

Abstracts of the 6th Cachexia Conference, Milan, Italy, December 8–10, 2011

Published online: 8 November 2011

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1-01

Leucine modulates the effects of the Walker tumour's proteolysis-inducing factor on gene expression and cells activity in C₂C₁₂ myotubes

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Cancer cachexia involves a severe weight lost, particularly from waste of skeletal muscle, which can arise from the increase on protein catabolism and/or the decrease on protein synthesis. The protein degradation in skeletal muscle of cancer patients is mediated by the cytokine proteolysis-inducing factor (PIF), produced by tumour, which increases the expression of the ubiquitin–proteasome pathway, and the skeletal muscle protein synthesis in these patients can be affected by several agents, including nutrient signalling. Some nutrients, such as branched-chain amino acid leucine, can decrease the expression of the ubiquitin–proteasome pathway and improve the protein content in skeletal muscle of cachectic animals. In this study, we investigated the effects of leucine on cell viability, morphology, functional proteasome activity, enzymatic activities, and protein synthesis and degradation in C₂C₁₂ skeletal muscle cells exposed to the PIF-like protein, purified from the Walker tumour-bearing rat. The Walker factor (WF), immunologically identical to PIF and with the same molecular weight, presented no cytotoxic effect in myotube cells, which had no alterations in their morphological characteristics in the presence of WF and/or leucine. However, increased phosphatase alkaline activity was observed, especially at low WF concentrations. At higher WF concentrations, chymotrypsin-like activity and also the 20S proteasome gene expression were increased, as well as the cathepsin B activity trended to increase. Adding leucine previously to WF-treated myotubes cells, proteasome activity decreased and phosphatase alkaline activity increased. Total protein synthesis was decreased in WF-treated cells in parallel to increase in protein degradation. These changes were minimized or reverted after leucine exposure. Taken together, these results suggested an important modulatory effect of leucine under the WF actions in C₂C₁₂ myotube cells.

1-02

Habitual skeletal muscle protein fractional synthetic rate in healthy individuals as determined by a novel oral tracer technique

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Background/aims: Using current methodology skeletal muscle fractional synthetic rate (FSR) is measured over short time periods in response to specific stimuli (e.g. feeding/exercise) resulting in wide variation in FSRs. In clinical studies, interventions occur over weeks or months and measures over longer periods may be more representative. We aimed to develop a novel method to determine skeletal muscle protein FSR to estimate habitual FSR in healthy individuals, over timescales comparable with clinical interventions, avoiding intravenous amino acid tracer.

Method: Four healthy males, median (range) age: 37 years (32–52), height 179 cm (177–185), weight 80 kg (71–87) were given 100 g water enriched to 70 atom% with 2H₂O as a single oral bolus. Vastus lateralis biopsies were performed using a Bergstrom needle at intervals 4–12 days post-dose. Serum was collected at baseline and three time-points between 3 and 14 days, measuring body water enrichment and analysis of plasma alanine enrichment (GC–pyrolysis–IRMS). Myofibrillar protein was isolated, acid hydrolysed and 2H-alanine enrichment measured. Skeletal muscle protein FSR was calculated (% day⁻¹) both using free 2H-alanine and body water to predict precursor enrichment.

Results: Body water 2H increased to 1520 ppm excess (1,435–1,582). Elimination half time was 8 days (7–10). The r₂ for the natural log of 2H enrichment against time was >0.999 in each individual. Plasma alanine was labelled in a predictable manner (in theory, four atoms become labelled; 3.1–4.2 were measured). Skeletal muscle FSR was calculated from six biopsies in four individuals as 1.18% day⁻¹ (0.94–1.59). For the two individuals with two biopsies each at different times, the differences in estimates of FSR were 14.3% and 20%.

Conclusions: This is the first study to describe skeletal muscle protein FSR in free-living healthy individuals over 4–12 days. Using a single oral 2H₂O bolus, endogenous labelling of alanine occurs in a predictable manner giving estimates of FSR comparable with published values.

1-03

Environmental conditions in muscle tissue culture alter responses to atrophy signals

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Muscle atrophy is a powerful determinant of poor prognosis, impaired physical function, and debility in all chronic diseases, including cancer. Laboratory models of muscle wasting have suggested a number of different mechanisms which can contribute to cancer-related muscle atrophy. However, paradoxically, very little progress has been made in developing therapies which are clinically effective in treating muscle wasting in cancer patients. Muscle tissue culture still has huge potential to help develop new treatments but the factors which have impeded the effective translation of basic research into clinical treatments need to be identified and addressed. For this reason, we studied the effects of using tissue culture conditions that reproduce the *in vivo* physiological environment of muscles. We first established two highly defined and reproducible muscle tissue culture systems using C2C12 myotubes under standard and modified conditions. These modified conditions have been chosen to attempt to mimic certain key aspects of the *in vivo* environment experienced by muscle in cancer patients, on the assumption that this may promote more representative adaptive responses. We then assessed whether such modified conditions alter the responses of mature myotubes to distinct atrophy-inducing treatments. After 3 days of treatment, we observed a 20–30% reduction in protein and myotube diameter but a 46% increase in myotube number in modified conditions compared to standard culture conditions. Only small differences in most atrophy-related gene expression or protein levels for markers of muscle growth and phenotype were observed but modified conditions did induce a 70% reduction in IGF-1 mRNA levels. However, surprisingly, the modified culture conditions offered partial or complete protection against further atrophy due to added atrophy-inducing treatments. This protection was observed despite similar changes in the transcript levels of selected atrophy-related genes. These studies confirm the potential for environmental conditions to modulate muscle responses to atrophy-inducing conditions *in vivo*.

1-04

During early recovery the worsening of immobilization-induced rat tibialis anterior muscle atrophy is associated with sustained activation of proteolytic and apoptotic pathways

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Background and aims: Muscle atrophy induced by immobilization stabilized in the shortened gastrocnemius muscle (GA), but worsened in the lengthened tibialis anterior muscle (TA) after 10 days of recovery. Thus, important remodeling within the TA following immobilization could condition the initial steps of recovery and may depend on the position of immobilization. The ubiquitin–proteasome system (UPS) and the mitochondria-associated apoptosis being involved in GA atrophy their role in muscle remodeling were studied during the very early stages of recovery in both the TA and GA.

Methods: Adult rats were subjected to unilateral hindlimb immobilization and the contralateral non-immobilized leg served as the control. After 8 days of immobilization (I8), casts were removed and animals were allowed to recover for 1 (R1) to 10 (R10) days. UPS-dependent proteolysis and apoptosis were evaluated in both the TA and GA.

Results: Muscle atrophy rapidly worsened after cast removal as soon as R1 and up to R6 in the TA previously immobilized, but stabilized in the GA from R1. In addition, a more pronounced and sustained activation of proteasome and apoptosome activities prevailed in the immobilized TA from I8 until R10, but was normalized at R6 in the previously immobilized GA.

Conclusions: Altogether, the data suggest that UPS-dependent proteolysis and mitochondria-associated apoptosis are involved in the muscle remodeling during recovery and may be responsible for the worsening of muscle atrophy observed in the TA. Alterations of connective tissue being differentially altered upon stretching during immobilization (Mattiello-Sverzut et al. *Histol Histopathol* 2006; 21:957–964), we hypothesized that this may impact muscle proteolytic signaling pathways.

1-05

MSY-3/Csda coordinates repression of myogenic genes in muscle wasting

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Background and aims: The Y-box protein MSY-3/Csda regulates postnatal repression of the myogenic transcription factor myogenin. In postnatal muscle, myogenin plays a key role in regulating pathways involved in muscle maturation, degeneration and eventually cachexia. MSY-3 binds a highly conserved DNA *cis*-acting element located upstream of the myogenin promoter. We wanted to find other possible targets of MSY-3 regulated in concert during muscle maturation and atrophy.

Methods: ByChIP assay, we analyzed MSY3/Csda binding *in vivo* in adult muscle and in C2C12. We tested the MSY-3/MyoD conserved site transcriptional activity by luciferase assay and *in vivo* electroporation. By high-throughput technology, we genome-wide analyzed MSY-3 DNA binding in adult muscle and expression profile of wt and MSY-3 knockout mice and denervated muscle.

Results: We found a conserved regulative *cis*-element in the MyoD locus, that matches with the myogenin *cis*-motif and by ChIP assays we verified that both MyoD and MSY-3/Csda bind to it *in vivo*. Luciferase assays show that the MYOD site, is sufficient for high levels of expression in C2C12 and MSY-3 acts as a repressor. *In vivo* muscle electroporation demonstrates that the MYOD site is required for MyoD postnatal repression. Furthermore, in combined genome-wide analysis of MSY-3 DNA binding and global RNA expression, we found other candidate genes possibly repressed by MSY-3 during adult muscle maturation, as well as myogenin, MYOD, AChRs and HDAC4.

Conclusions: This study suggests that both MyoD and myogenin are controlled by the same repressor complex (MSY-3/Csda), recognizable by a similar *cis*-motif. This motif is responsible for MYOD auto activation/maintenance during muscle differentiation and its repression MSY-3-mediated in postnatal muscle, suggesting a developmental stage depending competition for the two transcription factors at the same site. This strongly suggests that MSY-3 regulates in concert MYOD and myogenin expression. Moreover, the genome-wide analysis highlights a group of genes possibly modulated by the same regulatory complex during muscle maturation and degeneration. These findings indicate MSY3/Csda as a potential target for muscle wasting caused by neurogenic atrophy, sarcopenia, and cachexia.

1-06

Resveratrol blocks glucocorticoid-induced muscle wasting in L6 cultured myotubes through a SIRT1-dependent mechanism

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Background and aims: Muscle proteolysis during sepsis and other catabolic conditions is, at least in part, regulated by glucocorticoids. Dexamethasone-treated myotubes are a commonly used *in vitro* model of muscle wasting. We reported recently that decreased expression and activity of histone deacetylases (HDACs) including sirtuin (SIRT) 1 are involved in the development of muscle wasting. Here, we tested the hypothesis that treatment with the SIRT1 activator resveratrol would prevent glucocorticoid-induced protein degradation and muscle atrophy, and that glucocorticoid-induced expression of atrogin-1 and MuRF1 is, at least in part, regulated by SIRT1.

Methods: L6 myotubes were treated for 24 h with 1 μ M dexamethasone in the absence or presence of resveratrol (100 μ M). After treatment for 24 h, myotubes were harvested for determination of atrogin-1 and MuRF1 mRNA and protein levels, protein degradation, and myotube diameter. In additional experiments, the effects of resveratrol treatment were tested in myotubes transfected with non-targeting or SIRT1 siRNA.

Results: Treatment with dexamethasone increased atrogin-1 and MuRF1 expression and protein degradation and reduced myotube diameter. Resveratrol treatment inhibited the dexamethasone-induced increase in atrogin-1 and MuRF1 expression and protein degradation as well as the reduction in myotube size. SIRT1 siRNA abolished the effect of resveratrol on atrogin-1 and MuRF1 expression.

Conclusions: Results suggest that resveratrol treatment may prevent glucocorticoid-induced muscle wasting through a SIRT1-dependant mechanism. SIRT1 activation may be a novel therapeutic approach in combating muscle wasting conditions. Supported by R01 DK37908 from the NIH.

1-07

Decreased NADPH oxidase expression and antioxidant activity in cachectic skeletal muscle

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Background: Cancer cachexia is the progressive loss of skeletal muscle protein that contributes significantly to cancer morbidity and mortality. Evidence of antioxidant attenuation and the presence of oxidised proteins in patients with cancer cachexia indicate a role for oxidative stress. This study aimed to investigate the superoxide generating NADPH oxidase (NOX) enzyme and antioxidant enzyme systems in murine adenocarcinoma tumour-bearing cachectic mice.

Method: Superoxide levels, mRNA levels of NOX enzyme subunits, and the antioxidant enzymes were measured in the skeletal muscle of mice with cancer and cancer cachexia. Protein expression levels of NOX enzyme subunits and antioxidant enzyme activity were also measured in these samples.

Results: Superoxide levels increased 1.4-fold in the muscle of mice with cancer cachexia, and this was associated with a decrease in mRNA of NOX enzyme subunits, NOX2, p40phox, and p67phox along with the antioxidant enzymes SOD1, SOD2, and GPx. Cancer cachexia was also associated with a 1.3-fold decrease in SOD1 and 2.0-fold decrease in GPx enzyme activity.

Conclusion: Despite increased superoxide levels in cachectic skeletal muscle, NOX enzyme subunits, NOX2, p40phox, and p67phox, were downregulated along with the expression and activity of the antioxidant enzymes. Therefore, the increased superoxide levels in cachectic skeletal muscle may be attributed to the reduction in the activity of endogenous antioxidant enzymes.

1-08

Differential adaptation of glycolytic and oxidative muscle to hypoxia

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Background: Hypoxia may be a trigger of skeletal muscle atrophy in acute and chronic respiratory disease. In chronic obstructive pulmonary disease patients, muscle fiber atrophy is most pronounced in glycolytic compared to oxidative muscle fibers. Therefore, we hypothesized that hypoxia preferentially affects glycolytic muscles.

Methods: Mice were maintained under normoxia or hypoxia. During 48 h, oxygen levels were reduced stepwise to 8%, which was maintained for 21 days. Food intake was monitored daily and mice were sacrificed at days 4 and 21.

Results: Pair-feeding experiments revealed that the reduction in oxidative (Soleus) muscle weight was solely attributable to hypoxia-induced reduced food intake (hypophagia), whereas hypoxia caused a greater decrease of the glycolytic muscle weight, which was not accounted for by hypophagia. Furthermore, cross-sectional area (CSA) of the individual fibers revealed reduced CSA in glycolytic muscle, whereas no reduction was seen in the oxidative muscle. Expression levels of genes involved in proteasomal protein degradation (Atrogin-1), lysosomal protein degradation (Lc3B, Atg5), endoplasmic reticulum (ER) stress signaling (Atf4 and Gadd34), and glucocorticoid receptor (GR) signaling (Gluc and GR) were increased by hypoxia in both muscles. Interestingly, only in glycolytic muscle, the upregulation involved a hypoxia-specific component and was not merely explained by hypophagia. Furthermore, capillary contacts per muscle fiber CSA, increased in both muscles in response to hypoxia. In the oxidative muscle, this was the result of a reduction in interstitial space, whereas this adaptation was accounted for by decreased fiber CSA in the glycolytic muscle.

Conclusions: Muscle mass, proteolysis, ER stress, and GR signaling in the glycolytic muscle are more affected than in the oxidative muscle by hypoxia. In addition, in oxidative, but not in the glycolytic muscle, these alterations are accounted for by hypophagia, indicative of an intrinsic sensitivity of glycolytic muscle to hypoxia, which may involve a differential adaptive capacity of the vascular bed.

1-09

The incidence of sarcopenia and its effects on body composition, physiological function, nutrition, and fatigue in patients before allogeneic hematopoietic stem cell transplantation

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Background and aims: Cachexia in patients with haematological malignancies is often related to sarcopenia. We believe that allogeneic hematopoietic stem cell transplant (allo-hematopoietic stem cell transplantation (HSCT)) patients are often stricken by sarcopenia prior to transplantation. The aim of this study is to investigate the incidence of sarcopenia and the relationship of sarcopenia with body composition and physiological function, nutrition, and fatigue in patients before allo-HSCT.

Methods: Study participants included patients who had undergone allo-HSCT from May 2007 to July 2011. One hundred and sixty-four patients were included in this study (median 50 years). Body composition was evaluated using bioelectrical impedance analysis. Evidence of sarcopenia was calculated using the male (<8.87 kg of skeletal muscle mass/m²) and female (<6.42 kg of skeletal muscle mass/m²). Physiological functions were evaluated by handgrip, knee extensor strength, and 6-min walk test (6MWT).

Results: Eighty-three patients (50.6%) enrolled in our study had sarcopenia prior to allo-HSCT. Lean body mass (LBM) strongly correlated with handgrip and knee extensor strength ($r=0.72\text{--}0.75$, $p<0.01$) and weakly correlated with 6MWT ($r=0.34$, $p<0.01$). Body weight and fat body mass correlated with fatigue ($r=-0.15$ and $r=-0.2$, respectively, $p<0.05$). Patients with sarcopenia experienced decreased muscular strength and increased fatigue compared with patients without sarcopenia (handgrip= -12% , knee extensor strength= -12% , fatigue= $+22\%$, $p<0.05$).

Conclusion: Sarcopenia has been reported to occur at a rate of 5% (2 SD) in the elderly. In this study, roughly half of the patients had sarcopenia before allo-HSCT. Therefore, patients may have decreased muscle mass prior to allo-HSCT. In the evaluation of body composition, we discovered that muscle strength and LBM showed a high correlation. Patients with sarcopenia also have decreased muscle strength due to muscle atrophy and we believe that this causes increased fatigue in these patients.

1-10

Oleic acid accelerates myogenic differentiation through modulation of Notch and beta-catenin pathways in muscle stem cells

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Background and aims: Marked changes in fatty acid composition occur during myogenic differentiation of muscle stem cells. In the present study, we demonstrated an increase in content of unsaturated fatty acids (as oleic acid) and delta-9-desaturase activity suggesting synthesis of oleic acid during myogenesis. The effects of oleic acid in muscle stem cell proliferation and myogenesis were investigated.

Methods: The effect of oleic acid in muscle stem cell proliferation was evaluated by ^{14}C -thymidine incorporation. The content of markers of notch and beta-catenin pathways in undifferentiated muscle stem cells and expression of myogenic regulator factors (MyoD, myogenin, and myf-6) in differentiating myotubes were evaluated by western blotting.

Results: The addition of oleic acid to myogenic differentiation medium led to an increase in myoblast fusion, myotube diameter, and length and in expression of MyoD, myogenin, and myf-6. At 8 days of culture in myogenic differentiation medium, increased diameter of skeletal muscle cells and content of myosin heavy chain were also observed due to oleic acid addition. The treatment of undifferentiated muscle stem cells with oleic acid decreased expression of HES-1 and cell proliferation and raised p38-MAPkinase phosphorylation, NF-kappaB activity, and the content of phospho(Ser9)-GSK3beta and beta-catenin.

Conclusion: These results support the proposition that oleic acid accelerates myogenesis by mechanism associated to increased p38-MAPkinase/NF-kappaB pathway, beta-catenin stabilization, and decrease in Notch signaling.

1-11

Oleic acid accelerates myogenic differentiation through modulation of Notch and alpha-catenin pathways in muscle stem cells

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Marked changes in fatty acid composition occur during myogenic differentiation of muscle stem cells. Increased content of unsaturated fatty acids (as oleic acid) and delta-9-desaturase activity were observed suggesting synthesis of oleic acid during myogenesis. The addition of

oleic acid to myogenic differentiation medium led to an increase in myoblast fusion, myotube diameter, and length and expression of myogenic regulator factors (MyoD, myogenin and myf-6). At 8 days of culture in myogenic differentiation medium, increased diameter of skeletal muscle cells and content of myosin heavy chain were also observed due to oleic acid addition. The treatment of undifferentiated muscle stem cells with oleic acid decreased expression of HES-1 and cell proliferation and raised p38-MAPkinase phosphorylation, NF-kappaB activity and the content of phospho(Ser9)-GSK3beta and alpha-catenin. These results support the proposition that oleic acid accelerates myogenesis by mechanism associated to increased p38-MAPkinase/NF-kappaB pathway, alpha-catenin stabilization and decrease in Notch signaling.

1-12

Dexamethasone-induced increase in FOXO1 activity and expression of atrogen-1 and MuRF1 in cultured muscle cells is PPAR β/δ -dependent

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Background and aims: Muscle wasting during various catabolic conditions is at least in part mediated by glucocorticoids. Corticosteroids upregulate FOXO1 activity, expression of atrogen-1 and MuRF1, and muscle proteolysis. Recent studies suggest that activation of the transcription factor PPAR β/δ upregulates the expression and activity of FOXO1 and the expression of atrogen-1 and MuRF1. The role of PPAR β/δ in glucocorticoid-regulated muscle wasting, however, is not known. We hypothesized that activation of PPAR β/δ results in increased protein degradation and muscle atrophy and that glucocorticoid-induced FOXO1 activation, expression of atrogen-1 and MuRF1, and protein degradation are at least in part regulated by PPAR β/δ .

Methods: Cultured L6 myotubes (a rat skeletal muscle cell line) were treated for 24 h with the PPAR β/δ agonist GW0742 (0.1 μM) or dexamethasone (1 μM) in the absence or presence of the PPAR β/δ antagonist GSK0660 (0.1 μM) followed by measurement of FOXO1 DNA-binding activity, mRNA and protein levels for atrogen-1 and MuRF1, protein degradation, and myotube diameter. In additional experiments, the effects of dexamethasone were tested in myotubes transfected with non-targeting or PPAR β/δ siRNA.

Results: Treatment of the myotubes with GW0742 increased FOXO1 DNA-binding activity, atrogen-1 and MuRF1 mRNA and protein expression, and protein degradation, and reduced myotube diameter. Dexamethasone stimulated PPAR β/δ and FOXO1 activity, atrogen-1 and MuRF1 expression, and protein degradation, and reduced myotube diameter. The effects of dexamethasone were blocked by GSK0660 or PPAR β/δ siRNA.

Conclusions: Results suggest that PPAR β/δ activates the atrophy program in skeletal muscle and that glucocorticoid-induced muscle wasting is at least in part regulated by PPAR β/δ . The transcription factor PPAR β/δ may be a target for the treatment and prevention of muscle wasting.

