influencing inflammation and insulin resistance and consequently protein turnover but has hardly been investigated in population studies 1,2. This study investigated the cross-sectional relationship between dietary fat, as percentage energy (%fat) and fatty acid profile, with indices of skeletal muscle mass in the population setting.
**Methods**: Body composition (fat free mass (FFM kg) and fat free mass index (FFMI, FFM/height2)) was measured using dual-energy X-ray absorptiometry in 2689 women aged 18–79 years from the TwinsUK Study. FFM and FFMI were calculated according to quintile of dietary fat (as a percentage of energy, measured by Food Frequency Questionnaire) after multivariate adjustment (for age, physical activity, smoking habit, energy mis-reporting and total fat mass).
**Results**: A higher polyunsaturated to saturated fat (P:S) ratio and a lower intake of %fat, saturated fatty acids,

monounsaturated fatty acids and trans fatty acids was associated with better indices of FFM. For %fat the difference between extreme quintiles of intake was 0.6 kg for FFM and 0.28 kg/m2 for FFMI, and for the P:S ratio was 0.6 kg and 0.28 kg/m2, respectively.

**Conclusions**: This is the first population based study to demonstrate an association between a comprehensive range of dietary fat intake and FFM. Compared with the decline in FFM that occurs over 10 years the associations found were of a similar magnitude. These findings indicate that a dietary fat profile already associated with CVD protection may also be beneficial for conservation of skeletal muscle mass.

### 6–02
### The effect of nutrient delivery profile, the nitric oxide precursor arginine, and post-prandial leucine "spiking" on microvascular blood flow and muscle protein metabolism in youth and older age

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**Background and aims:** Impairments in anabolic responses to nutrition may promote sarcopenia/cachexia. We characterised the effects of distinct oral nutrient delivery profiles on muscle anabolism in youth and older age and also tested the efficacy of novel strategies aimed at enhancing fed-state anabolism in older age.

**Methods**: 16 younger men received 15 g EAA ($N$=8, 20±2y; "BOLUS") or 4×3.75 g, each dose at 45 min intervals ($N$=8, 20±2y; "PULSE"). Two additional groups of older men ($N$=8/group; 70±2y) were fed identically. A further two additional groups of older men received either 15 g EAA+3 g L-Arginine (a nitric oxide synthase (NOS) substrate)) to modulate blood flow or 15 g EAA with 3 g leucine provided 90 min post-BOLUS to provide a late prandial "leucine spike" ($N$=8/group, 70±2y). MPS was measured via 13C6-Phe bound to myofibrillar proteins, anabolic signals via immunoblotting and muscle microvascular blood-flow by (Sonovue™) contrast-enhanced ultrasound. Statistical analysis was undertaken via 2-way RM-ANOVA.

**Results**: In BOLUS groups feeding achieved rapid aminoacidemia (peak +250 % by +45 min; $P$<0.001), mTORC1 activation (peak ~4-fold increases in P70S6K; $P$<0.001) and muscle capillary recruitment (peak microvascular blood volume (MBV) (+36 %; $P$=0.008 in YOUNG, only). Response to PULSE were dampened (peak EAA +150 % by +115 min, P70S6K and MBV unchanged). Despite these radical differences, post-feed MPS was identical (young-BOLUS, 0.069±0.005 %.h−1 vs. young-PULSE, 0.078±0.009 %.h−1, $P$=0.23 N; old-BOLUS, 0.056±0.005 %.h−1 vs. old-PULSE, 0.058±0.006 %.h−1, $P$=0.62). Independent of feeding strategy "anabolic-resistance" was observed in old age (young 0.075±0.05 %.h−1 vs. old 0.058±0.004 %.hr−1, $P$=0.001). Temporally, BOLUS increased MPS only 90–180 min post feed (both old and young) while old-PULSE showed sustainment of MPS beyond 180 min. While arginine restored microvascular responses (peak MBV +29 %, $P$=0.04 vs. fasting) this failed to offset anabolic resistance (0.052±0.006 %, $P$=0.001 vs. young), as did "leucine spiking".

**Conclusions:** Dose-dependent mechanisms underlie muscle-full. Similar anabolism between feeding regimens endorses pursuing optimization of inter-feed intervals, not rates of aminoacidemia.

### 6–03
### Important role of neuropeptide Y in food restriction-induced hyperactivity in female adolescent mice

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**Background and aims:** Anorexia nervosa (AN) is an eating disorder characterized by self-imposed food restriction and paradoxical motor hyperactivity, representing a clinical condition where motivation prevails homeostatic rules with a failure of the brain to adjust motor activity to energy storage. Neuropeptide Y (NPY) is critically involved in both homeostatic control and reward/motivation and in this study we utilised NPY−/− mice to investigate homeostatic and behavioral abnormalities associated with AN.