This work was supported by NIH R01 DK37908 (POH) and by a postdoctoral fellowship from Gobierno Vasco BFI2010-240 (EC).

1-13

Bed rest-induced muscle wasting is associated with modulation of myogenic markers

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Background/aims: Bed rest is associated with loss of skeletal muscle mass and strength, mainly due to protein hypercatabolism. In addition, inactivity has been shown to be associated with inflammation and oxidative stress that may lead to modulation of transcriptional factors that regulate myogenesis. In the present study, we investigated the relationship between prolonged muscle immobilization and gene expression of factors involved in myogenesis, such as MyoG, MyoD, Myf5 and Pax7.

Methods: Twenty healthy young male volunteers were recruited for a period of strict bed rest (33 days). All daily activities were performed in horizontal clinostatic conditions. For each volunteer, three biopsies were performed in the vastus lateralis muscle; the first, 1 day before bed rest (control), the second and the third after 7 and 33 days from the beginning of immobilization, respectively. Muscle biopsies were used to assess gene expression of MyoG, MyoD, Myf5 and Pax7 by real-time PCR.

Results: Pax7 and Myf5 expression is reduced of about 20–25% at both days 7 and 33 of immobilization. As for Myf5, at day 33, there is a tendency to restore control values. MyoD gene expression increases of about 50% after 7 days of bed rest, and returns to basal levels at day 33. Finally, MyoG expression is increased, though not significantly, due to sample variability, after both 7 and 33 days of immobilization.

Conclusions: Our results show that bed rest-induced muscle wasting is associated with downregulation of Pax7 and Myf5 mRNA levels. This could indicate a reduced satellite cell population, resulting either from enhanced differentiation, as suggested by increased MyoG expression, or by satellite cell death, due to inflammation and oxidative stress associated with inactivity. Further studies are in progress to clarify this point.

1-14

Muscle actin is polyubiquitinated in C2C12 myotubes and human muscle biopsies and targeted for breakdown by the E3 ligase MuRF1 Cécile Polge¹, Anne-Elisabeth Heng¹, Daniel Béchet¹, Lydie Combaret¹, Bernard Monsarrat², Daniel Taillandier¹, Didier Attaix¹ (¹INRA, UMR 1019, Unité de Nutrition Humaine, CRNH Auvergne, F-63000 Clermont-Ferrand, France; ²CNRS, Institut de Pharmacologie et de Biologie Structurale, 205 route de Narbonne, F-31077, Toulouse, France)

Background and aims: Elaborating new strategies to prevent muscle wasting requires information on the precise mechanisms of the breakdown of contractile proteins. Experiments were performed (1) to demonstrate that actin is polyubiquitinated in muscle cells and human biopsies and thus degraded by the 26S proteasome and (2) to identify the ubiquitin (Ub) protein ligase E3 responsible for actin recognition and polyubiquitination.

Methods: Chimeric flag-actin was stably transfected in C2C12 myotubes treated or not with 1 μM dexamethasone (Dex). Poly-Ub conjugates in C2C12 cells and human muscle biopsies were purified using an affinity matrix and deubiquitination as described (Ventadour et al. J. Biol. Chem. 2007, 282:5302–9). We used glutathione S-transferase pull-down and ubiquitination assays to detect MuRF1-actin interaction and MuRF1-dependent actin polyubiquitination. mRNA levels of E3 ligases were measured by qRT-PCR analysis in C2C12 myotubes treated or not with Dex. C2C12 myotubes were transfected with siRNAs to induce MuRF1 knockout.

Results: Chimeric flag-actin was destabilized and polyubiquitinated in stably transfected and Dex-treated C2C12 myotubes and only proteasome inhibitors blocked its breakdown. Poly-Ub actin was also detected in human muscle biopsies from control and cancer patients. An increase in MAFbx/atrogen1, Trim32 and Ozz mRNAs was observed in Dex-treated C2C12 cells, while Nedd4, E4b, MuRF1 and MuRF3 mRNAs did not change significantly. However, MuRF1 protein content increased in Dex and Dex+MG132 treated C2C12 cells and in muscles from early-stage

cancer patients. Actin was polyubiquitinated by MuRF1 in an in vitro ubiquitination assay. Accordingly, MuRF1 siRNA stabilized the breakdown of flag-actin.

Conclusions: These data demonstrate that actin is polyubiquitinated in muscle cells and human biopsies, and that MuRF1 is implicated in this process. This further supports the need for new strategies blocking specifically the activation of the UPS and/or MuRF1 in wasting diseases.

Work supported by the Association Française contre les Myopathies.

1-15

Role of ubiquitin E3 ligases in sarcopenia: towards an integrative understanding of their roles in muscle wasting

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Background and aims: Progressive loss of sarcomeres, leading ultimately to a state referred to as sarcopenia, is induced by a variety of atrophy-inducing signals, including physical inactivity, cytokines and inflammation, and metabolic disorders. It has been postulated that the induction of E3-type ubiquitin ligases represents an initial and rate limiting step in the degradation of muscle proteins, thereby providing attractive targets for atrophy attenuation.

Methods: Here, we have studied mice that were either deficient for the E3 ligase MuRF1 (MuR1-KO), or that overexpressed MuRF1 under the control of a CK promoter (MuRF1-CK-TG).

Results: We characterized their responses to a variety of atrophy inducing stimuli, including denervation, hindlimb suspension, and injection of TNF-alpha. Our results indicate that inactivation of MuRF1 leads to potent protection from muscle atrophy, and that protection occurred preferentially in fast fiber types. Intriguingly, we also noted a protection of force-bearing bones from demineralization in MuRF1-KO mice and thus in a context where MuRF1 is not known to be expressed. Consistent with systemic regulatory effects of MuRF1 during atrophy, we noted metabolic effects on lipid and glucose oxidation, and circulating amino acid levels.

Conclusions: Future studies are warranted if additional humoral pathways regulated by MuRF1 may contribute to sarcopenia protection in addition to the known intra-muscular pathways involved in the degradation of structural muscle proteins.

1-16

Mice lacking the USP19 deubiquitinating enzyme show less muscle wasting in response to several catabolic conditions due to suppression of ubiquitin ligases and autophagic genes and enhanced rates of protein synthesis

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Background and aims: Although many enzymes involved in ubiquitination are activated in atrophying muscle, little is known about the role of deubiquitinating enzymes (DUBs). We previously showed that the USP19 DUB is induced in various conditions of muscle atrophy including cancer and that silencing of USP19 in muscle cells increases expression of myofibrillar proteins. To test the relevance of this in vivo, we inactivated USP19 in mice and characterized the wasting response induced by denervation, fasting, and cancer.

Methods: USP19 KO or WT mice were subjected to the above catabolic conditions. Muscle mass, myofiber area, protein synthesis (flooding dose method), mRNAs encoding ubiquitin ligases MuRF1, atrogin-1, and autophagy genes Bnip3, Atg4, Gabarap and LC3 were measured.

Results: USP19 KO mice showed 26–39% less muscle wasting than WT mice. Myofiber area measurements in the denervation studies confirmed that this was due to less myofiber atrophy in the KO mice. In the fed state, fractional rates of muscle protein synthesis were similar in WT, KO. However, in fasting, fractional synthesis rates were 260% and 135% higher in the KO in the sarcoplasmic and myofibrillar fractions respectively. In all conditions, WT, KO muscle showed levels of expression of MuRF1, Atrogin1 and autophagy genes that were similar in basal state. However, in the catabolic states, MuRF1 and Atrogin-1 and some autophagy genes in the KO were <50% that in WT. This regulation by USP19 appeared to be muscle intrinsic as silencing of USP19 in muscle cells also abrogated the increase in MuRF1 induced by dexamethasone.

Conclusions: USP19 is essential for the full catabolic response to diverse wasting conditions including cancer cachexia. USP19 suppresses protein synthesis and enhances protein degradation (ubiquitin proteasome system and autophagy) suggesting that it acts on an upstream signalling pathway(s). These results identify USP19 as a potential drug target in cachexia.

1-17

IL-6 receptor signaling antagonism maintains muscle oxidative capacity during the progression of cachexia in the ApcMin/mouse: a role for exercise

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Background and aims: Cancer cachexia is associated with the loss of skeletal muscle. Muscle mitochondria function and content have been suggested to be as key regulators of muscle protein turnover, and we have demonstrated that muscle oxidative capacity is reduced in severely cachectic ApcMin/mice. Exercise and anti-cytokine therapies have been shown to be effective in preventing the progress of cachexia tumor-bearing mice. However, limited studies have explored the effect of these therapies on alterations in muscle oxidative capacity. Therefore, the purpose of this study is to determine regulation of muscle oxidative capacity during the progression of cachexia and whether IL-6 inhibition or exercise can preserve muscle mass through the maintenance of muscle oxidative capacity.

Methods: Three experiments were conducted using ApcMin/mice. Experiment 1 stratified mice by body weight loss. Experiment 2 administered IL-6 receptor antibody for 2 weeks after the onset of cachexia. Experiment 3 examined if moderate intensity treadmill training could attenuate changes induced by systemic IL-6 over-expression.

Results: Muscle mitochondrial content was not reduced during the onset of cachexia, while protein expression related to biogenesis and fusion was repressed. As cachexia progressed, mitochondrial content decreased and biogenesis- and fusion-related proteins were further repressed, while fission protein expression increased. IL-6 receptor antibody administration attenuated mitochondrial content loss, rescued the repression of biogenesis and fusion proteins, and prevented the induction on fission proteins. Exercise training prevented the onset of cachexia due to IL-6 over-expression, and was associated with an increase in mitochondrial biogenesis and fusion protein expression, while repressing fission protein expression.

Conclusions: The loss of muscle oxidative capacity occurs during late stages of cachexia, while repressed expression of proteins regulating mitochondrial biogenesis and dynamics occur early in the development of cachexia. Attenuation of IL-6 receptor signaling or exercise training can reverse these changes.

1-18

Optimization of macrophage-secreted myogenic factors and promotion of muscle regrowth

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Background and aims: Muscle wasting is a common side effect of many pathologies and conditions such as AIDS, cancers, chronic heart diseases, aging, prolonged bed rest, space flight, etc. Using an animal model of hypogravity, we previously showed that regrowth of atrophied muscles is associated with an inflammatory reaction and that the presence of macrophages is necessary for an optimal recovery. A growing body of evidence indicates that macrophages possess important myogenic capacities raising the question of whether the activity of macrophages can be optimized to promote muscle repair and regrowth. The objective of this study was thus to determine the effect of modulating the concentration and activation of macrophages on muscle regrowth from atrophy.

Methods: Mice were hindlimb unloaded for a period of 10 days, which induces a 50% decrease in soleus muscle force and mass, followed by 3, 7, or 14 days of reloading. At the day of reloading, mice were injected with macrophage-colony stimulating factor (M-CSF, 10 µg/ml) or phosphate-buffered saline (PBS) directly into the right and left soleus muscles, respectively. In addition, we developed an in vitro co-culture system in which myotubes atrophied by dexamethasone (1 µg/ml) were exposed or not to bone marrow-derived macrophages (BMDM, 25,000 cells/ml) and/or M-CSF (100 ng/ml).

Results: Compared to PBS injection, M-CSF induces a twofold increase in macrophage concentration in soleus muscles, which is associated with a 20% increase in muscle force and a 10% increase in muscle fiber diameter at day 7 of reloading ($p < 0.05$). In vitro, our data indicate that contrary to BMDM or M-CSF alone, the combination of BMDM and M-CSF increased myotube diameters by 20% and decreased the expression of the catabolic protein MuRF-1 by 25% ($p < 0.05$) relative to control.

Conclusion: Together, these results suggest that the myogenic capacities of macrophages can be optimized through M-CSF to promote muscle regrowth.

1-19

SOCS3 overexpression counteracts the IL-6/STAT3 signaling pathway and improves muscle wasting in experimental cachexia

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Background and aims: Elevated serum interleukin (IL)-6 correlates with muscle wasting and mortality in both cancer and severe burn injury. Systemic administration of IL-6 induces wasting, while its

inhibition can partially rescue muscle mass. The signaling pathways responsible for muscle wasting downstream of IL-6 are not completely known. IL-6 binds to its receptor, thus inducing the recruitment of gp130 and, in turn, the activation of STAT3. We recently reported that STAT3 is associated with tumor-induced muscle wasting. Since SOCS3 is one of the inhibitors of the IL-6/STAT3 signaling, here we aim at investigating whether SOCS3 can antagonize the IL-6/STAT3 pathway, thus improving muscle wasting in cancer- and burn-associated cachexia.

Methods: C2C12 myotubes were used to investigate the IL-6/STAT3 pathway in vitro. MLC-SOCS3 C57BL/6 transgenic mice served to isolate the effects of SOCS3 on the skeletal muscle. CD2F1 mice were injected with the Colon-26 (C26) adenocarcinoma to study cancer-associated cachexia, while C57BL/6 wild-type were burned to evaluate burn-induced cachexia. SOCS3 was overexpressed by means of in vitro adenoviral infection or in vivo electroporation.

Results: C2C12 overexpressing Ad-GFP-SOCS3 proved resistant to IL-6 induced wasting, while SOCS3 alone induced hypertrophy in control myotubes. Muscle weight was increased in female MLC-SOCS3 transgenics only, thus suggesting a gender-specific function of SOCS3. STAT3 nuclear translocation was reduced in gastrocnemius muscle of MLC-SOCS3 mice, consistent with decreased p-STAT3 levels. Both C26 tumor-bearers and burned mice exhibited reduced muscle CSA and wasting, associated with increased levels of p-STAT3. Localized overexpression of SOCS3 in the tibialis muscle inhibited STAT3 activation and prevented muscle wasting in C26 and burn cachexia.

Conclusions: These data support the use of SOCS3 manipulation as a therapeutic approach in cachexia of high IL-6, enhancing both muscle mass and fiber size. Among the likely targets of SOCS3 activity is STAT3.

1-20

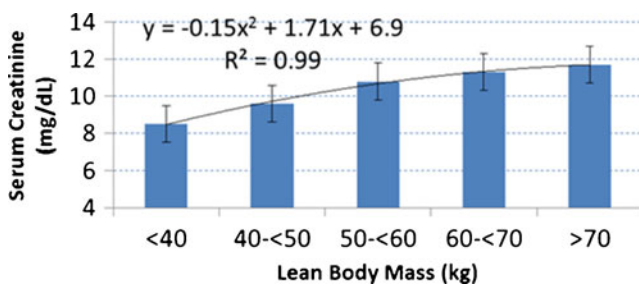
Serum creatinine is a biomarker of skeletal muscle mass in chronic hemodialysis patients

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Background: Skeletal muscle mass is a major component of lean body mass (LBM) and an important nutritional measure in people with chronic kidney disease including those who undergo maintenance hemodialysis (MHD) treatment. We hypothesized that serum creatinine concentration in stable MHD patients with adequate dialysis represents skeletal muscle mass.

Methods: In the “Nutritional and Inflammatory Evaluation in Dialysis” Study cohort, we examined 725 randomly selected patients receiving MHD at eight DaVita dialysis clinics in the Los Angeles South Bay area. Near-infrared interactance (NIR) in all patients and dual energy X-ray absorptiometry (DEXA) in a subset of randomly selected 118 patients were used as the reference standards for LBM measurements over a 5-year period. The index test was 3-month averaged serum concentrations of creatinine (SCr). The reference test was LBM measured via the difference between body mass and body fat assessed by NIR in the main cohort and by DEXA in the subcohort. **Results:** Both DEXA and NIR measured LBM correlated significantly with SCr ($r=0.65$ to 0.71 , $p<0.001$). SCr in women had even stronger correlation with LBM. Across increments of 10 kg from <40 to >70 kg LBM, an incremental increase in SCr was observed which can be expressed by a polynomial equation ($r=0.99$):

Lean body mass (kg)	<40 (n=97)	40 to <50 (n=226)	50 to <60 (n=234)	60 to <70 (n=115)	≥70 (n=75)	p Value
Creatinine (mg/dL)	8.5±2.4	9.6±2.8	10.8±3.3	11.3±3.3	11.7±3.9	<0.001



Conclusions: Three-month averaged SCr is a good correlate of LBM, as determined from DEXA or NIR measured LBM, in MHD patients.

1-21

Total-body creatine pool size and skeletal muscle mass determination by D3-creatine dilution in rats

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Background and aims: There is currently no direct, facile, and inexpensive method to determine total-body skeletal muscle mass for the routine diagnosis and treatment of skeletal muscle wasting conditions such as sarcopenia, cachexia, and disuse. We sought to validate in rats the hypothesis that the enrichment of creatinine-(methyl-d3) (D3-creatinine) in a spot urine sample after a single defined oral tracer dose of D3-creatine can be used to determine total-body creatine pool size and skeletal muscle mass.

Methods: We determined (a) an oral tracer dose of D3-creatine that was completely bioavailable with minimal urinary spillage and sufficient enrichment in the body creatine pool for detection of D3-creatine in muscle and D3-creatinine in urine, and (b) the time to isotopic steady state. We then used cross-sectional studies in growing (9–22 weeks of age) rats to compare creatine pool size determined by the D3-creatine dilution method to lean body mass determined by quantitative magnetic resonance.

Results: D3-creatine (1 mg/kg) was 103±14% bioavailable, and the specific dose used in these studies (0.475 mg/rat) averaged <0.5% urinary spillage. Isotopic steady state was achieved 24–48 h after giving D3-creatine. Creatine pool size, calculated from urinary enrichment of D3-creatinine 72 h after D3-creatine administration, significantly increased with muscle accrual during rat growth, significantly decreased in response to dexamethasone-induced skeletal muscle atrophy and was highly correlated with lean body mass ($r=0.9517$; $p<0.0001$). Enrichment of D3-creatine in muscle was greater in muscle with predominantly oxidative

versus glycolytic fibers. Creatine pool size calculated from muscle D3-creatine enrichment and converted to skeletal muscle mass based on muscle creatine content yielded expected skeletal muscle composition.

Conclusions: A novel, facile, direct, noninvasive D3-creatine dilution method has been validated in rats for the determination of total-body creatine pool size and skeletal muscle mass, and holds promise for routine clinical application.

1-22

An investigation of potential skeletal muscle protein biomarkers of cancer cachexia

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Background and aims: There remains an unmet clinical need for diagnostic biomarkers/therapeutic targets in cancer cachexia. This study evaluated several skeletal muscle biomarkers (selected from previous animal/human studies) in a cohort of cachectic upper gastrointestinal cancer (UGIC) patients.

Methods: One hundred twenty patients (18 controls, 102 UGIC patients) were recruited. Mean (SD) weight loss of UGIC patients was 7.7 (9.2)%. Cachexia was defined as weight loss $\geq 5\%$. Immunoblotting of protein homogenates of rectus abdominis muscle biopsies obtained at surgery was performed probing for Akt ($n=52$), phosphorylated-Akt ($n=52$), FOXO1 ($n=59$), FOXO3a ($n=59$), LC3 ($n=32$), beta-dystroglycan (BDG, $n=52$), beta-sarcoglycan (BSG, $n=52$), calmodulin-kinase II (CAMKII, $n=59$), phosphorylated-CAMKII ($n=59$), and myosin heavy chain ($n=47$). ImageJ was used to calculate densitometry and results analysed using SPSS v15.0. Follow-up of UGIC patients was for a median of 663 days (range, 450–1,955 days). Candidate biomarkers were assessed for: (1) differences between controls and UGIC patients, (2) diagnostic biomarkers of cancer cachexia, and (3) prognostic biomarkers of survival (lower vs. upper third).

Results: Compared with controls, UGIC patients had lower Akt levels (0.49 (0.31) vs. 0.89 (0.17), $p=0.001$), lower total/phosphorylated-Akt ratio (1.73 (1.77) vs. 4.38 (2.62), $p=0.002$) and a trend towards higher CAMKII levels (0.77 (0.25) vs. 0.56 (0.30), $p=0.053$). Compared with noncachectic patients, cachectic patients had higher BDG levels (1.01 (0.16) vs. 0.87 (0.20), $p=0.007$) and a trend towards higher BSG levels (0.63 (0.28) vs. 0.55 (0.55), $p=0.052$). Survival was reduced in UGIC patients with lower vs. higher ratio of total/phosphorylated-Akt (median survival 483 vs. 640 days, $p=0.020$).

Conclusions: We suggest that Akt is suppressed and CAMKII is increased in skeletal muscle of UGIC patients. Few other potential diagnostic/prognostic biomarkers were revealed. This may reflect lack of sensitivity using immunoblotting, broad diagnostic criteria, or a heterogeneous patient group including individuals at different timepoints during the cachexia journey.

1-23

Altered skeletal muscle ultrastructure and myocyte enhancer factor (MEF) 2 C gene expression in the colon 26 carcinoma model of cachexia: a potential link?

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Background and aims: Cachexia is a highly debilitating paraneoplastic disease observed in more than 50% patients with advanced cancers and directly contributes to 20–30% of cancer deaths. Loss of skeletal muscle is a defining and often fatal characteristic of patients with cancer cachexia. Pathological mechanisms of cachexia are complex and multifactorial, which limits the development of an effective means of predicting, preventing, or treating it. This study aims to identify transcriptional regulators underlying cancer-induced skeletal muscle wasting.

Methods: The colon 26 (C26) carcinoma mouse model of cachexia was established and skeletal muscle was isolated for gene and protein expression studies by real-time qPCR and Western blotting; ultrastructural studies were performed using transmission electron microscopy.

Results: The present study has identified a potential novel role for MEF2C as a regulator of skeletal muscle wasting in C26-bearing mice. MEF2C, an important transcription factor in controlling skeletal muscle maintenance and regeneration, was found to be downregulated at both transcript and protein levels during cancer-induced skeletal muscle wasting. Myogenic genes including myoglobin and myomesin, also displayed reduced expression due to cachexia; suggesting a potential link to MEF2C. These altered molecular effects imply compromised oxygen transport capacity in addition to distorted structural integrity. Changes in mitochondrial integrity were also evident in electron micrographs showing ultrastructural alterations.

Conclusion: Together, these effects may limit sarcomeric contractile ability and lead to skeletal muscle weakness and fatigue in cancer patients. Moreover, these may also predispose to structural instability in skeletal muscle and thus promote degradation along the course of disease progression. We believe that the identification of MEF2C is important as it appears to be a potential regulator of skeletal muscle loss in cancer cachexia. Targeting MEF2C could better direct intervention strategies at early stage disease and thus minimise or delay skeletal muscle wasting in cancer patients.

1-24

Effect of plasminogen activator inhibitor 1 (PAI-1) in rodent cancer cachexia and sarcopenia

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The plasminogen activator (PA) system has been shown to play an important role in various diseases including muscle wasting conditions. In particular, plasminogen activator 1 (PAI-1), a serine protease inhibitor (aka serpin E1) that targets urokinase-type (uPA) and tissue-type plasminogen activator (tPA), was shown to play an important role in muscle regeneration and hypertrophy. For example, PAI-1 knockout mice show improved muscle regeneration in cardiotoxin-induced injury model (Koh et al. 2005) and PAI-1 is upregulated during acute resistance exercise (Chen et al. 2002) as well as cancer, cardiovascular diseases, and diabetes. Moreover, PAI-1 expression is modulated by a variety of pathways known to be involved in muscle growth and regeneration such as pro-inflammatory cytokines, corticosteroids, IGF-I, and TGF- β proteins. We have further studied PAI-1's role and mode of action in a human skeletal muscle assay system (HuSKmC) as well as in rodent cancer cachexia and sarcopenia models. We show here that HuSKmC cells increase during differentiation PAI-1 mRNA expression and secrete active PAI-1 measured by enzyme-linked immunosorbent assay. Treatment with TGF- β proteins further increase PAI-1 expression and secretion. Inhibition of PAI-1 by pharmacologic

and genetic tools increased HuSKmC differentiation indicating a tonic effect of secreted PAI-1. On differentiated myotubes, both PAI-1 targets uPA and tPA concentration-dependently induce increase in myotube diameter. Increased PAI-1 levels were detected in circulation of the mouse cancer cachexia model; in addition, cultured cancer cell lines, which are known to induce cachexia *in vivo*, actively secrete PAI-1. Moreover, expression of PAI-1 was increased in muscles of the cancer cachexia xenograft mouse model and sarcopenic rats. These data revealed further insights into the role and mode of action of PAI-1 in muscle cell differentiation and wasting conditions.

1-25

Autonomous CaMKII activity and SRF phosphorylation are decreased in skeletal muscle during experimental cancer cachexia

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Background and aims: Muscle wasting is an essential component of cancer cachexia. Studies suggest that calcium/calmodulin-dependent protein kinase II (CaMKII) is involved in the regulation of muscle mass. A unique feature of CaMKII is its autophosphorylation after calcium/calmodulin activation, resulting in calcium-independent autonomous activity. The transcription factor serum response factor (SRF) is an important substrate of autonomous CaMKII activity. Recent studies suggest that increased autonomous CaMKII activity and phosphorylation of SRF(Ser103) result in muscle hypertrophy. Based on those observations, we hypothesized that cancer-induced muscle atrophy is associated with reduced autonomous CaMKII activity and SRF (Ser103) phosphorylation.

Methods: Cancer cachexia was induced in male Wistar rats by *i.p.* inoculation of AH-130 cells. Control rats were injected with corresponding volume of saline. After 7 days, gastrocnemius muscles were harvested for determination of phosphorylated CaMKII(Thr286/287) and SRF(Ser103) by Western blotting and measurement of autonomous CaMKII activity using a commercially available CaMKII kinase activity kit. In addition, muscle weight was measured and mRNA levels for atrogin-1 and MuRF1 were determined by real-time PCR.

Results: Muscle weight was reduced by 25% and atrogin-1 and MuRF1 mRNA levels were increased three- and fourfold, respectively, in tumor-bearing rats. Muscle levels of p-CaMKII(Thr236/237) and autonomous CaMKII activity were reduced by 30% in tumor-bearing rats. These changes were accompanied by a 25% decrease in nuclear levels of p-SRF (Ser103).

Conclusions: Muscle wasting in cancer cachexia is associated with reduced autonomous CaMKII activity and SRF(Ser103) phosphorylation. Stimulation of autonomous CaMKII activity and SRF phosphorylation may prevent cancer-induced muscle wasting.

Supported in part by NIH R01DK37908 to POH. ZA was supported by the Department of Clinical Medicine, Sapienza, University of Rome, Rome, Italy

1-26

Sex differences of cytokine profiles and muscle loss in pancreatic cancer patients

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Background: Cancer cachexia is a devastating wasting syndrome characterized by fat and muscle loss that affects the majority of advanced cancer patients. This syndrome, however, is poorly understood.

Objective: The objective of the study was to examine the extent to which muscle area changes over time in pancreatic cancer patients.

Methods: Forty newly diagnosed or recurring cancer patients of all stages (16 women (40%); age, 58 (SD: 10)years old) from McGill University Health Center and the Jewish General Hospital, with an Eastern Cooperative Oncology Group score of 0–3 and a life expectancy greater than 3 months were included in the study. Muscle area was assessed from computed tomography images, performed for clinical indications, of the third lumbar vertebrae with Slice-O-Matic. Cytokines were measured from plasma samples with the Bio-PlexR system and data output being analyzed with the Bio-Plex ManagerTM software. Group-based trajectory modeling was used to identify trajectories of muscle area over time.

Results: Twenty (50%) patients in the study were losing muscle over time, 13 (81%) women compared to seven (29%) men. Four trajectories of muscle area were identified: one losing muscle area over time and three with stable muscle area over time. Therefore, the losing weight group included 13 women and 7 men, while the other three groups included a total of 3 women and 24 men (z value 3.23, p value=0.001). Women had differing cytokine profiles compared to men at baseline.

Conclusion: Muscle loss occurs in half of the pancreatic cancer patients in this study, the majority of which are women. Future studies on cancer cachexia should explore the effects of sex differences among pancreatic cancer patients, especially in regard of the influence of cytokine profiles.

1-27

microRNA-451: a human specific regulator of adaptation to exercise training

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Background: Human skeletal muscle demonstrates remarkable heterogeneity in capacity to adapt to external loading, such that gains in muscle strength or mass during resistance exercise training (RET) vary greatly between individuals. Our objective was to explore relationships between adaptive heterogeneity and abundance of miRNAs—the key regulators of translation of protein-coding mRNA.

Methods: Muscle biopsies were obtained from young (18–28 years old, $n=14$), middle-aged (45–55 years old, $n=20$) and older (65–75 years old, $n=17$) men and women before and after 20 weeks fully supervised whole-body RET. Using miRNA arrays that detect ~700 human miRNAs, we hypothesised that miRNA abundance would differ between the highest ($n=5$) and lowest ($n=5$) responders (in terms of muscle strength) to RET. Results were confirmed using the entire cohort ($n=51$), with TaqMan-based miRNA detection technology.

Results: Baseline physiology did not differ between high and low responders. We provide independent validation of a previous observation that mir-451 is specifically upregulated in young male low-responders to RET, and extended this observation to middle-aged and older male subjects. No such association was found in female subjects. Intriguingly, mir-451 is not expressed in murine C2C12 cells and its expression is absent or 10–50-fold lower in mouse/rat than in human skeletal muscle extracts. Targets of mir-451 are limited in number (~20) and represent conserved protein coding genes, including Hamartin (TSC1), F-box protein 33 (FBXO33) and calcium-binding protein 39 (CAB39) which along with additional evidence that mir-451 can inhibit Serine/threonine-

protein kinase 11 (LKB1), places it in a central position to interact with numerous anabolic and catabolic related pathways.

Conclusion: We have validated a novel regulator of male human *in vivo* muscle growth. The fact that miR-451 demonstrates a human-specific expression pattern supports the notion that current preclinical murine or rodent models of muscle growth may be unable to fully mimic the human scenario.

2-01

Transcriptomic and proteomic profiling of livers from cachectic mice reveals dysfunctional metabolism

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

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

Results: The transcriptomic and proteomic datasets revealed high correlation between the three approaches, with very few instances of incongruity. Pathway analysis utilizing several software packages indicated that central metabolic processes including lipid handling, glycolysis/gluconeogenesis, amino acid metabolism, tricarboxylic acid cycle and mitochondrial electron transport chain are reduced in cachectic mice. Linking these metabolic pathways to upstream regulatory events, transcriptional activation is reduced within the RXR canonical pathway (e.g., LXR, FXR, TR, PPAR $\alpha/\delta/\gamma$), associated with cytokine signaling through activated JAK/STAT pathway, SOCS3, and IL-1/LPS-BP signaling. Repressed expression of genes and proteins in key energy generation pathways is counter-intuitive to the expected role of the liver in settings of food restriction/weight-loss—i.e., to adaptively utilize amino acids, carbohydrates and fatty acids, and activate ketone body production and gluconeogenesis. As a counterpoint to this dramatic disruption in metabolic pathways, we see significant acute phase protein production and a concomitant increase in protein translation, potentially mediated through phosphorylated 4E-BP downstream of mTOR.

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

2-02

Sarcopenia in cirrhotic patients with and without hepatocellular carcinoma

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Background/aims: Prognostic assessment of cirrhotic patients remains a challenge. Sarcopenia is defined as low levels of muscle mass; it may be present in chronic/malignant diseases. It is not well-studied/understood in cirrhotic and HCC patients. We aimed to establish sarcopenia frequency and if it predicts mortality in a cohort of cirrhotic patients with and without hepatocellular carcinoma (HCC).

Patients and methods: One hundred sixty-three patients with cirrhosis were consecutively evaluated for liver transplant and had computed tomography (CT) scan at the third lumbar vertebrae were selected. Skeletal muscle cross-sectional area was measured by CT to determine the third lumbar vertebrae (L3) skeletal muscle index (L3 SMI) defined as total lumbar muscle cross-sectional area adjusted for stature; sarcopenia was defined using previously published gender-specific cutoffs.

Results: One hundred eighteen patients were males (72%), mean age of 55 \pm 1 years, 51 patients (31%) had HCC at the time of CT. Median time of follow-up was 19 \pm 1 months. Sarcopenia was present in 61 patients (37%), and there was no difference in the frequency of sarcopenia among patients with and without HCC (31% vs. 40%, $P=0.3$). By univariate Cox analysis the bilirubin, creatinine, albumin, Model for End-Stage Liver Disease (MELD), Child–Pugh, sodium, L3 SMI, and sarcopenia were associated with mortality. The presence of HCC was not associated with increased mortality (HR 1.12, $P=0.6$). By multivariate Cox regression analysis, only MELD score (HR 1.08, $P=0.001$), and sarcopenia (HR 2.18, $P=0.001$) were independently associated with early mortality. Median survival for sarcopenic patients was 19 \pm 5 months, compared to 30 \pm 2 months in nonsarcopenic patients ($P=0.001$). There was a poor correlation between sarcopenia and MELD score ($r=-0.13$, $P=0.1$) and sarcopenia and Child–Pugh score ($r=0.04$, $P=0.6$).

Conclusions: Sarcopenia is present in 37% of patients with cirrhosis and is not associated with the presence of HCC. Sarcopenia constitutes a strong and independent predictor of mortality in cirrhotic patients and does not correlate with degree of liver dysfunction evaluated with conventional scoring systems. Further studies including sarcopenia with conventional scores may allow better mortality prediction among cirrhotic patients with and without HCC.

2-03

Metabolic derangements in the gastrocnemius and the effect of selective NF- κ B inhibition in a murine model of cancer cachexia

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Cancer cachexia is a severe wasting syndrome characterized by the progressive loss of lean body mass and systemic inflammation. Inhibiting NF- κ B signaling largely prevents such cancer-induced muscle wasting in murine models. We have previously shown the utility of compound A, a highly selective novel NF- κ B inhibitor that targets the I κ B kinase complex, to provide clinical benefit in cancer-induced skeletal muscle and cardiac atrophy. Using a metabolomics approach, we describe the changes found between cachectic and noncachectic gastrocnemius muscles, before and after compound A treatment. Of the 234 metabolites in the gastrocnemius, cachexia-induced changes in gastrocnemius metabolism reset the steady-state abundances of 42 metabolites ($p<0.05$). These changes, not evenly distributed across biochemical categories, are concentrated in amino acids, peptides, carbohydrates and energetics intermediates, and lipids. The gastrocnemius glycolytic pathway is markedly altered—changes consistent with tumor Warburg physiology. This is the first account of a Warburg

effect that is not exclusively restricted to cancer cells or rapidly proliferating nonmalignant cells. Cachectic gastrocnemius also displays tricarboxylic acid cycle disruptions, signs of oxidative stress, and impaired redox homeostasis. Interestingly, treatment with compound A has more profound effects on the metabolism of the tumor itself than on the gastrocnemius. In fact, compound A treatment has only a modest effect on the biochemistry of the cachectic gastrocnemius, failing to restore the gastrocnemius' baseline metabolic profile. Conversely, the biochemical changes in the tumor point to a direct anti-oxidant effect, but also a potential reduction in the growth rate of the tumor—consistent with the observation of tumor shrinking post-treatment. These changes give significant insight into the complex pathophysiology of cancer cachexia, shedding light on specific changes that might be avenues for targeted therapies.

2-04

Disrupted circadian regulation of metabolic pathways in livers of cachectic mice associated with tumour-derived IL-6

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Background: Circadian patterns of gene expression are intimately linked with control of metabolic pathways to coordinate diurnal cycles of feeding and activity. The molecular regulation of body clocks involves complex circuitry of negative feedback loops mediated by transcription factors BMAL1/CLOCK entwined with CRY, PER, and nuclear receptors ROR α and REVERB α . While perturbations to these intrinsic circadian regulators are associated with obesity, the impact of the converse setting of cachexia on their action is unknown.

Aim: In this study, we focused on the liver and hepatic metabolic pathways fundamental for the control of total body homeostasis. Given the integral role of nuclear receptors in regulating metabolic pathways, we characterized the diurnal expression pattern of nuclear receptors and genes they regulate in the livers of cachectic colon 26 tumour-bearing mice.

Results: Six-timepoint analysis by RT-qPCR of BMAL1, CLOCK, CRY1, PER2, REVERB α , and ROR α showed significant changes to their normal rhythmic expression patterns, particularly at the midpoint of the dark and light cycles. Western analysis of BMAL1 protein at six timepoints confirmed disruption of core circadian regulation. Analysis of liver samples by cDNA microarrays identified numerous genes that displayed rhythmic patterns in control mice (i.e., peaking at 2 am or 2 pm) many of which lost the normal diurnal rhythm in cachectic C26 livers. PPAR $\alpha/\delta/\gamma$ as well as ERR α all displayed markedly altered circadian expression patterns, along with their target genes in fatty acid β -oxidation (PBE, HADHA, and B), fatty acid uptake (LPL), lipogenesis (FAS, SCD1) and glucose/pyruvate metabolism (PEPCK, PDK4). Expression profiling demonstrated decreased expression of many other genes in key metabolic pathways indicating a profound disruption in circadian regulation of hepatic metabolism.

Conclusion: As cachexia in C26 mice is driven by tumour-derived factors such as IL-6, it is likely that chronic stimulation of cytokine-signaling in the liver disrupts hepatic metabolic pathways responsible for maintaining diurnal energy homeostasis throughout the body.

2-05

Disturbance of haematopoietic and immune systems of cachectic mice

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Background: Infection due to immunosuppression following chemotherapy in advanced cancer is an inherent problem with cytotoxic drugs. Cachectic cancer patients are particularly susceptible to excessive toxicities resulting in delay or termination of treatment and in some instances death. Systemic inflammation associated with cachexia is also linked with repression of drug clearance pathways, altering the pharmacokinetics of anticancer agents and thereby enhancing the potential for adverse toxicity.

The Colon-26 carcinoma is a widely used mouse model of cachexia mediated by tumour-derived IL-6 and a common preclinical model for evaluation of antineoplastic pharmaceuticals. Prior to assessment of susceptibility to myelosuppression by cytotoxic drugs, haematopoietic and immunological cell populations of cachectic colon-26 mice were compared with those of mice engrafted with a noncachectic variant of the same tumour, delineating effects of cachexia vs. those of a tumour per se. Changes attributable to anorexia were identified using pair-fed mice.

Results: Blood of cachectic mice displayed highly elevated erythrocyte sedimentation rate and thrombocytosis, consistent with systemic inflammation. Their bone marrows indicated developing anaemia with erythroblast microcytosis and elevated transferrin receptor (CD71) levels. Downregulated hepcidin and upregulated transferrin in the livers point to iron-deficiency anaemia rather than the expected anaemia of chronic disease. Production of new lymphocytes, both pre-B and pre-T cells, was abrogated, with obvious implications for susceptibility to infection. Natural killer cell populations were strongly ablated, which could affect innate anti-tumour response, particularly surveillance and suppression of metastases. Neutrophil and monocyte levels were increased in circulation and peripheral lymphoid organs. The blood neutrophil lymphocyte ratio was elevated 15–20-fold; high NLR in humans is strongly prognostic of poor outcome for many tumour types.

Conclusion: The progressive changes in the immune and haematological systems of cachectic mice associated with rises in plasma IL-6 indicate that the C26 tumour provides an excellent model for studying the links between cachexia, altered drug clearance, and bone marrow toxicity

2-06

Regulation of TNF/cachectin signalling

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Background: It is 26 years since the hormone cachectin was identified as tumor necrosis factor (TNF) and TNF-producing tumors injected intramuscularly produce significant weight loss and cachectic symptoms. Great strides have been made in understanding how TNF signals and studies on TNF have led to text book examples of receptor signalling but there is still much to learn.

Methods: Using a combination of genetics, biochemistry, mass spectrometry and small molecule inhibitors my lab has recently made significant contributions to understanding how TNF signals.

Results and conclusions: We have shown that cIAPs are critical regulators of the initial phase of TNF signalling, and identified new molecules HOIL-1/HOIP and Sharpin (Vince et al. 2009; Haas et al. 2009; Gerlach et al. 2011; Vince et al. 2007) that together form the Linear Ubiquitin Assembly Complex. Using multiple reaction monitoring mass spectrometry, we now demonstrate that linear ubiquitin chains are present within the endogenous TNF signalling complex and together with other data generated in my lab (Wong et al. 2010) present a new model for TNF signalling that emphasizes the exquisite regulation of TNF signalling.

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2-07

Cachexia and inflammation in patients with chronic obstructive pulmonary disease (COPD)

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Background and aims: Chronic obstructive pulmonary disease (COPD) is chronic inflammatory disease with specific loss of lean body mass determined cachexia. One of causes of cachexia in COPD can be excessive production of cytokines and molecules that are engaged in regulation of nutritional status. Study aim was: (1) Evaluation of IL-6 level and leptin concentration and assessment of the relation between blood level of these molecules in COPD patients. (2) Attempt to find the connection between IL-6 and leptin and nutritional status.

Material and methods: Fifty-five COPD patients—43 males, 12 females (mean age 62.31±11.08) and 32 controls (mean age 57.43±8.79) participated in the study. In study and control group, fat-free mass index (FFMI) as indicator of cachexia was measured using analyzer of body composition. Leptin and IL-6 concentration were measured in serum with a specific enzyme-linked immunosorbent assay methods.

Results: Average leptin blood concentration in study group for females and males was 358.38±66.57, 316.75±27.14, respectively; in control group 344.99±54.98, 309.58±19.36, respectively. Significant correlation between leptin and BMI (body mass index)—R Spearman=0.52, and FMI (fat mass index)—R Spearman=0.60, but not for FFMI was confirmed. Average IL-6 serum level in study and control group was 2.47±2.0, 1.30±1.51 pg/ml, respectively, and it was statistical difference between groups. IL-6 serum level did not correlate with leptin level as well as with indicators of nutritional status: BMI, FFMI, FMI. It was not observed IL-6 level difference between well-fed COPD subjects and patients with cachexia.