**Methods:** We developed an activity-based anorexia paradigm combining food restriction with running wheel access. Wild-type (WT) and NPY−/− female mice at the age of 5 weeks were randomly assigned to either the home-cage (HC) or running-wheel cage (RW) group. After a 7-day ad lib baseline period, mice were restricted for food access to the first 1 h of the dark phase for 8 days. Body weight, food intake and wheel-running activity were recorded during the ad lib baseline (ad lib) and food restriction period (RF).

**Results:** WT mice in both HC and RW groups showed rapid weight loss during RF with no significant difference between the two groups. In contrast, the weight loss in NPY−/− mice

during RF was significantly greater in RW compared to the HC group with an accelerated weight loss in the RW group at the second half of the RF period. Interestingly, during RF, WT in RW group showed similar food intake to that seen in the HC group, whereas NPYKO−/− mice in RW group showed a trend to decreased food intake compared to that in the HC group. Importantly, NPY−/− mice showed significantly increased wheel-running activity during RF from their baseline level, whereas no such RF-induced hyperactivity could be observed in WT mice. Moreover, the weight loss in NPY−/− during RW showed significant negative correlation with their wheel-running activity, whereas no such correlation was observed in WT mice.

**Conclusion:** These results suggest NPY plays a critical role in adjusting motor activity in response to feeding and energy homeostatic disturbances, which may have important implication in the hyperactivity observed in AN patients.

### 6–04
### Anorexia assessment in hospitalized patients with digestive and non-digestive tumors

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**Background and aims:** Anorexia has long been recognized as a complex and common symptom in cancer patients leading to malnutrition. Its assessment may be difficult and its prevalence among patients with cancer with different organ involvement has not been widely documented before treatment. Therefore, our aim was to study the prevalence of anorexia among hospitalized patients with digestive and non-digestive tumors at the time of diagnosis.

**Methods:** Patients were self-administered a questionnaire including information on weight loss and self-perception of food eaten (appetite change, % served-food eaten represented as a pie chart); the FAACT-ESPEN score, the anorexia questionnaire and VAS for anorexia. Data were compared by Student's t and chi-square tests, and differences were significant when a $p$ value was $<0.05$.

**Results:** 89 patients were included (56 non-digestive –ND- tumors, 33 digestive –D- tumors, age 54.6+16.9 years and 62+12.8 years, respectively). Patients with D tumors had significantly more metastases and had lost weight more frequently and in a greater amount than patients with ND tumors. Regarding anorexia, neither a difference on the FAACT-ESPEN or VAS were found, nor on the percentage of the food usually eaten (most patients with D or ND tumors referred eating 75–100 %), or on changes in taste, smell, vomiting or self-referred anorexia; however, patients with D tumors had a significantly greater self-referred weight loss, appetite decrease and early satiety.

**Conclusion:** Patients with D tumors may experience greater anorexia, which reflects on a greater weight loss.

### 6–05
### Effects of oral meal feeding on whole body protein breakdown and protein synthesis in cachectic pancreatic cancer patients

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**Background:** Pancreatic cancer is often accompanied by cachexia, a syndrome of severe weight loss and muscle wasting. A suboptimal response to nutritional support further aggravates cachexia. The influence of nutrition on protein kinetics in these patients is poorly understood.

**Aim:** To investigate the effect of feeding on protein kinetics in cachectic pancreatic cancer patients.

**Methods:** Eight cachectic pancreatic cancer patients and seven control patients received a primed continuous intravenous infusion of L-[ring-2H5]-Phenylalanine and L-[3,3-2H2-Tyrosine for 8 h. After 4 h oral feeding was started (0.083 g protein/kg/h). Whole body protein breakdown (PB) was measured as total rate of appearance (Ra) of Phenylalanine corrected for exogenously administered Phenylalanine. Protein synthesis (PS) was calculated as total Phenylalanine Ra minus rate of Phenylalanine hydroxylation. Net protein balance (NB) was calculated by subtracting PB

from PS. Results are given as mean ± standard deviation in μmol/kg lean body mass/h.