Conclusions: (1) COPD is an inflammatory disease with increased serum level of IL-6 and leptin. (2) Leptin and IL-6 are not directly responsible for state of nutrition in COPD patients. (3) It is necessary to find different new markers, which will be the connection between inflammation and cachexia in COPD patients

2-08

Gene expression of the NF- κ B signaling path in the subcutaneous adipose tissue of cachectic patients

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Cachexia, a paraneoplastic syndrome is frequently underlined by systemic inflammation, leading to dramatic wasting of lean and fat mass and to metabolic chaos. The adipose tissue is an endocrine organ and secretes a number of factors known as adipokines, many of which

are involved in the regulation of inflammatory processes. We have demonstrated that this tissue contributes to systemic inflammation during cachexia both in animal models and in patients (Machado et al., *Cell Tissue Res*, 2004, Dec 503–14; Batista Jr et al., *Med Sci Sports Exerc*, 2008, Mar 549–56). A key point in the expression of pro-inflammatory cytokines (including TNF- α) is the activation in cells of the nuclear transcription factor kappa B (NF-kappaB), which also plays a part in modulating the resolution of inflammation. In order to examine NF-kappaB gene expression in the subcutaneous adipose tissue, cachectic patients were selected according to the parameters described by Evans et al. (*Clin Nutr*, 2008, Dec 793–9), after signing a term agreeing to donate blood and tissue samples, both collected during surgery. Patients were divided into three groups: control (C, $n=13$), tumor with cachexia (TC, $n=13$), and tumor without cachexia (T, $n=12$). PCR real time for the NF-kappaB signaling pathway genes was performed and the results show a significant increase in the expression of p65/RelA ($p<0.03$) and Ikk β genes ($p<0.05$) and also a tendency of increased Ikk α gene expression, both in T. p50/p105, Ikb α , Ikb β and Ikk γ gene expression did not change significantly and a pronounced variation between individuals was found. These results show that TC individuals are probably less capable of modulation in the NFkB pathway, what may interfere with the resolution of inflammation, as increased cytokine content triggers this process.

2-09

Gender differences and changes in physiological function, nutrition, and fatigue in the early phase in patients undergoing allogeneic hematopoietic stem cell transplantation

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Background and aims: Hematopoietic stem cell transplant (HSCT) patients have decreased exercise capacity and muscle strength from before to after transplantation. However, there are no reports evaluating body composition in adult HSCT patients. The primary aim of this study is to investigate the change in body composition, physiological function, nutritional status, and fatigue pre- and post-allogeneic (allo) HSCT. Additional aims of this study include examining gender differences in physiological function, nutritional status, and fatigue pre and post allo-HSCT.

Methods: Study participants included patients who had undergone allo-HSCT from July 2007 to June 2011. Ninety-eight patients were included in this study (65 men, 33 women). The evaluation was performed up to 3 weeks before and 6 weeks after transplantation. Body composition was evaluated using a bioelectrical impedance analysis (BIA). Physiological functions were evaluated by handgrip, knee extensor strength, and 6-min walk test (6MWT).

Results: Body weight (BW) and fat body mass significantly decreased after transplantation (5% in BW and 20% in fat body mass, $p<0.01$). Moreover, there was no significant decrease in lean body mass before to after transplantation. Handgrip, knee extensor strength, 6MWT, all decreased significantly after transplantation (20% in both handgrip and knee extensor strength and 5% in 6MWT, $p<0.01$). There were no significant differences between men and women with respect to any of the tested parameters.

Conclusions: We found that, despite the fact that lean body mass did not decrease, muscle strength decreased significantly in allo-HSCT patients. Many patients receive intravenous drip therapy after transplantation. Thus,

despite decreased muscle mass, the lack of change in lean body mass may be a result of a corresponding increase in body water volume. Based on these facts, the BIA method may not be ideal for accurately assessing muscle mass in the early phases of allo-HSCT.

2-10

Prevalence of undernutrition, sarcopenia and cachexia in patients with inflammatory bowel diseases

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Aims: This paper aims to study the prevalence of undernutrition, sarcopenia, and cachexia in patients with inflammatory bowel diseases in acute phase.

Methods: Thirty patients with ulcerative colitis and 26 patients with Crohn's disease in acute phase had been inspected in hospital conditions. We studied the body weight, body mass index, body composition, muscle volume of arm, protein content in the blood, and number of lymphocytes in the blood. Content of fat body mass and fat-free mass was determined by methods of bioelectrical impedance and dual energy X-ray absorptiometry.

Results: Reduction of the body weight and body mass index (<18.5) was detected in 42% of patients with ulcerative colitis and 54% of patients with Crohn's disease. The diagnosis "cachexia" was installed in 10% of patients with ulcerative colitis and 8% of patients with Crohn's disease. Reduction of the muscle volume of arm (sarcopenia) has been revealed in 25% of patients with ulcerative colitis and 27% of patients with Crohn's disease. Hypoproteinemia and hypoalbuminemia took place in 16% of patients in both groups, reducing of number of lymphocytes in the blood in 14% of patients with ulcerative colitis and 27% of patients with Crohn's disease.

Conclusions: Undernutrition was detected in 42–54% of patients with inflammatory bowel diseases, however diagnosis of cachexia was installed in 8–10% of patients, sarcopenia took place in 25–27% of patients with inflammatory bowel diseases in acute phase.

2-11

Association between body condition and survival in dogs with naturally-occurring chronic kidney disease

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Obesity in people with chronic kidney disease (CKD) is associated with longer survival. The purpose of this study was to determine if a relationship exists between body condition and survival in dogs with naturally-occurring CKD, with the hypothesis that overweight and obesity are associated with longer survival times. Data regarding initial body weight and body condition score (BCS), clinicopathologic values and treatments were collected retrospectively from medical records and compared with survival times. One hundred dogs diagnosed with CKD (International Renal Interest Society stages II, III or IV) between 2008 and 2009 were enrolled in the study. For dogs with BCS recorded ($n=72$), 13 were underweight (BCS=1–3; 18%), 49 were moderate (BCS=4–6; 68%), and 10 were overweight (BCS=7–9; 14%). For dogs with at least two body weights recorded ($n=77$), 21 gained weight, 47 lost weight, and 9 had no change in weight. Dogs classified as underweight at the time of diagnosis (median survival=25 days) had a significantly shorter survival time compared to both moderate (median survival=190 days, $P<0.001$) and overweight dogs (median survival=365 days; $P<0.001$). There was no significant difference in survival between moderate and overweight dogs ($P=0.95$). Higher BCS at the time of diagnosis was significantly associated with improved survival. These results suggest that body condition is an important consideration in dogs with naturally-occurring CKD. Further studies are

warranted to evaluate the relationship between obesity and survival in dogs with CKD.

2-12

Vitamin D repletion and receptor activation ameliorate cachexia in chronic kidney disease (CKD)

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Background and aims: Vitamin D deficiency is prevalent and may be important in CKD-associated cachexia.

Methods: CKD was induced by 5/6 nephrectomy (N) in 8-week old c57BL/6 J mice.

Results: Both 25-vitamin and 1,25-vitamin D levels are significantly lower in N mice compared with sham (S) mice. N and S mice received 25-VitD (VitD25, 80 ng/kg, i.p., 3× per week), paracalcitol (PC, 150 ng/kg, i.p., 3× per week) or vehicle (V) for 2 weeks. N/V mice were fed ad libitum whereas N/VitD25, N/PC, S/V, S/VitD25, and S/PC mice were pair-fed to N/V mice. Serum BUN and creatinine was significantly higher in N/V, N/VitD25, and N/PC compared with S/V, S/VitD25, and S/PC mice ($p<0.01$). N/VitD25 and N/PC mice gained more weight than N/V mice (1.4 ± 0.2 and 1.2 ± 0.3 vs. 0.7 ± 0.3 g, $p<0.01$). Basal metabolic rate was higher in N/V compared with N/VitD25 and N/PC mice ($3,895.8\pm 234.7$ vs. 3415.2 ± 224.6 and $3,216.5\pm 124.4$, $p<0.01$). N/V mice lost lean and fat mass whereas N/VitD25 and N-PC mice gained lean and fat mass. Muscle strength, assessed by rotarod activity and grip strength, showed significant improvement in N/VitD25 (117.4 ± 183.5 s, $1,653.5\pm 126.4$ g/100 g) and N/PC (121.4 ± 21.5 s, $1,624.5\pm 125.6$ g/100 g) compared with N/V mice (68.8 ± 12.6 s, $1,243.2\pm 129.0/100$ g, $p<0.001$). mRNA of uncoupling proteins 1 and 2, which regulate energy expenditure, and proinflammatory cytokine IL-6 were upregulated in skeletal muscle and adipose tissue in N/V but normalized in N/VitD25 and N/PC mice. mRNA of myogenic pathway genes, IGF-I, MyoD, and PAX3 were all downregulated in the skeletal muscles in N/V but normalized in N/VitD25 and N/PC mice.

Conclusions: 25-Vitamin repletion and vitamin D receptor activation ameliorated cachexia as well as reversed cytokine over-expression in a mouse model of CKD-associated cachexia. Vitamin D deficiency may be an important factor in the pathogenesis of cachexia and inflammation in CKD.

2-13

Low selenium and inflammatory status in patients with heart failure with and without cachexia

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Introduction: Oxidative stress and chronic inflammation are striking features in chronic heart failure (CHF). Both may cause an impaired selenium (Se) metabolism characterized by decreased biosynthesis of selenoprotein-P (SEPP), a protein involved in Se transport and antioxidant defence in the blood. Concentrations of Se and SepP have not yet been studied in CHF.

Methods: We prospectively investigated 35 patients with CHF, 25 noncachectic (LVEF 31±2%, peak VO₂ 15±1 mL/kg/min, NYHA class 2.5±0.1) and 10 cachectic (LVEF 28±3%, peak VO₂ 13±1 mL/kg/min, NYHA class 2.9±0.1) and 30 control subjects (peak VO₂ 25±1 mL/kg/min, LVEF 66±2%). Blood concentrations of Se, SEPP, pro-inflammatory interleukin-22 (IL-22), and high sensitive C-reactive protein (hsCrP) were assessed by spectroscopy and enzyme-linked immunosorbent assay (ELISA), respectively. We measured in a subgroup of 17 patients and 12 controls interleukin-10 (IL-10) and tumor necrosis factor (TNF) blood cytokine excretion upon lipopolysaccharide (LPS) stimulation by ELISA.

Results: In CHF, we found lower blood concentration of both Se and SEPP compared to controls (70.4±2.6 vs. 80.3±2.4 µg/L [$p<0.008$] and 2.7±0.9 vs. 3.0±0.8 mg/L [$p<0.05$], respectively). Cachectic patients showed lowest blood concentration of Se compared to noncachectic patients and controls (66.4±5.5 vs. 73.5±3.2 vs. 79.9±2.3 µg/L, analysis of variance, $p<0.03$). Furthermore, cachectic patients had lowest SEPP blood concentration indicating a lack in antioxidant capacity in cardiac cachexia (2.4±0.1 vs. 2.9±0.1 vs. 2.9±0.1, $p<0.02$). Cachectic patients had highest blood concentration of IL-22 and hsCrP compared to noncachectic patients and controls (23.9±9.4 vs. 7.5±2.1 vs. 3.1±0.8 pg/mL [$p<0.0003$] and 0.8±0.2 vs. 0.3±0.1 vs. 0.2±0.1 pg/mL [$p<0.03$]). The concentration of IL-22, which exerts antimicrobial properties, correlated with lower levels of SEPP ($r=0.40$, $p<0.03$) and Se ($r=0.50$, $p<0.03$) and higher levels of anti-LPS-Immunoglobulin-M-antibodies in CHF ($r=0.65$, $p<0.007$). Upon blood LPS stimulation, CHF patients showed a higher IL-10 and TNF release (78±11 vs. 37±6 and 919±80 vs. 516±79 pg/mL, $p<0.01$). Higher hsCrP levels in CHF correlated with lower SEPP and Se blood concentrations ($r=0.47$, $p=0.01$ and $r=0.40$, $p=0.055$, respectively).

Conclusion: Decreased selen status (i.e., serum Se and SEPP levels) are seen in CHF patients. The subgroup of CHF patients with cachexia displayed the lowest blood concentrations indicating limited anti-oxidative potential. Low selen status correlated with higher IL-22 and hsCrP levels. Chronic inflammation may constitute a causal factor for the observed relative deficiency in both Se and SEPP concentrations in cardiac cachexia and could be a therapeutic target.

2-14

Peripheral tissue endothelial dysfunction and relationship to exercise capacity in patients with chronic heart failure by non-invasive arterial tonometry methodology

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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 (CHF) by a novel non-invasive and easily applicable method. We aimed to assess the relationship of ED to exercise capacity and clinical status in CHF patients with and without cachexia.

Methods: We studied 75 patients with CHF [age 65±11 years, 24% female, body mass index (BMI) 28.6±5.5 kg/m², New York Heart Association (NYHA) class (I/II/III/IV, 4/41/24/3), left ventricular ejection

fraction (LVEF) 36±11%, pVO₂ 16.6±5.0 ml/min/kg (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 symptom limited treadmill spirometric exercise test (modified Bruce protocol) and six-min walk test (6MWT). For comparison, we studied 20 healthy controls (CON) of similar age and gender distribution.

Results: Compared to controls (RHI 2.14±0.62), endothelial function was 16% lower in CHF (RHI 1.80±0.36, $p=0.007$) and decreased stepwise with advancing disease severity (NYHA I+II/III+IV 1.89±0.37/1.63±0.33, analysis of variance, $p<0.005$). RHI was more reduced in CHF patients with ischaemic aetiology than in non-ischaemic CHF (ischaemic CHF vs. non-ischaemic CHF, 1.64±0.3 vs. 1.96±0.5; $p<0.05$). In linear regression analyses, lower RHI was associated with lower pVO₂ ($r=+0.30$, $p<0.05$) as well as lower 6-min walking distance ($r=+0.40$, $p=0.01$). 6MWT distance was reduced in CHF vs. CON (414±146 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 CHF. 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 using reactive hyperaemia is a simple and easily applicable method for the use in ambulatory and clinical settings, and can be used in patients with and without cachexia.

2-15

The association of body mass index and presence of cachexia with survival and disability after stroke: data from 1,521 hospitalised patients with 30 months follow-up

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Background: Overweight and obesity are established risk factors for cardiovascular disease including stroke. In patients with established cardiovascular disease, increasing evidence suggests an inverse relationship between body mass index (BMI) on outcome, which has been termed obesity paradox. The impact of body weight in general and presence of cachexia specifically on outcome after stroke is not well established. We aimed to investigate the relationship between BMI and mortality as well as functional outcome in patients after stroke.

Patients and methods: We analysed data from of the Telemedical Project for Integrative Stroke Care project. In 1,521 patients suffering from an acute stroke or TIA data on BMI at time of hospital admission and on subsequent outcomes were available. Patients were grouped by BMI as cachectic (BMI ≤18.5), reference group (BMI >18.5 to 25) overweight (BMI >25 to 30), mild obesity (BMI >30 to 35) and advanced obesity (BMI >35, all kg/m²). Outcome measures after 30 months included all-cause mortality, recurrent stroke, need for institutional care, and dependency (institutionalisation, Barthel index <60 or modified Rankin score >3).

Results: During 30 months of follow-up, 401 patients (27%) died. Mortality rates at 30 months were 61%, 33%, 24%, 18%, and 13% in

the BMI subgroups ≤ 18.5 , $>18.5-25$, $>25-30$, $>30-35$, and >35 kg/m², respectively ($P < 0.001$). Rates for the combined endpoint of death or recurrent stroke were 64%, 40%, 31%, 22%, 18%, respectively ($p < 0.001$). Also, institutional care and dependency followed the same stepwise pattern with lowest rates in obese subjects (all $p < 0.001$). BMI was a significant inverse predictor for poor outcome after multivariable adjustment for age, sex, co-morbidity, living in partnership, and stroke severity: Compared to BMI 18.5–25 (HR 1.0, i.e., reference group) risk for death or recurrent stroke was higher in patients with BMI < 18.5 (HR 2.74, 95%CI 1.23–6.03), but lower in patients with overweight (HR 0.79; 95%CI 0.60–1.03, $p = 0.08$), mild obesity (HR 0.56, 95%CI 0.37–0.86; $p < 0.01$) and advanced obesity (HR 0.51, 95%CI 0.27–0.97; $p < 0.05$). When patients' obesity (BMI > 30) are considered as reference group, risk for death or recurrent stroke more than five times increased in patients with BMI < 18.5 , and two times increased in patients with BMI 18.5–25. Similarly, the risk for death or institutional care or and dependency was highest in patients with cachexia (BMI < 18.5), and decreased stepwise with increasing BMI being lowest in obese and very obese patients.

Conclusion: Patients hospitalised for stroke or TIA with low BMI (BMI < 25) and particularly with cachexia show increased subsequent morbidity and mortality. In contrast, obese patients have better outcome for mortality or recurrent stroke, need for institutional care, and dependency than patients with normal BMI. A better outcome after stroke in obese patients is in contrast to data from primary prevention studies, but concurs with observations of an obesity paradox in other cardiovascular diseases including heart failure and diabetes mellitus. Treatment strategies to increase or maintain body weight in patients with stroke or TIA should be tested.

2-16

Stroke-induced local activation of skeletal muscle apoptosis in the paretic leg depends on direct denervation: data from a MCAO stroke mouse model

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Background: Weight loss is a common observation after stroke and nutritional status contributes to post-stroke recovery and rehabilitation progress. Weight loss due to impaired feeding and disuse atrophy are clinically recognised. However, mechanisms of catabolic stimulation in skeletal muscle in relation to the degree of brain damage and muscle denervation have not been investigated in detail.

Methods: We investigated skeletal muscle apoptotic activation in a model of acute focal cerebral ischaemia produced by temporal occlusion of the mouse middle cerebral artery (MCAO). Caspase 3 (C3) and caspase 6 (C6) activity of the gastrocnemius muscle were assessed 3 and 7 days after MCA occlusion of both the paretic and non paretic legs. Global body composition (fat and lean tissue by nuclear magnetic resonance) and gastrocnemium muscle were assessed in relation to infarct size.

Results: Activity of caspase 3 and caspase 6 were upregulated after stroke compared to sham in the paretic and nonparetic leg at day 3 (C3 +548% and +454%; C6 +145% and +134%, respectively; both $p < 0.001$) and still increased at day 7 (C3 +194% and +248%, $p < 0.05$). This was accompanied by progressing wasting of the gastrocnemius muscle until day 7 (paretic leg, -20%; nonparetic leg, -19%; both, $p < 0.01$) and global

lean tissue loss (day 3, -17%; day 7, -12%, $p < 0.05$ vs. baseline). Infarct volume directly related to C3 and C6 activity only in the paretic leg (C3, $r = 0.72$; C6, $r = 0.78$; $p < 0.01$) but not in the nonparetic leg ($p > 0.5$).

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

2-17

The pathophysiological course of burn-injury associated cachexia: a new murine model

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Background: Burn injury is characterized by chronic inflammation (increased chemokines, cytokines, and acute phase proteins), hypermetabolism, and hypercatabolism (increased resting energy expenditure and caloric requirements) resulting in a unique profound and prolonged loss of lean body mass (LBM) with concurrent hepatosplenomegaly. Several animal burn models have been developed, however no murine models have been shown to faithfully reproduce the pathophysiological response of human burn injury.

Methods: Adult male C57BL/6 mice were shaved, Naird[®], and subjected to full-thickness burns using heated brass plates. Tissues were flash frozen for analysis and muscle sectioning. Piximus was used for quantification of LBM, fat mass, bone mineral density, and bone mineral content.

Results: Survival and weight loss were dependent upon total body surface area (TBSA) burned and brass plate contact time, with 20% TBSA burn (5-s contact time) resulting in 60% survival over 30 days. In burned mice, body weight, LBM, individual muscle masses remained significantly reduced and liver and spleen weights were significantly increased out to 30 days. Body weight nadir in burned mice was 14 days, with significantly reduced total body mass (-7.4%), LBM (-11.5%), individual muscle masses [quadriceps, (-25.1%); gastrocnemius, (-20.3%); tibialis, (-30%)], and muscle fiber cross-sectional area (-12%) compared to shams. At 14 days, burned mice had significantly reduced fat mass (-18.5%), bone mineral density (-7.5%), bone mineral content (-17.5%), and increases in spleen (+44.4%), and liver masses (+17.5%). At 14 days, burned mice had significantly elevated cytokine, chemokine, and acute phase proteins consistent with results in human burn subjects. Recovery of initial body weight occurred at 35 days, however significant hyperphagia and polydipsia persisted out to 80 days.

Discussion: This murine model accurately reproduces human pathophysiology following severe burn injury, providing a robust framework for future pharmacological and genetic approaches to dissect out the molecular mechanisms underlying burn pathophysiology.

3-01

Examining the consensus classification of cancer cachexia in pancreatic cancer patients

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A hallmark of pancreatic cancer (PC) is weight loss (WL) and cachexia. However, cachexia is difficult to define. According to the new international consensus classification of cancer cachexia patients who have one or more of the following three criteria should be diagnosed as cachectic: (a) >5% loss of body weight (BW) during the past 6 months, or (b) a body mass index (BMI) <20 kg/m² and ongoing WL >2%, or (c) sarcopenia and ongoing WL >2%.

Aim: The study aims to use this new cancer cachexia definition to diagnose cachexia in an unselected group of patients with advanced PC referred for palliative treatment.

Methods: Thirty-nine patients with PC were consecutively recruited, and asked to recall pre-illness stable BW and duration of WL. Anthropometric measurements (height, BW, skinfold thickness) were performed at inclusion and after 1 month. Sarcopenia was assessed with anthropometry (mid-upper arm muscle area <32 cm² for men and <18 cm² for women).

Results: Median age was 62 years and median Eastern Cooperative Oncology Group Performance Status status was 1. Median survival was 5 months. Upon inclusion, 92% had weight loss >5%, 28% had BMI <20 kg/m², and 10% had sarcopenia. Median survival among patients meeting one criteria (*n*=25) or two criteria (*n*=9) was 7 months while patients meeting three criteria (*n*=3) had a median survival of 2 months. According to the definition, three patients did not have cachexia, and the survival in this group was >8 months. After 1 month, 30 (88%) of the patients were evaluated and 16 (53%) patients were found to have cachexia.

Conclusion: Almost all patients (92%) with advanced PC were cachectic at inclusion according to the new definition. After 1 month, about half of the patients (53%) fulfilled the criteria for the diagnosis, probably indicating an effect of the palliative treatment.

3-02

From expert opinion to patients: classifying people with cancer cachexia

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Background and aims: The publication of an expert consensus on the diagnostic criteria and classification of cancer cachexia (Fearon et al., *Lancet Oncology*, 2011) stimulated us: (1) to apply the published classification system to real patient data; (2) to determine if the primary tumour site influenced the proportions of patients with “precachexia” or “cachexia”.

Methods: One hundred ninety-eight persons with a recent diagnosis of advanced cancer of various origins from the McGill University Health Center and the Jewish General Hospital, in Montréal, Canada were evaluated prior to treatment. Self-reported weight loss over the past 6 months and anorexia/associated symptoms were obtained. Serum C-reactive protein (CRP) levels were collected and measured. Patients were classified as normal, with precachexia or with cachexia.

Results: We evaluated 81 women (40.9%) and 117 men (59.1%). In our sample, 107 (54%) persons were classified as “normal”, 8 (4.0%) with precachexia, and 83 (41.9%) with cachexia at the time of diagnosis. People with pancreatic cancers had the higher rates of cachexia (58.0%), followed by colorectal (50.0%), upper GI (42.3%), hepatobiliary (41.2%), lung (34.5%), breast (26.3%), ENT (26.1%), and prostate cancers (33.3%). High serum CRP levels (>10 mg/L) were less prevalent on average in normal patients (40.0%), followed by patients with precachexia (71.4%) and cachexia (50.8%), but this difference was not statistically different (*p*=0.12).

Conclusions: The Lancet Oncology classification system enabled us to identify patients with cachexia. However, while the <5% weight loss criteria for precachexia is clear, factoring in the other criteria (anorexia and catabolic change) is imprecise. We recommend that clearer definitions for anorexia and catabolic load be established.

3-03

The evolution of clinical trial design in cancer cachexia: a systematic review based on the novel classification and definition criteria

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Background: Many challenges exist in the design of clinical trials for cancer cachexia particularly in patient selection and identification of appropriate outcomes. A consensus framework for the definition and classification of cancer cachexia was proposed in 2010; the main goal to help improve the design of clinical trials.

Objective: To evaluate published and ongoing/unpublished clinical trials according to patient selection (cachexia stage, oncology treatment profile), and outcomes related to the four domains of the consensus framework: domain I, depletion of reserves (body weight, muscle mass, strength); domain II, limitations to nutritional intake (food intake, nutrition impact symptoms); domain III, catabolic drive (systemic inflammation, altered metabolism, response to chemotherapy); domain IV, functional/psychosocial effects of cachexia (physical function, quality of life, distress, fatigue).

Methods: A systematic literature review identified all clinical trials from 2000 to August 31, 2011. Databases searched: Medline, Embase, PsycInfo, CINAHL (published), and www.clinicaltrials.gov (ongoing/unpublished). A total of 3,030 citations were identified, review of titles and abstracts excluded 2,206 citations not related to cancer, cachexia, or clinical trials. Ninety-three full-text articles and 64 registered clinical trials were selected and reviewed by two researchers.

Results: Sixty-five published and 34 ongoing/unpublished clinical trials were included. Patient population—patients were included based on criteria known to define cachexia that was inconsistently applied: weight loss (70%), good functional status (43%), life expectancy >3 months (25%), reduced food intake/anorexia (26%), and inflammation/altering metabolism (12%). When compared to the criteria for cachexia stage, all studies likely included patients with cachexia but also pre-cachexia (*n*=42) and refractory cachexia (*n*=78). The oncology treatment profile: no treatment (31%), active treatment or no treatment (24%), active treatment (21%), or not reported (23%). It was not clear whether no treatment referred to a break from current active treatment or no further treatment options were available. A description of supportive care was reported in 25% of studies. Outcomes related to the four domains are listed in Table 1.

Table 1. Outcomes and relevant measures used in cachexia clinical trials

	Number of Studies	%
Domain I. Depletion of Reserves	86	87%
Body Weight	72	73%
Body Composition	60	61%
CT	2	2%
DEXA	9	9%
Anthropometrics	14	14%
BIA	33	33%
Muscle Strength	21	21%
upper limb hand-grip dynamometry	14	14%
lower limb extension	3	3%
Domain II. Limitations to Nutritional Intake	72	73%
Food Intake	33	33%
Patient-reported food records (calculated)	26	26%
Subjective categorical classification	7	7%
Nutrition Impact Symptoms	53	54%
Appetite	43	43%
Domain III. Catabolic Drive	40	40%
Inflammation	32	32%
CRP	21	21%
Altered Metabolism (REE, indirect calorimetry)	13	13%
Response to chemotherapy	5	5%
Domain IV. Functional/Psychosocial Effects	73	74%
Physical Function	41	41%
Physician reported (ECOG, KPS, WHO, etc.)	27	27%
Objective measures (measured PA, exercise capacity)	12	12%
Patient-reported	8	8%
Quality of Life	53	54%
Fatigue	18	18%
Distress (depression, anxiety, mood, well being)	9	9%

Conclusion: There is a lack of consistency in defining criteria for patient selection into clinical trials, given the variability observed. Outcomes as proposed in the conceptual framework are unfortunately rarely included, specifically for muscle mass and measures of physical function. Further work in the clinical trials in the cancer cachexia working group of SCWD will propose implementation steps based on this review.

3-04

Diagnostic stages for cancer cachexia: are they clinically relevant?

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Background: Cancer cachexia stages (CCS; Fearon et al. 2011) were applied to the Human Cancer Cachexia Database via consensus among the co-authors. **Methods:** In 208 patients with advanced non-small cell lung or gastrointestinal cancers, we tested the relationship between the noncachexia (NC), sarcopenia (S), pre-cachexia (PC), cachexia (C), and refractory cachexia (RC) stages with selected outcomes.

Results:

	NC	S	PC	C	RC
	n=50	n=10	n=29	n=49	n=70
ECOG PS* (0-4; 4worst)	0.7±1.0^	0.6±0.5	1.1±0.3	1.2±0.7	2.5±0.8
McGill QoL Total Score (0-10; 0 Worst)**	7.0±1.2	7.2±1.0	5.2±1.3	5.3±1.5	4.9±1.5
# Hospital days/follow-up days(%)***	4.6±11.2	6.1±13.9	13.5±25.5	4.9±11.6	30.8±35.8
Chemotherapy Dose Reduction†	4/17 (23.5%)	1/1 (100%)	3/9 (33.3%)	7/16 (43.8%)	8/16 (50%)
Total fat mass by DXA (kg) ††	26.1±11.7	19.8±10.9	19.9±10.7	17.1±9.4	13.0±7.6
Total lean mass by DXA (kg) ‡	10.9±2.1	7.3±2.3	10.3±2.8	8.3±1.7	9.6±2.2
Survival (wks) ‡‡	69.4	62.8	35.0	39.4	11.1
Median (95% CI)	(48.1–90.8)	(42.5–83.2)	(13.9–56.1)	(28.4–50.4)	(5.9–16.3)

^mean ± SD. * NC different from PC, C, RC; PC different from S, RC; C different from RC; RC different from S; ** NC and S different from PC, C, RC; *** RC different from NC, S, PC, C; ANOVA p<0.05. † Chi-square test: p<0.05. †† NC different from C, RC; ‡ S different from NC, PC; ANOVA p<0.05. ‡‡Log-rank test: p<0.01.

Conclusions: The proposed CCS appears to have specific clinical relevance. Additional studies are needed to confirm their applicability.

3-05

A review on psychosocial effects of cancer cachexia

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Background: Cancer cachexia (CC) is frequent in advanced cancer, associated with high morbidity and mortality. Without anticancer treatment options, the course is usually fatal. Psychosocial effects (PSE) of CC are recommended to be looked for and psychosocial support should be provided.

Objectives: This study aims to review the literature on PSE of CC with the aim of identifying misbehaviour which could be modified.

Methods: A systematic literature search in MEDLINE was performed. Keywords for the topics “advanced cancer”, “anorexia/cachexia” and “psychosocial effects” were used for the search string. The search was amended by hand search.

Results: Eighteen studies were included. The analysis revealed causes of, presentations of, and coping strategies for PSE of CC for patients and carers. Few do not manage the effects constructively, adverse reactions have been identified. The main causes of PSE are a lack of knowledge CC is usually irreversible and inappropriate attempts to increase nutritional intake. Depending on the coping strategies of affected ones and their carers, PSE can increase or decrease. Identification of PSE of CC offers potential for psychosocial interventions to improve quality of life of those affected. Our analysis generated a conceptualisation of PSE of CC. A number of suggestions for psychosocial interventions were detected.

Conclusion: The concept of PSE in CC could help to sensitize healthcare professionals to cachexia-related problems and inform the management of CC.

3-06

Neural invasion induces cachexia and pain in pancreatic cancer

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Background and aims: Pancreatic cancer is characterized by a high frequency of cachexia, pain, and neural invasion (N-inv). Neural damage is occurred by N-inv and modulates pain and muscle atrophy in the connected spine. N-inv, thus, may affect cachexia in pancreatic cancer. The aim of this study is to elucidate the role of N-inv in cachexia and pain using clinical data and experimental N-inv model of pancreatic cancer.

Methods: Radiological N-inv, body composition, and pain were assessed using dynamic computed tomography, bioelectrical impedance analysis, and MD Anderson symptom inventory, respectively, in 50 patients (29 women, 21 men; median age, 66.5 years; range, 44–85; primary tumor sites in the pancreatic head/body/tail, 22/23/5) with treatment-naive advanced pancreatic cancer who were scheduled to undergo chemotherapy in National Cancer Center Hospital East. Patients with liver metastasis were excluded. The relationships between radiological N-inv and other factors were evaluated. N-inv model was made by the injection of Capan-1, human pancreatic cancer cell, into a left sciatic nerve of severe combined immunodeficiency mice. To investigate the effects of sham operation and subcutaneous tumors, the PBS group and the SC group were produced by injecting PBS into the sciatic nerve and by subcutaneous injection of cancer cells into the left flank, respectively. Allodynia was measured by Von-Fray test. Food intake and body weight were recorded. All mice were euthanized at 6 weeks after surgery. Bilateral epididymal fat tissue, right gastrocnemius muscle, and the tumor were harvested and weighed.

Results: Clinical studies in patients with pancreatic cancer have revealed that N-inv is related to cachexia and pain. N-inv mice showed allodynia and a loss of body weight without appetite loss, which are compatible with an animal model of cancer cachexia.

Conclusion: This study provides the evidence that N-inv induces cachexia and pain in pancreatic cancer.

3-07

Physical function at the time of diagnosis: the role of cancer cachexia

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Background and aims: We have recently applied the international consensus classification system for cancer cachexia (Fearon et al. 2011) on people with advanced cancer. Our main objective was to determine the extent to which cachexic state predicted physical function at the time of diagnosis. A second aim was to verify the role of systemic inflammation in predicting physical function.

Methods: One hundred ninety-eight persons with a recent diagnosis of advanced cancer of various origins from the McGill University Health Center and the Jewish General Hospital (JGH) in Montreal, Canada were evaluated prior treatment. Cachexic state was determined by applying the guidelines suggested by Fearon et al. Physical function was measured by the 2-min walk test (2MWT), the Timed-Up and Go (TUG), comfortable gait speed over 5 m, and by the physical function subscale of the SF-36 (PFI). Serum C-reactive protein (CRP) levels were collected and measured. Multiple linear regressions were used to analyze the relationship between the variables.

Results: Cachexic state was not a significant predictor of physical function regardless of how physical function was measured, holding age, gender, primary tumor site, and CRP levels constant. The TUG and 2MWT ($R^2=0.21$ and 0.22) were significantly predicted by age ($b=0.06\pm 0.02$ and -0.89 ± 0.33 , respectively) and CRP levels ($b=0.02\pm 0.01$ and -0.41 ± 0.15 , respectively). Age ($b=-0.01\pm 0.002$) was the only significant predictor of comfortable gait speed ($R^2=0.28$). Sex ($b=11.31\pm 5.15$) and CRP levels ($b=-0.23\pm 0.09$) significantly predicted self-reported PFI ($R^2=0.25$).

Conclusions: At the time of diagnosis, systemic inflammation seems to be an important predictor of physical function. Although physical function is on average lower in cachexic and precachexic patients than in normal patients, cachexia in itself does not seem to be a significant predictor of physical function at diagnosis. Physical function levels during the disease progression could however be influenced by cancer cachexia.

3-08

The clinical management of the emotional aspects of cancer cachexia

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Background: The prevention or alleviation of the symptoms of cancer cachexia, such as weight loss and poor appetite, would relieve much cachexia-related suffering. However, whilst the research community works to achieve this goal there is another important task: to help people with cancer cachexia to adapt and live with the symptoms. This is now acknowledged internationally by experts in the field of cancer cachexia research¹.

Purpose: The study aims to discuss the psychosocial support of people affected by cancer cachexia, drawing on recent publications.

Methods: Searches were conducted of PubMed, EMBASE, PsycINFO and CINAHL databases for publications about the clinical management of the emotional impact of cancer cachexia. Limits were English language; 3/09 to 9/11.

Findings: Recent studies describe the problem of distress in response to symptoms of cancer cachexia syndrome. Some authors suggest ways of alleviating the problem and others urge the development of psychosocial interventions. Preliminary work is now testing supportive interventions.

Conclusion: Despite accounts over many years of cachexia-related distress, little attention has been paid to the potential for psychosocial support to aid patient self-management and improve emotional health outcomes. Emergent thinking is that psychosocial support for cancer cachexia can have benefit for both patients and their family members, but this is yet to be demonstrated empirically in robust trials.

¹Fearon et al. Definition and classification of cancer cachexia: an international consensus. The Lancet Oncology 12(5): 489–495.

3-09

Healthcare professionals' response to cachexia in advanced cancer

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Background: Cancer cachexia is a complex metabolic syndrome characterised by severe and progressive weight loss which is predominantly muscle mass. It is a devastating complication of advanced cancer with profound bio-psycho-social implications for patients and their families. At present, there is no curative treatment for cachexia in advanced cancer therefore, the most important healthcare response entails the minimisation of the psycho-social distress associated with it. However, the literature

suggests healthcare professionals' are missing opportunities to respond to the multi-dimensional needs of this population.

Aim: The objective of this study was to explore healthcare professionals' experience, understanding and perception of need of patients with advanced cancer who have cachexia and their families.

Methods: An interpretative qualitative approach based on symbolic interactionism was adopted. A purposive sample of doctors, nurses, specialist nurses, and dieticians were recruited from a cancer centre in a large teaching hospital in Northern Ireland. Data collection consisted of two phases: focus group interviews followed by individual semi-structured interviews.

Results: Findings from the focus group interviews were used as a framework for the semistructured interview schedule. Results centred on the influence of a variable combination of knowledge, culture, and resources on the management of cachexia in advanced cancer. Data revealed that variation in healthcare professionals' perceptions of cachexia in advanced cancer, along with their professional ethos, influenced their response to it in clinical practice.

Conclusions: This study has revealed that cancer cachexia is a complex and challenging syndrome which needs to be addressed from a holistic model of care to reflect the multidimensional needs of patients and their families. Effective management will require a combination of knowledge, a supportive culture, and adequate resources.

3-10

Energy requirement in elderly cachectic patients with cancer

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Background: Cachexia is a well-known adverse effect of cancer and is associated with poor prognosis, impaired physical function and reduced tolerance to anticancer treatments. Despite understanding of cachexia has progressed, the clinical management remains complex and few data about energy requirement is available in elderly patients with cancer cachexia despite its implication for nutritional support.

Aims: The present study evaluated measured resting energy expenditure (mREE) and predicted energy expenditure (pREE) in elderly patients with cancer cachexia.

Methods: Fifty-nine elderly patients from our consultation of nutrition and addressed by the service of oncology were consecutively included (76.88±9.17 years). They all had weight loss >5% or BMI<20 and weight loss >2%, over the past 6 months according to Fearon's criteria. mREE was measured with indirect calorimetry and pREE was calculated from the Harris and Benedict equations.

Results: Mean weight was 57.86±12.88 kg and mean weight loss was 7.13 kg±3.46 at 6 months; mean BMI was 21.93±4.25; mean albuminemia: 30.71±7.19 g/l and CRP 35.96±40.07 mg/l. Mean MNA score was 17.68±4.87. mREE was 1,296.57±341.11 kcal/day and pREE was 1,135.07±199.13 kcal/day. Of the patients, 31.7% showed mREE more than 20% pREE; while caloric intake was 1,361 kcal±572/day and 23.52 kcal/kg/day. Anorexia was present in 43% of the patients.

Conclusion: As expected, patients had inflammatory process and malnutrition criteria. Elderly patients with cancer cachexia show rather similar values for mREE and pREE that do not really confirm an elevated REE in the majority of our patients as commonly held. Loss of weight seems to be mainly explained by insufficient caloric intake since caloric intake was largely under energy requirement that may be estimated at least at 1.4 REE in these patients.

3-11

Neuropeptides, metabolic disorder and inflammation in colon cancer patients: contributing to the cachexia syndrome

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Background and aims: Cancer cachexia, a devastating wasting syndrome, affects around 50% of all cancer patients and up to 80% of those with the advanced form of the disease. Inflammation plays a pivotal role in cachexia affecting the control of central energy balance, tissue metabolism and function, and modulation of appetite. This study aimed to examine plasma lipid profile, neuropeptides, and inflammatory marker levels in cancer patients with and without cachexia.

Methods: Forty-five subjects (control $n=15$, tumour-bearing patients without cachexia $n=15$, and tumour-bearing cachectic patients $n=15$). Samples were obtained from male and female patients, 18–100 years with gastrointestinal tumour, who underwent surgery at University Hospital (University of São Paulo). Approximately 10 mL of blood were collected in the surgical procedure within the venous access procedure of anaesthesia. The plasma lipid profile (Triglycerides, HDL-cholesterol, total cholesterol, NEFA), glucose, insulin, HOMA-IR, PAI-1, endotoxin, MSH, MCH and NPY levels were determined (Kits ELISA and colorimetric methods).

Results: Total cholesterol levels were increased in tumour-bearing (without and with cachexia) patients ($p<0.05$). In addition, NEFA and PAI-1 levels were increased in cachectic patients only ($p<0.05$). In contrast, all neuropeptides assessed (MCH, MCH, and NPY) were decreased in cachectic tumour-bearing patients, as compared with the control group ($p<0.05$).

Conclusion: These results indicate that cachectic cancer patients exhibit alterations in metabolism, inflammation and modified neurohormonal control of food intake, favouring the aggravation of the syndrome.

3-12

Serum IGF-1 levels help detect and predict skeletal muscle loss in cancer patients

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Background and aims: Skeletal muscle wasting is a cardinal feature of cancer cachexia and an important prognostic sign. Unfortunately, measurement of skeletal muscle mass for clinical purposes can be challenging using standard bedside techniques. Effective treatment of cachexia in cancer patients requires accurate assessment of skeletal muscle mass to determine both the success of interventions and to identify patients at high risk of further muscle loss. The objective of this study was to establish whether serum markers can help in detecting and predicting changes in skeletal muscle mass.

Methods: Cancer patients ($n=28$, mean age 65.6, 11 (39%) female, 26 (90%) advanced stage) were recruited during or after chemotherapy treatment. Each patient had measurements of skeletal muscle mass performed by either CT image analysis or DEXA on two occasions (mean(SD) interval: 50 (18)days) and serum samples collected at the same time as measurement of skeletal muscle mass were analysed for cortisol, CRP, IGF-1, myoglobin, and creatine kinase.

Results: At first visit, skeletal muscle mass corrected for height (SMI) was weakly correlated with IGF-1 only ($R=0.39$, $p=0.04$), and IGF-1 was also the only factor predictive of imminent change in SMI ($R=0.63$, $p<0.001$). At the second visit, IGF-1 was the only factor correlated with SMI ($R=0.59$, $p=0.003$) and was also highly correlated with recent change in skeletal muscle mass ($R=0.66$, $p<0.001$). Myoglobin level was also inversely related to recent change in SMI ($R=-0.40$, $p=0.03$). Of the factors tested IGF-1 was most strongly related to muscle mass. Furthermore, loss of muscle mass was most clearly seen in the 45% of patients with IGF-1 levels <15 nmol/l (age-adjusted reference range 6.8–23.3).

Conclusion: Even low-normal IGF-1 levels are insufficient to maintain stable skeletal muscle mass in cancer patients with advanced disease.

3-13

Functional impairment and distinct metabolic adaptations in skeletal muscle of pre-cachectic and cachectic patients with non-small cell lung cancer

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Background: A recent study demonstrated that exercise capacity is already decreased in patients with early stages of non-small cell lung cancer (NSCLC) cachexia, i.e. pre-cachexia. As physical performance is an important determinant of quality of life and mortality, we further focused on the molecular basis of this observation.

Aim: This study aims to investigate whether histochemical and metabolic properties of skeletal muscle are altered in pre-cachectic and cachectic patients with NSCLC.