**Results:** Baseline PB and PS were higher in cachectic patients compared with controls (breakdown: 63.1±18.2 versus 43.9±4.6, $p=0.049$; and synthesis: 59.1±17.5 versus 39.3±5.8, $p=0.021$). During feeding, PB decreased significantly in both the cachexia group (63.1±18.2 to 30.7±5.9, $p=0.012$) and the control group (43.9±4.6 to 17.1±11.6, $p=0.018$). This decrease was similar in both groups ($p=0.487$). PS was not affected by feeding in cachectic patients (59.1±17.5 to 58.6±18.6, $p=1.000$), but was stimulated in controls (39.3±5.8 to 49.1±3.1, $p=0.018$), which was significantly different ($p=0.021$). Both groups achieved a positive NB during feeding: 28.0±13.1 (cachexia) and 32.0±7.0 (control), $p=0.487$.

**Conclusion:** Cachectic pancreatic cancer patients have a higher protein turnover at baseline. Both cachectic patients and controls are able to achieve a comparable positive NB during feedings. In cachectic patients this is modulated by reduced PB, whereas in controls both PB and PS are modulated. Impaired nutritional stimulation of PS in pancreatic cancer cachexia should be a topic in future studies.

### 6–06
### Overweight and obesity is associated with improved functional outcome after stroke

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**Background** Obesity is a common risk factor in the development of cardiovascular diseases such as stroke. The impact of obesity on functional outcome after stroke is less well established. The aim of this study was to evaluate the functional outcome after stroke and effect of hospitalized post stroke-rehabilitation according to body weight.

**Methods** 101 patients with acute ischemic or haemorrhagic stroke (age 70±11y, BMI 26.7±5.7 kg/m$^2$, all mean ± SD) admitted to in-patient rehabilitation centre were studied. Patients were categorized as underweight (BMI <18.5, $n=3$), normal (BMI 18.5 to <25, $n=43$), overweight (BMI 25 to <30, $n=33$), obesity (BMI 30 to <35, $n=17$) and advanced obesity (BMI ≥35 all kg/m$^2$, $n=4$). Physical examinations performed at admission (23±18 days after acute stroke) followed by follow up (FU) examinations (27±5 days) included functional assessment scores: Barthel Index [BI], modified Rankin Score [mRS], and Rivermead Motor Assessment [RMA].

**Results** Obese patients had better functional status at admission: BI 72±25, mRS 3.1±1.0, RMA 6.4±2.6 than overweight patients: BI 64±22, mRS 3.5±0.8, RMA 5.7±2.3 and patients with normal BMI: 50±22, mRS 3.8±0.7, RMA 4.7±1.9. The worst functional status presented in underweight patients (BI 42±15, $p<0.05$, mRS 4.0, RMA 4.6±0.6). Functional scores improved after rehabilitation in the total cohort and in all subgroups. The most improvement was seen in overweight group (BI 82+19, mRS 3.0+1.0, RMA 8.3+2.9, all $p<0.0001$) Still, at discharge from hospitalized rehabilitation obese patients presented best functional outcome (BI 81±18, mRS 2.9±11, RMA 7.1±3.0) than any other BMI group followed by overweight patients (BI 82+19, mRS 3.0+1.0, RMA 8.3+2.9, all $p<0.0001$) and patients with normal weight (BI 67±22, mRS 3.5±0.9, RMA 6.7±2.3, all <0.001).

**Conclusion** Obese and overweight patients have better functional outcome after stroke. This finding holds true before and after post stroke rehabilitation.

### 6–07
### Parenteral Nutrition Survey

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**Background and Aims:** The role of parenteral nutrition (PN) in patients with incurable cancer and malignant bowel obstruction remains controversial. We aim to investigate current practice of PN by survey selected groups from ESMO, MASCC, ESPEN and EAPC.

**Methods:** Internet-based survey investigating decisions on PN in patients with malignant bowel obstruction based on two case-vignettes. We categorized participants into 3 groups: group 1: recommendation of PN in both cases, group 2: recommendation of PN in 1 case and group 3: no recommendation. For statistical analysis we assembled group 1 and 2 and compared them with group 3. Statistical analysis was performed with chi-square test, http://quantpsy.org.
**Results:** Eighty-one participants (56.6 %) completed the survey. Nine (11 %) recommended PN in both cases, 37 (46 %) in one case and 35 (43 %) did not in any case. Contrary to a higher amount of clinical work (>75 %: 44 %, 57 % and 60 %) and number of incurable patients (>75 %: 11 %, 22 % and 23 %) the impact of PN in routine care decreased in group 1,2 and 3 (very important: 56 %, 22 % and 0 %; important: 22 %, 19 % and 11 %). Cachexia stage (case 1: cachexia, case 2: refractory cachexia) was estimated correctly by 11 %, 54 % and 11 %, cachexia classified routinely by 44 %, 35 % and 23 %. By comparing group 1 and 2 ($n$ =46 (57 %)) with group 3, in group 1 and 2 there was a lower amount of oncologists (26 % vs. 51 %, $p$ =0.019) and palliative care specialists (13 % vs. 32 %, $p$ =0.023), a lower number of participants who would recommend PN in refractory cachexia (17 % vs. 49 %, $p$ =0.003) and a higher number who would recommend PN in pre-cachexia (57 vs. 6 %, $p$ =0.000). A Karnofsky index >70 % was presupposed by 26 % compared to 49 % in group 3 ($p$ =0.036).
**Conclusions:** A large heterogeneity of views, attitudes and understanding of cachexia affects prescribing modalities of experienced clinicians.