Methods: In this prospective study, 22 healthy controls and 16 cachectic and 10 pre-cachectic patients with NSCLC were studied. Physical performance was assessed using quality of life (QLQ-C30) and physical activity (SF-20) questionnaires. Quadriceps muscle biopsies were analyzed by immunohistochemistry to distinguish muscle fibers containing type I (oxidative), type IIa (mixed) or type IIx (glycolytic) myosin heavy chain isoforms. Furthermore, expression and activity of an important regulator (PGC1 α) and enzymes (citrate synthase and 3-Hydroxyacyl-CoA dehydrogenase) of oxidative metabolism were assessed.

Results: Although skeletal muscle mass was only significantly decreased in patients with cachexia ($p<0.001$), both patients groups showed decreased physical performance ($p<0.001$). Interestingly, both patients with pre-cachexia and cachexia demonstrated a fiber type I to >II shift compared with healthy controls. While pre-cachectic patients showed a higher percentage of type IIx ($p=0.015$), cachectic patients showed a higher percentage of type IIa ($p=0.036$) fibers. mRNA expression of PGC1 α was decreased in both patients groups and decreased activity of oxidative enzymes was observed in muscle of patients with cachexia.

Conclusions: The shift from types I to II fibers and alterations in oxidative metabolism observed in both patients with pre-cachexia and cachexia may lie at the basis of exercise intolerance in these patient groups. The relative shift to type IIa or IIx fibers in cachexia and pre-cachexia, respectively, implicates that metabolic adaptations are distinct in successive stages of cachexia.

3-14

The prevalence and prognostic value of cachexia in patients with stage III NSCLC

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Background and aims: Cachexia frequently occurs in patients with lung cancer, and is associated with reduced physical function, intolerance to anti-cancer therapy and shorter survival. Our aim was to study the prevalence and the prognostic value of cachexia and to explore parameters associated with cachexia in patients with stage III non-small cell lung carcinoma (NSCLC). **Methods:** In 40 patients at diagnosis of stage III NSCLC, weight loss, FFM, handgrip strength, anorexia, nutritional intake and serum biochemistry were assessed. The ESPEN SIG and Fearon criteria were used to define pre-cachexia and cachexia, respectively. Cachexia was also defined by the Evans criteria. Additionally, quality of life was assessed by the EORTC-QLQ30 questionnaire. Differences between groups were analysed by independent t tests and ANOVA, and survival by Cox regression, adjusted for sex and tumour stage (IIIa/IIIb).

Results: According to the SIG and Fearon criteria, pre-cachexia was present in 9 (23%), cachexia in 7 (18%), and no cachexia in 24 (60%) patients. Survival between these groups was not significantly different, but patients with cachexia reported a lower quality of life ($p=0.03$). According to the Evans criteria, cachexia was present in 11 (28%) patients and no cachexia in 29 (72%) patients. The cachexia group showed a significantly shorter survival than the no-cachexia group (HR=4.4, $p=0.001$). Patients with cachexia had higher levels of CRP and IL-6, and lower Hb and serum albumin than patients without cachexia ($p<0.01$), and all inflammatory parameters were significantly correlated (Pearson r : 0.5–0.7, $p<0.01$). Patients with cachexia tended to report a lower quality of life ($p=0.08$).

Conclusions: Pre-cachexia and cachexia are prevalent at diagnosis of stage III NSCLC, but criteria find different prevalences. Cachexia seems to be associated with shorter survival and a lower quality of life. Further studies are warranted to more extensively explore these new criteria in cancer patients.

3-15

Impact of cachexia on circulating inflammatory cytokines on gastrointestinal cancer patients: IL-6 as a potential biomarker

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Background and aims: Cancer cachexia genesis and development have been proposed to be triggered by complex network of peripheral mediators involving IL-6, CPR and adiponectin, cytokines involved on inflammatory and body weight homeostasis. Thus, we proposed to determine whether the upregulation of the aforementioned cytokines, reported in gastrointestinal cancers (GI), is associated with substantial but involuntary weight loss in cancer patients (cachexia). We also aimed to evaluate the relationship between these cytokines and the clinic-pathological variables found to affect the incidence of cachexia in cachectic GI cancer patients.

Methods: IL-6, IL-8, TNF- α , IL-10, CPR and adiponectin were assessed by multiplexed microsphere cytokine immunoassay (Millipore®) in 59 patients, which were divided into four groups: stable weight control (WS, $n=10$) and tumor control (T, $n=12$), and control cachectic (C, $n=6$) and tumor cachectic (TC, $n=31$). All the data were related to the effect of cachexia and/or cancer.

Results: Increased levels of CRP and adiponectin were found in TC group when compared with WS (1.4- and 7.1-fold, $p<0.01$, respectively), demonstrating a cachexia effect (ANOVA, $p<0.01$). In T and TC patients, IL-6 levels were also increased when compared with WS (1.3- and 11-fold, $p<0.001$, respectively), demonstrating a tumor effect and interaction between the variables (ANOVA, $p<0.05$). Association between adiponectin and body weight loss showed a positive relationship ($r=0.668$, $p<0.001$), as well as increased levels of IL-6 were found in different cancer T stages (I/II— 34 ± 10 , III— 115 ± 59 , IV— 215 ± 83 , $p<0.001$) in cachectic GI patients.

Conclusions: A novel finding of this study was that some molecules seems to be disrupted only during cachexia state while some others increase in both states (tumor x cachexia). In addition, our results suggest that IL-6 may have an important role as a biomarker during cancer cachexia development.

Financial support: FAPESP 2007/52782-1, 2008/51094-9 and 2010/51078-1.

3-16

Low prevalence of precachexia and cachexia in patients with head and neck cancer scheduled for treatment with primary radiotherapy
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Background and aims: Recently, an international group of experts achieved consensus on the definition, classification and staging of cancer cachexia¹. It has been assumed that cachexia is present in many cancer patients. The aim of this study was to assess the prevalence of precachexia and cachexia in patients with head and neck cancer, scheduled for treatment with primary radiotherapy.

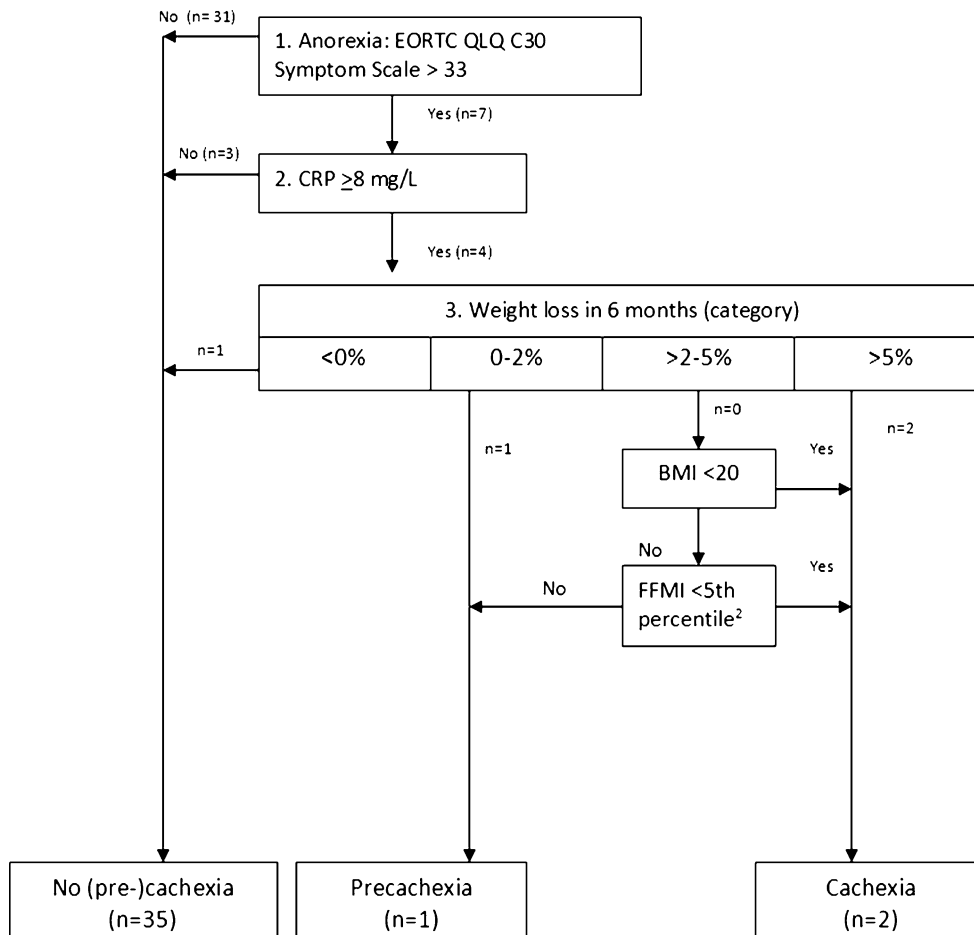
Methods: Of all patients with head and neck cancer who were scheduled for treatment with primary radiotherapy, all items needed for the classification of (pre) cachexia were assessed before start of the antitumor treatment: anorexia symptom score with use of the EORTC QLQ C30 (symptom scale >33), C-reactive protein (CRP; ≥ 8 mg/L), weight loss history in the year prior to treatment, body mass index (BMI; <20) and fat free mass index (FFMI; <5 th percentile²).

Results: Thirty-seven patients were studied (76% men). Eighty-nine percent of the tumours were T-stage I or II. Sixty percent of the patients had gained body weight, 16% of the patients had lost 0–2% body weight, 11% of the patients had lost >2 –5% body weight and 14% of the patients had lost more than 5% body weight. Median CRP was 4.0 mg/L (2–47) and 12 patients (32%) had a high CRP according to the hospital cut-off value (≥ 8 mg/L). Prevalence rates for precachexia were 2.7% and for cachexia 5.4%. Figure 1 shows the classification of (pre)cachexia in this group of patients with head and neck cancer, with use of a decision tree.

Conclusion: Prevalence of precachexia and cachexia is low in patients with head and neck cancer, scheduled for treatment with primary radiotherapy.

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1. Fearon K, et al. Lancet Oncol 2011; 12(5):489–495
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- Figure 1. Prevalence of precachexia and cachexia for head and neck cancer patients using a decision tree



3-17

Assessing body composition using computed tomography imaging: findings in UK patients with thoracic cancer

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Background and aims: Cancer cachexia adversely affects treatment response, quality of life and survival. Effective treatments are lacking and progress is hindered, in part, by the lack of reliable assessments of body composition. The use of computed tomography (CT) images for body composition analysis is one potential way forward. This study examined the inter- and intra-observer reliability of this technique and findings in a UK cohort of patients with thoracic cancer.

Methods: CT scans were analysed using Slice-O-Matic software to calculate cross-sectional area of skeletal muscle and adipose tissues at the level of the third lumbar vertebrae, from which whole-body fat and fat-free mass were estimated. Intra- and inter-observer reliability was assessed in the first 10 of 100 patients examined. Intra- and inter-class correlation coefficients and descriptive statistics were used to assess reliability and the main cohort findings respectively.

Results: CT image analysis was found to be highly reliable both within and between observers for the assessment of all tissue types with intra- and inter-class correlation coefficients of 1.00 (lower 95% confidence intervals 0.98–1.00). Of 100 patients (52 female, mean (SD) age 70(9)years, BMI 25.0(4.6)kg/m²) one fifth were sarcopenic. Of this group, only one third were classified as malnourished using clinical criteria.

Conclusion: CT image analysis is a highly reliable method of assessing body composition in patients with thoracic cancer. It can be used to accurately identify patients with severe muscle wasting, who may be overlooked by current clinical criteria, and may play an important role in the future management of cancer cachexia.

3-18

Exploring physical activity level in patients with thoracic cancer: implications for use as an outcome measure

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Background and aims: Cachexia is common in patients with thoracic cancer impairing physical function and quality of life. New approaches which target muscle tissue are emerging and activity monitors could provide an objective assessment of their effect on physical function. We have collated data from three studies involving the use of one such monitor in order to benchmark aspects of physical activity for patients with thoracic cancer, explore how these relate to physician-rated performance status, and consider the implications for future studies.

Methods: Patients with thoracic cancer and an Eastern Cooperative Oncology Group Performance Status (ECOG PS) of 0–2 wore an ActivPAL™ monitor for 1 week. The mean time spent each day in a range of activities, e.g. standing or stepping, or their frequency, e.g. number of sit-to-stand transitions, steps taken, were calculated and compared according to ECOG PS using tests of difference.

Results: Data from 84 patients (54 male, mean (SD) age 66 (9)years) were collated. Each day, patients spent a mean (SD) of 4.3 (2.0)h upright, completed 45 (17) sit-to-stand transitions, and took 4,246 (2,983) steps. There was wide variation in each activity examined. All but the number of sit-to-stand transitions differed significantly between ECOG PS categories.

Conclusion: These data provide a detailed insight into how physical activity levels change with declining PS. The wide variation in physical activity within each ECOG PS suggests that performance scales lack sufficient sensitivity to evaluate new cachexia treatments. Our data help inform future work in this area.

3-19

Relationship between nutritional status, Glasgow prognostic score and the presence of complications in patients with cancer of the esophagus and stomach undergoing treatmentJacqueline B. Silva¹, Sílvia F. Maurício¹, Tatiana Bering¹, Maria Isabel T. D. Correia² (¹Food Science Post Graduation Program, Pharmacy School, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil; ²Alfa Institute of Gastroenterology; Hospital of Clinics, Medical School, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil)

Background and aims: The relationship between weight loss and the presence of inflammation has been described in patients with cancer. Cancer cachexia is a consequence of multiple factors, including production of cytokines due to the increased acute phase response which also leads to higher concentration of C-reactive protein and lower albumin levels. The presence of inflammation contributes to loss of lean mass and body fat. Both nutritional status and severity of inflammation may be associated with the development of complications during cancer treatment. Thus, the assessment of nutritional status and inflammation could be used as tools to screen patients who may benefit from early nutritional interventions.

Methods: In the present study, the relationship between nutritional status, defined by subjective global assessment (SGA), and the severity of inflammation as defined by the Glasgow prognostic score (GPS) and, its relation to complications during cancer treatment (defined by common toxicity criteria) were assessed.

Results: This study enrolled 43 patients with cancer of the esophagus and stomach with a mean age of 64.7±12.0 years. The nutritional status, according to the three categories of SGA was associated with the three categories of the Glasgow prognostic score ($p<0.05$), and both the SGA and the GPS were associated with the presence of complications, but the GPS seems to be more accurate in identifying complications than the SGA as established by the area under the curve (AUC) (GPS: AUC=0.77, $p<0.05$, CI=0.580, 0.956; SGA: AUC=0.679, $p<0.05$, CI=0.426, 0.931).

Conclusions: GPS may potentially be used as a nutrition screening tool, and both GPS and SGA may identify those at risk of complications undergoing treatment, but the GPS has higher accuracy than the SGA.

3-20

Serum interleukin-15 levels, body weight, and muscle mass in cancer patients with cachexia at the time of diagnosis and after 8 weeksPedro L. Martínez-Hernández¹, Angel Hernanz-Macias², Carmen Gómez-Candela³, Cristina Grande-Aragón², Jaime Feliu-Battle⁴, Javier Castro-Carpeño³, Isabel Martínez-Muñoz², Laura Zurita-Rosa³, Marta Villarino-Sanz³, Concepción Prados-Sánchez⁵, Joaquín Sánchez García-Girón⁶ (¹Internal Medicine Department, Hospital Universitario “La Paz”, Madrid, Spain; ²Department of Biochemistry, Hospital Universitario “La Paz”, Madrid, Spain; ³Department of Clinical Nutrition, Hospital Universitario “La Paz”, Madrid, Spain; ⁴Department of Medical Oncology, Hospital Universitario “La Paz”, Madrid, Spain; ⁵Department of Pneumology, Hospital Universitario “La Paz”, Madrid, Spain; ⁶Department of Thoracic Surgery, Hospital Universitario “La Paz”, Madrid, Spain)

Background: Interleukin-15 (IL-15) has important anabolic effects on muscle protein metabolism through a decrease in the ATP-ubiquitin-dependent proteolytic pathway. The role of IL-15 in human cancer cachexia is unknown.

Objective: To assess the relationship between IL-15 and body weight and muscle mass in cancer patients with cachexia at diagnosis of malignancy and 8 weeks later.

Design: Observational study of 21 cancer patients and eight healthy subjects. Cancer patients were divided into those with and without

cachexia (loss of $\geq 10\%$ or $< 10\%$ of the initial body weight, respectively). Body composition was measured by leg-to-leg impedance. Serum IL-15 levels were assessed at baseline and after 4 and 8 weeks.

Results: Baseline IL-15 values were similar in cancer patients and in healthy subjects. Five patients showed an increase of 3.7 kg at the end of the study (5.4% of body weight) and showed a mean increase of IL-15 of 1.32 pg/mL (121%) at 4 weeks and 2.32 pg/mL (197%) at 8 weeks, as compared with mean decreases of -4.1 kg (-5.3%) and -0.09 kg (-2.5%) and 0.6 pg/mL (40.8%) in the 13 patients who lost weight ($P=0.001$ and $P=0.022$, respectively). Changes of IL-15 at 4 and 8 weeks as compared with baseline were directly associated with changes in body weight, body mass index, fat-free mass, and muscle mass ($P<0.05$).

Conclusions: IL-15 may have a physiopathologic role as regulator of body weight and muscle mass in cancer patients independently of the presence of cachexia.

3-21

Prevalence of cachexia in head and neck cancer patients: an explorative study

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Background and aims: Already at diagnosis, head and neck cancer patients are at risk for malnutrition. Local symptoms, such as swallowing problems are a major cause of malnutrition in these patients. Additionally, malnutrition may result from changes in smell and taste/aversion and loss of appetite¹. Presence of these systemic symptoms at diagnosis may be indicative for the cachexia syndrome. Therefore, we tested the hypothesis that head and neck cancer patients suffer from cachexia.

Methods: Twenty-six adult patients (21 male and 5 female) with newly diagnosed squamous cell carcinoma in the oral cavity ($n=9$), pharynx ($n=11$) or larynx ($n=6$), using a solid diet, were included. Cachexia was assessed 1 week prior to start of cancer treatment, according to the criteria of Fearon et al.²: appendicular skeletal muscle index < 7.26 kg/m² in men and < 5.45 kg/m² in women, as measured by dual energy X-ray absorptiometry, and any degree of weight loss $> 2\%$. Additionally, inflammatory markers (CRP, IL-6 and Hb) were assessed.

Results: Prevalence of cachexia was 31% (8/26) (95% CI: 17%; 50%). Of the cachectic patients, six had elevated levels of serum CRP (> 5 mg/l), one had an elevated level of IL-6 (> 4.0 pg/ml) and two had anemia (Hb < 8.5 mmol/l in men, < 7.5 mmol/l in women). None of the cachectic patients had disturbed levels of all inflammatory markers.

Conclusions: About one third of newly diagnosed head and neck cancer patients suffers from cachexia. In the strategy to prevent further deterioration of nutritional status of this patient group, treatment of cachexia should be a specific target.

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3-22

Association of lower limb power output and functional ability in cancer cachexia

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Background and aims: Lower-limb extensor power (LLEP) is important for the daily performance of dynamic lower-limb activities that require rapid rates of muscle contraction. However little is known about the relationship between muscle power and functional ability nor the influence of weight loss on power output in cancer cachexia. We hypothesized that (1) LLEP would be associated with power-related functional ability and (2) LLEP would be influenced by degree of weight loss in patients with upper gastrointestinal cancer (UGC) cachexia.

Methods: Fifty-four patients ($n=35$ men) with UGC were recruited. Mean (SD) age was 65(11)years and average weight loss was 8.4(9.4)%. LLEP of the dominant limb was measured using a Nottingham Power Rig (highest of five measurements) and timed-up-and-go (TUG) and sit-to-stand (STS) times (fastest of three efforts) were measured as indicators of functional ability. Statistical analysis was by Pearson's product moment using SPSS v 15.0.

Results: Mean LLEP was 1.29 (0.55)W kg⁻¹ (1.45 (0.60)W kg⁻¹ men, 0.99 (0.26) kg⁻¹ women). Mean TUG was 7.28 (1.80)s (7.24(1.83)s men, 7.37 (1.81)s women) and mean STS time was 0.66 (0.20)s (0.65 (0.20)s men, 0.68 (0.19)s women). There was a significant negative correlation between LLEP and both TUG ($r=-0.51$, $p<0.001$) and STS ($r=-0.58$, $p<0.001$). Furthermore, percentage weight loss was significantly negatively correlated with muscle power ($r=-0.32$, $p=0.021$) and significantly positively correlated with STS ($r=0.38$, $p=0.011$) but not with TUG ($r=0.26$, $p=0.089$).

Conclusions: We have shown evidence of an association between lower limb power, functional ability, and weight loss. This supports a rationale for developing multimodal anti-cachexia interventions to improve power output in patients with cancer.

3-23

Suppression of skeletal muscle turnover in cancer cachexia: evidence from the transcriptome in sequential human muscle biopsies

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Background and aims: Although muscle wasting in cancer cachexia is thought to arise from an imbalance between synthetic/proteolytic pathways, direct evidence in human cancer cachexia is limited. This study examined the transcriptional profile of quadriceps muscle in patients before and after potentially curative surgery for upper gastrointestinal cancer (UGIC).

Methods: Baseline quadriceps muscle biopsies were performed on 12 UGIC patients undergoing curative resection (mean weight loss 7.4%) and 6 healthy controls (HC). UGIC patients had a follow-up biopsy an average of 260 days post-surgery (clinically disease-free, weight-stable preceding 2 months). The global transcriptome of these biopsies was analysed using

microarrays. Significance analysis of microarrays (SAM), gene ontology (GO) and ingenuity pathway analysis (IPA) were carried out. Muscle function (quadriceps strength/lower limb power/hand grip dynamometry) and physical activity (step count/24 h) were measured in UGIC patients.

Results: SAM analysis of baseline compared with follow-up UGIC patient biopsies identified 1,747 downregulated and 121 upregulated genes (false discovery rate (FDR) $\leq 6\%$; fold change $\geq 30\%$). RT-qPCR validated selected genes. GO analysis showed downregulation of genes at baseline. IPA revealed enrichment for skeletal and muscle disorders, PPAR α /RXR α activation, IL-6 signalling and glucocorticoid receptor signalling. Nine hundred forty-one genes had lower expression (FDR $< 10\%$) in baseline UGIC patients' compared with HC muscle. Of these downregulated genes, 558 were also regulated between baseline and follow-up UGIC patient samples. All except one (TFDP2) were higher at follow-up. Comparison between HC and UGIC follow-up samples revealed a transcriptomic profile indistinguishable from healthy muscle. Muscle function and physical activity were not different at follow-up compared with baseline indicating transcriptomic differences were due to tumour removal rather than varying physical activity levels.

Conclusions: This study shows that cancer and weight-loss leads to a suppression of transcriptional activity for genes involved in both anabolism and catabolism supporting the idea that muscle turnover is suppressed in cancer cachexia.

3-24

An investigation of microRNAs in the skeletal muscle of cancer patients with cachexia

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Background and aims: MicroRNAs (miRNAs) represent powerful mechanisms for regulating tissue phenotype. This study set out to investigate the role of miRNAs in the skeletal muscle of patients with cancer cachexia.

Methods: Eighteen upper gastrointestinal cancer patients (average weight loss, 8.9%) undergoing potentially curative surgeries were recruited. Three controls undergoing surgery for benign disease were also recruited. Rectus abdominis muscle biopsies was taken at the time of surgery and RNA extracts prepared. qPCR and microarrays (Exiqon LNA mercury platform) were used to study expression level of miRNAs in cancer cachexia. miRNAs differentially expressed in human muscle tissue and those that changed with $> 10\%$ weight loss were identified using significance analysis of microarray. Differentially expressed miRNAs with a fold change of $\geq 30\%$ and a false discovery rate $< 10\%$ over ≥ 3 probes were chosen.

Results: Microarray revealed five miRNAs (miR-29b, miR-143, miR-100, miR-193b, miR-768-3p) that were upregulated with $> 50\%$ fold change and one miRNA (miR-208a) which was downregulated with $> 50\%$ fold change. qPCR validation of selected differentially expressed miRNAs indicated that miR-29b correlated with weight loss ($R=0.5$; $p=0.03$) and miR-424 showed a trend ($R=0.4$; $p=0.06$). miR-195 was also related to weight loss ($R=0.4$; $p=0.08$). Investigation of the 'myomirs' (miR-1, miR-133a and miR-206), which are highly expressed in human muscle and play a role in muscle development/physiological responses showed that weight-loss significantly co-varied with miR-133a ($R=0.6$, $p<0.01$) but not with miR-1 and miR-206.

Conclusions: This study has identified novel miRNAs involved in muscle wasting in cancer cachexia which may represent potential biomarkers and targets for future therapeutic intervention development.

3-25

Expression profiles of regulatory and proteolytic pathways in skeletal muscle of patients with non-small cell lung cancer cachexia

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Background: Experimental research has indicated that increased muscular nuclear factor kappa B (NF- κ B) and ubiquitin (Ub) proteasome system (UPS)-mediated proteolysis plays a pivotal role in cancer-induced muscle wasting. Currently, only limited data on these pathways is available in clinical cachexia. This hampers progress in human cachexia and therefore, translation to clinical cachexia is essential at this moment.

Aim: The study aims to investigate NF- κ B activity and expression of E3 Ub-ligases in skeletal muscle of cachectic patients with NSCLC.

Methods: Sixteen cachectic and 10 pre-cachectic patients with NSCLC were compared to 22 healthy controls. Body composition was assessed by DXA and plasma and quadriceps muscle biopsies were obtained. Transcriptional levels of I κ B α were determined as indirect measure of NF- κ B activity and ex vivo plasma transfer to stable NF- κ B-luciferase reporter muscle cells were used to assess NF- κ B inducibility. Proteolytic activity was evaluated by measurement of mRNA expression of four E3 Ub-ligases (NEDD4, Atrogin-1/MAFbox, TRIM32, MuRF1) and autophagy-related markers (MAP1LC3B, BNIP3).

Results: Cachectic patients demonstrated significant weight loss (mean 13.1%) and decreased muscle mass ($p<0.001$), while mean weight loss in pre-cachectic patients was limited (2.1%) and muscle mass was not altered. Increased muscular mRNA expression of I κ B α was evident in cachectic but not in pre-cachectic patients. Interestingly, ex vivo exposure to plasma of pre-cachectic and cachectic patients showed induction of NF- κ B luciferase activity in cultured muscle cells. E3 Ub-ligase expression was not significantly altered in both patients groups, whereas transcript abundance of autophagy-related genes was increased in cachectic patients.

Conclusions: This study reveals that NF- κ B activity, as observed in muscle tissue, is the consequence of a factor contained within the circulation of cachectic patients. The absence of Ub-ligase transcriptional activity suggests that decreased protein synthesis or other proteolytic systems, such as autophagy as found here, may contribute to muscle atrophy in human cancer cachexia.

4-01

Establishment of novel animal models of cancer cachexia by transplantation of human gastric cancer cell lines and effects of rikkunshito, a traditional Japanese medicine, on the cancer cachexia models

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Background and aims: Cancer cachexia, observed in 80% of advanced cancer patients, is a multifactorial syndrome characterized by anorexia, body weight loss, loss of adipose tissue, and skeletal muscle accounting for at least 20% of deaths in cancer patients. Cancer cachexia is

responsible not only for a poor quality of life (QOL) but also poor responses to chemotherapy. Therefore, development in the treatment and amelioration of cancer cachexia is required for the improvement of the decreased QOL and effective chemotherapy. It has been reported that patients with gastric cancer have the highest frequency (83%) of weight loss. The aims of the present study are to establish cancer cachexia model by transplantation of human gastric cancer cell and examine the effects of rikkunshito, which is a traditional Japanese medicine that prescribed to treat various gastrointestinal tract disorders, on the cancer cachexia models.

Methods: Novel two cell lines (MKN45clone85 and 85As2) were originally established from human gastric cancer cell line MKN45 by repeated transplantation followed by sorting of the clones causing significant weight loss in mice. These cells (1×10^6 – 10^7 cells) were subcutaneously transplanted into nude rats, and then body weight, food intake, body composition and plasma inflammatory makers were measured. The effects of oral administration of rikkunshito into rats transplanted with 85As2 cell were also examined.

Results: In nude rats transplanted these cell lines, weight loss, anorexia, low fat-free mass and increased plasma inflammatory makers were observed (the degree of cachexia:85As2>MKN45clone85). Oral administration of rikkunshito significantly ameliorated severe symptoms of cancer cachexia–anorexia in rats transplanted with 85As2 cells.

Conclusions: These findings suggest that the novel animal models established here are useful for the study of cancer cachexia including the pathogenesis and development of potential therapies. Further, rikkunshito would contribute to the improvement of QOL of patients with cancer cachexia.

4-02

The Animal Cachexia Score (ACASCO): a new tool for the staging of cancer cachexia in experimental animals

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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.

Methods: The score considers five main different factors: body weight and lean body mass loss (BWC), anorexia (ANO), inflammatory, immunological and metabolic disturbances (IMD), physical performance (PHP) and quality of life (QoL). The score's scale goes from 0 to 100: mild cachexia (less than 25), moderate (more than 25 and less than 50), severe (more than 50 and less than 75) and terminal phase (more than 75 and up to 100).

Results: In the study, we analyzed the aforementioned factors in tumour-bearing rats (Yoshida AH-130 ascites hepatoma) at different days after the tumour inoculation in order to validate the ACASCO. The parameters analyzed were the following: body and muscle weights (BWC); plasma levels of tumour necrosis factor- α , serum amyloid A, albumin, lactate, urea, hematocrit and lymphocyte proliferation assay (IMD); food intake (ANO); grip strength test and total physical activity (PHP); signs of distress (temperature alterations, closed eyes, piloerection, diarrhea, constipation, chomodacryorrhea, lack of movement); and the intruder-resident paradigm and forced swim tests (QoL).

Conclusions: The present score facilitates cachexia staging in experimental animals bearing tumours and therefore will allow for a more appropriate measurement of the degree of cancer wasting.

4-03

Changes in the expression of melanocortin receptors and pro-opiomelanocortin in the hypothalamus in a rat model of cancer cachexia

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The cancer cachexia is frequently seen in patients with advanced cancer and is characterized by anorexia and weight loss. It is well-known that such advanced cancer stages are scarcely responsive to available pharmacological treatments, suggesting that once a critical point is reached, improvement of the metabolism and availability of nutrients may be hard to achieve. The central melanocortin system directly controls nutrient intake and energy metabolism. In the present study, we investigated the changes in the expression of melanocortin 3 and 4 receptors (MC3R and MC4R) and pro-opiomelanocortin (POMC), which encodes α -melanocyte-stimulating hormone, within the rat hypothalamus in both early- and late-stage of cancer cachexia. A tumor xenograft model was prepared in F344/N-rnu rat using human gastric cancer cells, 60As6. At 8 weeks after tumor inoculation, when rats exhibited slight anorexia and weight loss, both MC3R and MC4R expressions in the hypothalamus were significantly reduced compared to control rats. In contrast, the dramatic increases in the expression of those receptors were observed in the hypothalamus of tumor-bearing rats 16 weeks after tumor inoculation, when rats exhibited severe wasting associated with significant reduction of both adipose tissue and muscle mass. Further, under the same conditions, remarkable decrease in POMC expression in the hypothalamus of tumor-bearing rats was also observed. These findings suggest that the dramatic upregulations of MC3R and MC4R possibly caused by downregulation of POMC in the hypothalamus may contribute to the metabolic alterations and reduced availability of nutrients under late-stage of cancer cachexia.

4-04

Myostatin gene inactivation reduces skeletal muscle mass loss and tumor growth, and increases lifespan in mice with cancer cachexia

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Background: Cancer cachexia is a devastating wasting syndrome that affects up to 50% of patients, and accounts for >20% of cancer-related deaths. Wasting results from depletion of adipose tissue and skeletal muscle. We tested the hypothesis that inactivation of myostatin, a potent inhibitor of skeletal muscle mass, would prolong survival and attenuate muscle atrophy and adipose tissue loss.

Methods: Fourteen-month-old C57Bl/6 J wild-type (WT) mice and myostatin knock-out mice (KO) received a subcutaneous injection of 5×10^6 Lewis lung carcinoma (LLC) cells to induce cachexia or vehicle alone

($n=10$ per group). A second cohort was used for a survival analysis on WT and KO mice injected with LLC cells as described above.

Results: Five weeks later, myostatin gene inactivation significantly increased food intake ($p<0.01$; WT-LLC: -9.3% and KO-LLC: $+6.52\%$). WT-LLC mice had a greater tumor weight than KO-LLC mice ($+30\%$; $p<0.05$). Adipose tissue loss was partially prevented in KO mice, with a fat loss that reached 80% in WT-LLC mice vs. 60% in KO-LLC mice ($p<0.05$). LLC injection induced skeletal muscle atrophy in WT mice as evidenced by the decrease in tibialis anterior and soleus muscle weights (TA and SOL; -25%), extensor digitorum longus (EDL; -30%) and gastrocnemius (GAS; -20%) compared to control WT. Myostatin gene inactivation significantly reduced the magnitude of this loss. Skeletal muscle mass was only reduced by 10% in TA and EDL muscles ($p<0.05$ and $p<0.001$; WT-LLC-injected mice vs. KO-LLC-injected mice) and 6% both in SOL and GAS muscles ($p<0.01$ and $p<0.05$; WT-LLC-injected mice vs. KO-LLC-injected mice). Finally, myostatin gene inactivation prolonged lifespan from 32 to 41 days ($p<0.001$).

Conclusion: Myostatin gene inactivation potently prolongs animal survival together with attenuating skeletal muscle atrophy and protecting adipose stores, while increasing food intake and reducing tumor growth. These findings highlight the therapeutic potential of myostatin inhibition for cancer cachexia.

4-05

Cancer cachexia's metabolic fingerprint in a murine model confirms a distinct entity

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Despite recent consensus definitions of cancer cachexia, lack of specific diagnostic biomarkers remains a major hurdle towards more accurate diagnosis of cancer cachexia, but also towards the understanding of cachexia as a separate entity from other wasting syndromes. In a previous pilot study, we have shown that cancer-cachectic mice have a unique metabolic fingerprint with distinct glucose and lipid alterations when compared to healthy controls. Further, ¹H NMR-based metabolomics studies were carried out to investigate the differences in metabolic profiles of cancer-cachectic mice when compared to tumor-bearing non-cachectic mice, calorie-restricted mice, and surgically treated cancer-cachectic mice. Male CD2F1 mice were divided into five groups: (1) cachexia group received cachexia-inducing C26 undifferentiated colon carcinoma cells; (2) tumor-burden group received, non-cachectic, P388 lymphoma cells; (3) starvation group remained cancer-free but were subjected to calorie-restriction; (4) surgery group was similar to cachexia group but tumors were resected mid-experiment; and (5) control group was left to age intact. Baseline, mid-experiment and final serum samples were collected for ¹H-NMR spectroscopic analysis. After data reduction, unsupervised principal component analysis and orthogonal projections to latent structures demonstrate that the unique metabolic fingerprint is independent of tumor burden and distinct from profiles of starvation and aging. Furthermore, mice that underwent surgical tumor removal have a metabolic profile that differentiates itself from that of cachectic mice, seemingly reverting to a profile more congruent with healthy controls, indicating a return to a normal state with cure in murine models. Hyperlipidemia, hyperglycemia, and reduced branched-chain amino acids, distinguish the metabolomic profile of cachexia from other groups. The findings that metabolomic

analysis of murine serum is able to completely differentiate cachexia from tumor burden and calorie-restriction warrant similar translational investigations in patients to better understand cancer cachexia's unique disease progression and treatment response.

4-06

Myocyte enhancer factor (MEF) 2C: a novel role in cancer-induced cardiopathology

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Background: Cardiac or respiratory failure is responsible for death in approximately 30% of advanced cancer patients with cachexia. Significant reduction in contractile function has been observed in mouse models of cancer cachexia. The reversal of this cardiac weight loss increased their longevity. These findings imply the importance in maintaining cardiac structure and function during cancer progression. The characterisation of structural and molecular changes in cardiac muscle during cancer cachexia would allow improved diagnosis and identification of potential therapeutic targets to improve quality of life and survival in cancer.

Aims: This study aims to investigate cardiac ultrastructure and gene expression of the myogenic transcription factor MEF2C and gene targets in cancer-bearing mice.

Methods: The colon 26 (C26) carcinoma mouse model of cachexia was established and whole hearts were isolated for gene and protein expression studies using real-time qPCR and Western blotting. Ultrastructure was studied with transmission electron microscopy.

Results: Significant weight loss (15–20%) was evident in hearts from C26 mice. Loss of structural integrity and altered mitochondrial morphology was seen in electron micrographs of cachectic cardiac muscles. Gene and protein expression of myocyte enhancer factor 2C, an important transcription factor in muscle maintenance and regeneration, was found to be downregulated in hearts from cancer bearing mice with concurrent suppression of downstream target gene transcription i.e. myoglobin and myomesin.

Conclusion: The distorted morphology observed in cardiac muscle of cancer bearing mice suggests compromised oxygen transport capacity and function. This deterioration of cardiac function due to cancer may contribute to other complications observed in cancer cachexia. The alterations in MEF2C gene expression in cardiac muscle due to cancer has not previously been described and may play an important role in the underlying pathogenesis of the disruption of cardiac sarcomeric integrity and energy homeostasis in cachectic hearts.

4-07

Shift of metabolic genes in cancer induced cardiac cachexia

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Background and aims: Patients suffering from heart failure or advanced cancer share several clinical features including limitation in exercise capacity, shortness of breath, early fatigue, and the development of cachexia. In both populations, cachexia is a major factor that reduces quality of life and is associated with an unfavorable

prognosis. The underlying mechanism of cancer-mediated cardiac cachexia is poorly understood. Here, we evaluated the expression of genes involved in fatty acid oxidation in a mouse cancer model associated with cardiac cachexia.

Methods and results: A solid peritoneal tumor was induced through intraperitoneal implantation of melanoma cells (10^6 B16-F10 cells). Tumor-bearing mice revealed markedly reduced heart weight/body weight (HW/BW: Cntr.: 5.1 ± 0.2 vs. tumor: 3.7 ± 0.2 mg/g; $p < 0.001$) and heart weight/tibia length ratio as compared to control mice (HW/TL: Cntr: 12.6 vs. tumor: 5.0 mg/cm; $p < 0.001$) 3 weeks after cell implantation. Echocardiographic analysis showed reduced systolic function of tumor bearing mice as measured by fractional shortening (FS: Cntr: $38.9 \pm 10.4\%$, $n = 12$ vs. tumor: $23.1 \pm 5.1\%$, $n = 9$; $p < 0.001$) and overall thinning of the wall thickness. This was associated with a high mortality in cancer animals (66% , $n = 25$ vs. 0% in control, $n = 17$; $p < 0.001$). QRT-PCR revealed increased mRNA expression of three peroxisome proliferator-activated receptor isoforms (PPAR α , δ and γ and their co-factor PGC1 α (PPAR α : $+71.4 \pm 19.2\%$; $p = 0.02$; PPAR δ : $+17.3 \pm 10.1\%$, $p < 0.04$; PPAR γ : $+70 \pm 20.6\%$, $p = 0.04$; PGC1 α : $+36.1 \pm 23.1\%$, $p = 0.01$)). Moreover, the mRNA levels of carnitine palmitoyltransferase-1, the rate-limiting enzyme that acts in β -oxidation, was significantly increased (CPT1 α : 114.2 ± 29.4 , $p < 0.001$; CPT1 β : 1648 ± 219 , $p = 3$).

Conclusion: These findings demonstrate that cancer-induced cachexia is associated with upregulation of components of the PPAR pathway involved in muscle fatty acid oxidative gene transcription. This observation suggests that cancer-mediated cardiac cachexia differs at the molecular and potentially also at the metabolic level from cardiac cachexia in endstage heart failure where this pathway has been reported to be upregulated.

4-08

Common pathways in cancer and cardiac cachexia

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Background and aims: Due to an aging population, cardiovascular and cancer diseases become an increasing problem. The morbidity and mortality of cancer and/or chronic heart failure patients is not only characterized by the progression of the disease-specific process, but also by cachexia which results in dramatic loss of lean mass and body fat. Despite the clinical importance, critical molecular mechanisms in cachexia development and potential common denominators between cardiovascular and cancer diseases remain unknown, prompting us to explore potential links between these two disease entities.

Methods: To induce cachexia, we transplanted Colon26 adenocarcinoma cells subcutaneously in Balb/c mice. Subsequently, mice were metabolically monitored by MRI technology and body weight and food intake were determined. Additionally, we investigated cardiac function by weekly echocardiography and PV-loop measurement. The heart weight/tibia length ratio, cardiac morphology, cardiomyocyte size and the degree of cardiac fibrosis were determined. Furthermore, the gene expression of the heart was analyzed by Taqman analysis and Affymetrix GeneChips.

Results: Cancer cachexia was induced in experimental mice as shown by significantly lower body weight, loss of adipose and skeletal muscle masses and anorexia. Analysis of gene expression pattern revealed a switch from an adult to a fetal gene program in the heart. Monitoring of heart function demonstrated a significantly reduced heart rate as well as impaired fractional shortening in tumor bearing mice. Additionally, the heart weight/tibia length ratio was lower in mice with cancer. This atrophic phenotype

was correlated with increased autophagy but not with the activation of the ubiquitin-proteasome system like in skeletal muscle.

Conclusions: We show that cancer cachexia causes an impairment of cardiac function and energy balance, leading to cardiac atrophy and insufficiency. Ongoing experiments will now address the molecular signaling pathways which induce the observed cardiac phenotype in response to tumor growth.