### 6–08
### Development of new diagnostic reagents of transthyretin and transferrin

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**Background**: Rapid turnover protein (RTP) including transthyretin (TTR) and transferrin (Tf) is known as an indicator of patients' nutritional status. The RTP has been measured mainly using nephelometers, and it was difficult to use general clinical analyzers. This raises the measured levels when measuring sera with turbidity. We have developed new diagnostic reagents of TTR and Tf which have the following advantages: (1) Adaptability to general clinical analyzers, (2)No influence with turbidity.
**Methods**: The assay principle is applied turbidimetric immunoassay (TIA) because of being adaptable to general clinical analyzers. All the reagents are ready-to-use. In the fundamental study, dynamic range, effects of interfering substances, accuracy and reproducibility were evaluated using Hitachi 7170 clinical analyzer. In the clinical study, 51 and 53 serum samples for TTR and Tf, respectively, were compared with those measured by nephelometry as a conventional method.
**Results**: The dynamic range for TTR and Tf was 2–60 mg/dL and 10–600 mg/dL, respectively. The interference of turbidity was not observed. In addition, bilirubin, hemolysis and rheumatoid factor did not influence the measured levels. The accuracy for TTR and Tf was 2.2 % and −0.5 %, respectively, with respect to certified values of the serum protein reference material IRMM ERM-DA470. The reproducibility for TTR and Tf was 0.8 % and 1.3 %, respectively. The correlation with conventional nephelometry was obtained to be y=1.05x 0.538 for TTR ($r$ =0.996, $n$ =51), and the correlation for Tf was y=1.00x 4.54($r$ =0.992, $n$ =53).
**Conclusions**: The newly developed reagents for TTR and Tf had good performance characteristics. The reagents are showed to be useful for nutritional monitoring of patients even if with turbidity.

### 6–09
### Prevention of inflammation-associated muscle atrophy and weakness with branched-chain amino acids as treatment for autoimmune myositis

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**Background and aims:** Polymyositis (PM) and dermatomyositis (DM) are autoimmune diseases characterized by muscle inflammation, atrophy, and weakness. Steroid myopathy, an adverse effect of steroid therapy, can occur during treatment of PM/DM. Although previous studies showed that branched-chain amino acids (BCAAs) prevent steroid-induced muscle atrophy, their efficacy for inflammation-associated muscle atrophy was unclear. The aim of this study was to clarify the efficacy of BCAAs for inflammation-associated muscle atrophy using C2C12 myotubes and mice with C protein-induced myositis (CIM), a murine model of PM.
**Methods:** In in vitro experiments, C2C12 myotubes, treated with TNFalpha or IL1beta in media containing either high or low BCAA concentrations, were evaluated for morphology, diameter, and gene expression. In in vivo experiments, CIM mice were administered BCAAs. Their muscle weight, gene expression, grip strength, and histological score were

measured to evaluate muscle atrophy, function, and inflammation status. Effects of BCAA in combination with prednisolone (PSL), a synthetic steroid, were also examined.
**Results:** Cultivation of C2C12 myotubes in media containing high BCAA concentrations inhibited pro-inflammatory cytokine-induced myotube atrophy and expression of atrogenes, as compared with media containing low BCAA concentrations. Correspondingly, BCAAs prevented muscle atrophy and weakness in CIM mice, while muscle inflammation was not affected by BCAAs. In contrast, treatment with a combination of BCAAs and PSL prevented not only muscle atrophy and weakness, but also muscle inflammation.
**Conclusions:** BCAAs have an anti-atrophic effect on inflammation-associated muscle atrophy in vitro and in vivo. BCAAs are likely to be effective for treating muscle atrophy and weakness of patients with PM/DM, even when accompanied by steroid myopathy.