4-09

Altered circadian rhythm and inflammatory signaling in white adipose tissue and lipid metabolites in cancer cachexia syndrome

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Involuntary weight loss among patients with cancer is often attributed to the cancer cachexia syndrome. The aetiology is multifactorial involving loss of skeletal muscle, adipose tissue and high systemic levels of inflammatory cytokines such as IL6. In the present study, we investigated the impact of the murine cachectic Colon 26 (C26) adenocarcinoma on white adipose tissue (WAT) and lipid metabolites in plasma and liver. Morphological analysis of WAT by light microscopy showed reduced size of white adipocytes in cachectic C26 tumour-bearing mice. Alterations in the mRNA levels, as well as diurnal rhythmic expression of REVERB α , BMAL1, PPAR δ , PPAR γ , C/EBP α and target genes PBE, ATGL, FAS, LPL and PERILIPIN, indicate perturbed diurnal pattern in circadian regulation of lipid metabolism. Furthermore, lipid mobilisation did not appear to be stimulated through classical hormone-induced PKA activation. These changes in WAT are accompanied by activation of IL6 signaling mediated primarily through phosphorylation of STAT3 rather than ERK1/2 and p38 MAP kinases. Additional Western analysis showed that the key mediator of metabolic homeostasis and sensor of low energy state AMPK is activated while downstream mTOR/4EBP1 signalling cascade, which has been implicated in the suppression of lipolysis, is inhibited. Lipid mobilization was evident in plasma with increased total free fatty acids. Extensive MS-based lipidomic analysis of liver and plasma of cachectic mice revealed distinctive changes in numerous lipid species including ceramides, sphingomyelins, phospholipids, cholesterol, TAGs and DAGs that were unaltered or opposite to levels apparent in pair-fed mice.

Conclusion: The dramatic depletion of adipose tissue in cachectic tumour-bearing mice is accompanied by aberrant lipid mobilization and metabolism that cannot be attributed to anorexia.

4-10

Activation of brown adipose tissue in cancer cachexia: an energetically wasteful process

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Background: Cancer cachexia/anorexia is a complex syndrome involving profound metabolic imbalance leading to muscle wasting and fat depletion and is the direct cause of death in 20–30% of all cancer patients. Brown adipose tissue (BAT) plays a key role in thermogenesis and energy balance

and may contribute to the physiological perturbations associated with advanced cancer including hypermetabolism, fever and cachexia. Therefore, we investigated the impact of the IL-6 producing cachectic Colon 26 (C26) tumour on BAT in mice.

Results: EM and light microscopy revealed profound delipidation and smaller brown adipocytes in cachectic C26 tumour-bearing mice. Circadian expression profiling of key regulators of lipid accumulation and fatty acid β -oxidation and their corresponding target genes revealed dramatic molecular changes indicative of active BAT. Increased Ucp1, Pbe and Cpt1 α expression at specific timepoints coincided with higher BAT and body surface temperatures during the dark cycle, indicative of tight temporal stimulation of thermogenesis in cachexia. Deiodinase 2, adenylate cyclase 3 and PGC1 α were increased at all diurnal timepoints. These changes persisted when cachectic mice were acclimatized to 28°C, confirming inappropriate stimulation of BAT despite thermoneutrality. Activation of BAT was accompanied by elevated levels of circulating IL-6 and enhanced cytokine signaling in BAT, shown by increased phospho-STAT3 and SOCS3 mRNA. Activation of BAT did not occur in mice bearing a non-cachectic variant of the C26 tumour that neither releases IL-6 into circulation nor elicits IL-6 signaling in BAT. While cachectic mice do reduce food intake, pair-feeding experiments demonstrated that activation of BAT could not be attributed to restricted nutrients. Indirect calorimetry via Oxymax showed that cachectic mice do not correspondingly reduce energy expenditure despite restricted calorie intake.

Conclusion: Our findings highlight a role for thermogenic activation of BAT associated with tumour-derived IL-6 as an energetically wasteful, maladaptive response to anorexia during the development of cachexia.

4-11

Depot modulation of interferon- γ in rat white adipose tissue by cancer-associated cachexia

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Background and aims: Cancer-associated cachexia is a progressive wasting syndrome characterized by an extensive loss of white adipose tissue (WAT) and skeletal muscle, which is regulated by inflammatory mediators produced by tumour and host. The WAT has been recognized as a source of many endocrine and inflammatory factors, which could contribute to cachexia-related inflammation. The WAT is a heterogeneous tissue, and therefore we sought to understand the role of inflammation in different WAT depots during cachexia progression.

Methods: Male Wistar male (210–230 g) were inoculated with Walker-256 tumour cells (2×10^7 cells) and sacrificed on days 0 (T0), 4 (T4), 7 (T7), 10 (T10) and 14 (T14) ($n=6-10$ /group), after inoculation. Retroperitoneal (RPAT), epididymal (EAT), mesenteric (MEAT) adipose tissue depots and serum of the tumour-bearing rats were collected. Total-cholesterol (Cho), triglycerides (TG), high density lipoprotein (HDL), and glucose were measured with enzymatic commercial kits, IFN- γ , TNF- α , and IL-1 β concentration were measured with ELISA and normalized by total protein.

Results: Tumour-bearing rats significantly decreased food intake on T10 (-8.7% ; $p<0.05$) and T14 (-10.7% ; $p<0.05$). A progressive decrease in serum glucose concentration was found starting on day 4 (-7% ; $p<0.05$) after tumour inoculation, and the higher reduction was observed on T14 (-16% ; $p<0.001$). Serum HDL concentration decreased (-6% ; $p<0.05$) and TG concentration augments on T14 ($+63\%$; $p<0.001$). No modulation was found in regard to TNF- α and IL-1 β in WAT. A progressive increase of INF- γ concentration was found in MEAT reaching the peak on T14 ($+88\%$; $p<0.05$). On the other hand, in RPAT INF- γ concentration decreased progressively with the lowest concentration on T14 (-46% ; $p<0.05$) and no alteration in INF- γ levels was observed in EAT.

Conclusion: IFN- γ was up-regulated in MEAT, suggesting a depot-specific of WAT regulation under cachexia. In addition, IFN- γ mediated inflammation in MEAT may favour the inflammatory milieu observed in cachexia progression.

4-12

Cancer cachexia modulates the expression of lipases in the epididymal adipose tissue of rats

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Background and aims: Increased fat cell lipolysis and hormone-sensitive lipase (HSL) overexpression are key factors underlying the loss of adipose tissue in cancer cachexia. However, the importance of novel lipolysis-related proteins [adipose triglyceride lipase (ATGL), comparative gene identification-58 (CGI-58) and perilipin A] on adipose tissue depletion in cachexia is still controversial. It was our aim to characterise the expression of these proteins during the progression of cachexia.

Methods: Animals were divided into: control (CTR), tumour-bearing (Walker 256-carcinoma) studied on the seventh day (TB7) and tumour-bearing studied on the fourteenth day (TB14) after tumour inoculation. We examined the gene (real-time PCR) and protein expression (Western blotting) of HSL, ATGL, CGI-58 and perilipin A in the epididymal adipose pad. Morphometrical analysis of adipocytes, plasma free fatty acids concentration (ELISA) and in vitro rates of lipolysis in isolated adipocytes were also determined.

Results: Adipocyte size was dramatically reduced in TB7 and TB14 (60% and 72% respectively), compared with control ($P<0.001$). HSL gene expression was higher in TB7, while other proteins were unaffected. At the end-stage of cachexia (TB14) HSL gene expression was similar to control values and the other proteins mRNA levels were reduced. However, protein expression showed a different pattern: ATGL and CGI-58 were reduced, while perilipin A presented a tendency for reduction ($P=0.06$ vs. CTR) in TB14. Plasma free fatty acids were increased in TB14 ($P<0.001$ vs. other groups). A decrease in isoproterenol-stimulated lipolysis was observed in isolated adipocytes from TB14 rats.

Conclusion: Chronic exposure of fat cells to TNF- α or sustained activation of the PKA pathway results in increased basal lipolysis and a markedly blunted response to stimulated lipolysis. We found a high plasma free fatty acid concentration in TB14, even with a reduction on ATGL and CGI-58 expression, suggesting the importance of basal lipolysis in terminal cachexia.

4-13

Adipokine expression in tumour-bearing rats during the progression of cachexia

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Background and aims: Cancer cachexia markedly affects the adipose tissue, an important endocrine organ, secreting the adipokines. Some authors suggest that the impaired response of adipokines to body weight loss may play a role in the pathogenesis of cancer-induced cachexia. However, the association between adipokine level and cancer-induced cachexia has not yet been fully elucidated. Therefore, it was our aim to examine adipokine expression during cachexia progression.

Methods: Animals were divided into three groups: control (CTR, $n=10$), tumour-bearing (Walker 256-carcinoma) on the seventh day (TB7, $n=6$) and tumour-bearing on the 14th day (TB14, $n=9$) after tumour inoculation.

We examined the protein expression (ELISA) of adiponectin and leptin in the epididymal adipose tissue (EAT), and evaluated plasma concentration of those factors. TNF- α content assessment in EAT was also carried out.

Results: Plasma leptin concentration was decreased in TB7 and TB14 ($P < 0.05$ vs. CTR). Adiponectin plasma concentration was diminished only in TB14. There was a decrease in leptin and adiponectin tissue content ($P < 0.05$ vs. CTR), in TB7. At the end-stage of cachexia (TB 14) the response was different: leptin tissue concentration was maintained and adiponectin showed an even more pronounced decrease ($P < 0.001$ vs. CTR and TB7). TNF- α tissue concentration was 2.0-fold higher in TB7 ($P < 0.05$ vs. CTR) and 2.5-fold higher in TB14 ($P < 0.05$ vs. CTR and TB7). Moreover, adiponectin was inversely correlated with TNF- α in EAT ($P < 0.01$).

Conclusions: Our results suggest that adiponectin has an important anti-inflammatory role in cancer cachexia and we conclude that modified adipokine tissue expression during cachexia progression may contribute to the aggravation of systemic inflammation viewed in this syndrome.

4-14

Erythropoietin administration counteracts adipose tissue depletion in murine cancer cachexia models

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Background/aims: While the relevance of adipose tissue depletion to the pathogenesis of cancer cachexia is controversial, recent reports show that mice genetically lacking triglyceride lipase are protected against cancer-induced cachexia [1] and that in lung cancer patients, obesity positively correlates with survival [2]. The aim of the present work was to test whether erythropoietin (EPO) administration could prevent fat wasting in mice bearing the Colon26 (C26) or the Lewis Lung carcinoma.

Methods: C26 mice received 5×10^5 tumor cells s.c. in the back, while LLC mice were injected i.m. in one hind leg with 5×10^5 cells. EPO (100 IU) was administered i.p. every 3 days. Animals were sacrificed 14 days after tumor implantation; blood and several tissues were collected for further analyses.

Results: Tumor growth in both C26- and LLC-bearing mice resulted in marked muscle and fat depletion, reduced food intake and anemia. In the C26 hosts, EPO treatment reversed the hematocrit reduction and partially prevented white adipose tissue (WAT) wasting, without affecting food intake or skeletal muscle mass. As for the LLC bearers, both the marked anemia and WAT depletion were only partially corrected by EPO, while no effects on muscle wasting and anorexia could be observed. EPO effect on WAT is likely exerted directly, as suggested by the increased levels of EPO receptor. The mechanism accounting for EPO-induced WAT preservation could rely on an increased clearance of circulating triglyceride, as suggested by the higher lipoprotein-lipase (LPL) activity found in the WAT of treated vs. untreated mice.

Conclusions: EPO administration might be beneficial in the treatment of cancer cachexia for its ability to counteract anemia and adipose tissue depletion.

1. Das SK et al., Science. 2011; 333(6039):233–8

2. Yang R et al., J Surg Res. 2011;170(1):e75–83

4-15

Perilipin expression in the subcutaneous adipose tissue in human cancer cachexia

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Background and aims: Cachexia is a wasting syndrome observed in severe clinical conditions such as neoplastic diseases, trauma, and infections. Loss of adipose tissue, which occurs primarily due to increased lipolysis, is a key feature of weight loss in cancer patients. Specific proteins located at the surface of lipids droplets, i.e. the perilipin family, have an important role in the control of lipolysis. In this study we verified perilipin A (PLIN) expression in the subcutaneous adipose tissue of cancer cachectic patients.

Methods: Samples were obtained from weight-losing tumour-bearing patients (Cx), weight-stable tumour-bearing patients (TB) and weight-stable non-tumour-bearing patients (CTR). The mRNA content (by real-time PCR) and protein expression (by Western blotting) of PLIN, as well as its tissue distribution (by immunohistochemistry) were studied. Data are presented as means \pm SE. Comparisons were made by one-way analysis of variance (ANOVA) followed by a post hoc test (Tukey). Differences were statistically significant at $P < 0.05$.

Results: PLIN gene expression was identical among the studied groups ($P > 0.05$). Cx patients presented a protein content 54.8% lower than CTR, but it was not statically significant ($p = 0.07$; CTR=93.15 \pm 18.91, Cx=54.81 \pm 10.34, TB=93.87 \pm 34.94 arbitrary units). Immunohistochemistry analysis showed that the distribution of PLIN in the adipose tissue was not modified by cachexia.

Conclusions: Loss of adipocyte perilipin content, with the concomitant loss of the protection of stored triacylglycerol from cytosolic lipases, is part of the mechanism by which TNF- α increases lipolysis. Our results suggest that a decreased PLIN content in subcutaneous adipose tissue may have a role in the higher lipolysis rate present in cachexia.

4-16

Heterogeneous time-dependent response of adipose tissue during the development cancer cachexia

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Background and aim: Several studies have demonstrated that cancer cachexia induces loss of fat mass that accounts for a large part of the dramatic weight loss observed both in humans and in animal models. To evaluate the onset of cancer cachexia in adipose tissue (AT) at morphological and molecular level, we evaluated the different WAT depots in cancer cachexia animal model at three different time-points after tumour implantation.

Methods: Male Wistar rats, 8 weeks old, were subcutaneously inoculated with 1 mL (2×10^7) of tumour cells (Walker 256). Samples of different AT depots were collected at days 0, 4, 7 and 14 and stored at -80°C (five animals per each day/group). Morphometric alterations were evaluated by light microscopy, and PPAR γ gene expression was quantified by RT-PCR (real time-PCR), and protein expression (PPAR γ) by Western blotting.

Results: In MEAT, sectional area showed an increase of 52% ($p < 0.05$) and 1.3-fold ($p < 0.001$), respectively, on day 14. Conversely, from RPAT and EAT were reduced (54% and 27%, $p < 0.05$), respectively, on day 14 compared to day 0. Gene expression of PPAR γ -2 fell significantly during cancer cachexia development in all three adipose tissues depots evaluated: MEAT 75.1% ($p < 0.001$) and TARP 57.4% ($p < 0.01$), respectively, on day 4. On day 14 (MEAT, 86.2%, RPAT, and 72.6% EAT, 81.9%, $p < 0.01$), all depots compared to day 0.

PPAR γ -2 protein and mRNA expression levels decreased on day 14 in all fat depots evaluated (MEAT, 76.1%, RPAT, and 56.5% EAT, 43.8%, $p < 0.01$).

Conclusion: In the present study, we demonstrated several changes at the morphological and molecular levels in AT of tumour-bearing animals resulting in depot-dependent adipose dystrophy. Down regulation of the master regulator of adipogenesis, PPAR γ , seems to have started earlier than any detectable morphological disruption, indicating the importance of PPAR γ in this setting. Financial Support: FAPESP 2007/52782-1, 2008/51094-9 and 2010/51078-1.

4-17

Adipose tissue extracellular matrix remodeling in cachectic cancer patients

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Background and aims: Profound loss of adipose tissue is a hallmark of cancer cachexia. During the progression of this syndrome, the morphological and biochemical characteristics of the adipose tissue are markedly modified. Nevertheless, the changes caused by cancer cachexia regarding adipose tissue extracellular matrix have not yet been fully described. The aim of the study was to evaluate the effects of cancer cachexia upon the expression of collagen VI $\alpha 1$ (COL6A1), fibronectin (FN1) and metalloproteinase 2 (MMP2) in the subcutaneous adipose tissue (SAT) of cancer patients, by real-time PCR and immunohistochemical analysis.

Methods: Patients of the University of São Paulo Hospital submitted to surgery were divided into two groups: cancer cachexia (CCx)—gastrointestinal cancer—and Control (C)—incisional hernia—($n = 14$ and 8 , respectively). The fresh sections of SAT from CCx and C were fixed in paraformol 4% and embedded in paraffin. The paraffin sections of $5 \mu\text{m}$ were processed for immunostaining. All slides were incubated with primary antibodies overnight at 4°C . The slides were incubated with DAB and counterstained with Meyers hematoxylin. Gene expression analysis was carried out by real-time PCR.

Results: The immunostaining for COL6A1 was dramatically modified in CCx, as the strong presence of collagen fibrils was evident in the interstitial space mostly in the fibrotic areas, but solely in CCx. The expression levels of COL6A1 were increased in CCx ($p < 0.05$). FN1 showed increased expression after immunostaining as well as augmented mRNA levels in CCx in comparison with the control. However, the expression of MMP2 was decreased in CCx, as evaluated by PCR and by immunostaining.

Conclusions: Cancer cachexia deeply affects the adipose tissue. The accumulation of components of the extracellular matrix in the adipose pad leads to remodeling. The increase of COL6A1 may be directly related with the emergence of tissue fibrosis

4-18

Contribution of the adipose tissue to cachexia-related systemic inflammation in gastrointestinal cancer patients

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Background and aims: Cachexia associated with cancer can be defined as a multifactorial syndrome in which there is continuous loss of muscle mass

(with a loss or no loss fat mass), which cannot be fully reversed by conventional nutritional support, leading to progressive functional impairment of the body. Over the last decade, WAT has been recognized as an important endocrine organ for its ability to synthesise and secrete adipokines such as TNF- α , IL-6, adiponectin or leptin. The aim of this study was to analyse the gene expression of inflammatory cytokines and their plasma profile in two fat depots (subcutaneous and visceral) of patients with cancer cachexia.

Methods and results: Cytokine concentration was assessed in plasma by ELISA and the gene expression in the subcutaneous and visceral adipose pads by real-time PCR obtained from cachectic patients, weight-stable cancer patients, and non-cancer patients, eight per group, CEP HU/USP: 752/07. TNF- α plasma levels in cachectic cancer patients were higher (42-fold, $p < 0.01$) than in weight-stable cancer patients and non-cancer patients. TNF- α (12-fold, $p < 0.01$) and CD68 (50-fold, $p < 0.001$) gene expression was increased in the subcutaneous adipose tissue of the cachectic group compared with the non-cancer group. However, gene expression in the visceral depot was unchanged.

Conclusion: Cancer-associated cachexia markedly affects the adipose tissue, inducing local and systemic inflammation.

4-19

Alterations in adipose tissue composition in colorectal cancer patients

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Background and aims: Colorectal cancer is the third most common cancer in the developed world. Advanced colorectal cancer is characterized by malnutrition and cachexia, which includes loss of muscle and adipose tissue. Mechanisms underlying fat loss are not well understood, and knowing the types of fat being lost from adipose tissue will enhance our understanding of aberrations of lipid metabolism in cancer and help define interventions to circumvent wasting. The aim of this study is to explore the differences in fatty acid composition between visceral adipose tissue (VAT) and subcutaneous (SAT) depots and relate this to body composition as well as changes in fat mass assessed using CT images in colorectal cancer patients.

Methods: Adipose tissue (VAT and SAT) was obtained intraoperatively from CRC patients ($n = 18$) and frozen at -80°C until analysis. Patient information including age, gender, weight, height, liver metastases, stage of disease, date of sample collection, types and dates of chemotherapy were obtained from patient records. To investigate amounts and types of fatty acids stored in adipose depots, adipose tissue triglyceride and phospholipid were isolated using Folch immediately followed by thin layer chromatography and gas liquid chromatography. CT image analysis was used to quantify SAT, VAT and intramuscular adipose tissue areas.

Results: The findings indicate that the majority of patients were overweight or obese at the time of diagnosis. VAT contained less saturated fatty acids than SAT, whereas mono-unsaturated fatty acids were higher in VAT than SAT. PUFAs amount did not significantly differ between depots. Subjects were stratified into normal and overweight/obese categories and no significant differences were observed in the fatty acid composition of the adipose tissue in these two groups.

Conclusions: The results demonstrated that there might be a relationship between the rate of adipose tissue loss and changes in fatty acid status during disease progression.

5-01

External validity of the European consensus on sarcopenia: mortality predictor

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Sarcopenia has an important impact in elderly. Recently, the European Working Group on Sarcopenia in Older People (EWGSOP) defined sarcopenia as the loss of muscle mass plus low muscle strength or low physical performance. Lack of clinical sounding outcomes (i.e., external validity), is one of the flaws of this algorithm. The aim of our study was to determine the association of sarcopenia and mortality in a group of Mexican elderly. Three hundred forty-five elderly were recruited in Mexico City and followed up for 3 years. The EWGSOP algorithm was integrated by: gait speed, grip strength and calf circumference. Other covariates were assessed in order to test the independent association of sarcopenia with mortality. Of the 345 subjects, 53.3% were women; with a mean age of 78.5 (SD 7) years. During the 3-year follow-up, a total of 43 (12.4%) subjects died. Age, MMSE score, Katz score, Lawton score, health self-perception, ischemic heart disease, and sarcopenia were associated in the bivariate analysis with survival, with an statistical significance of <0.1 (see table). Negative predictive value for sarcopenia regarding mortality was of 90%. Kaplan–Meier curves along with their respective log-rank test were significant for sarcopenia. The components of the final Cox regression multivariate model were age, ischemic heart disease, ADL and sarcopenia. Adjusted HR for age was 3.24 (CI 95% 1.55–6.78, $p=0.002$), IHD 5.07 (CI 95% 1.89–13.59, $p=0.001$), health self-perception 5.07 (CI 95% 1.9–13.6, $p=0.001$), ADL 0.75 (CI 95% 0.56–0.99, $p=0.048$) and sarcopenia 2.39 (CI 95% 1.05–5.43, $p=0.037$).

5-02

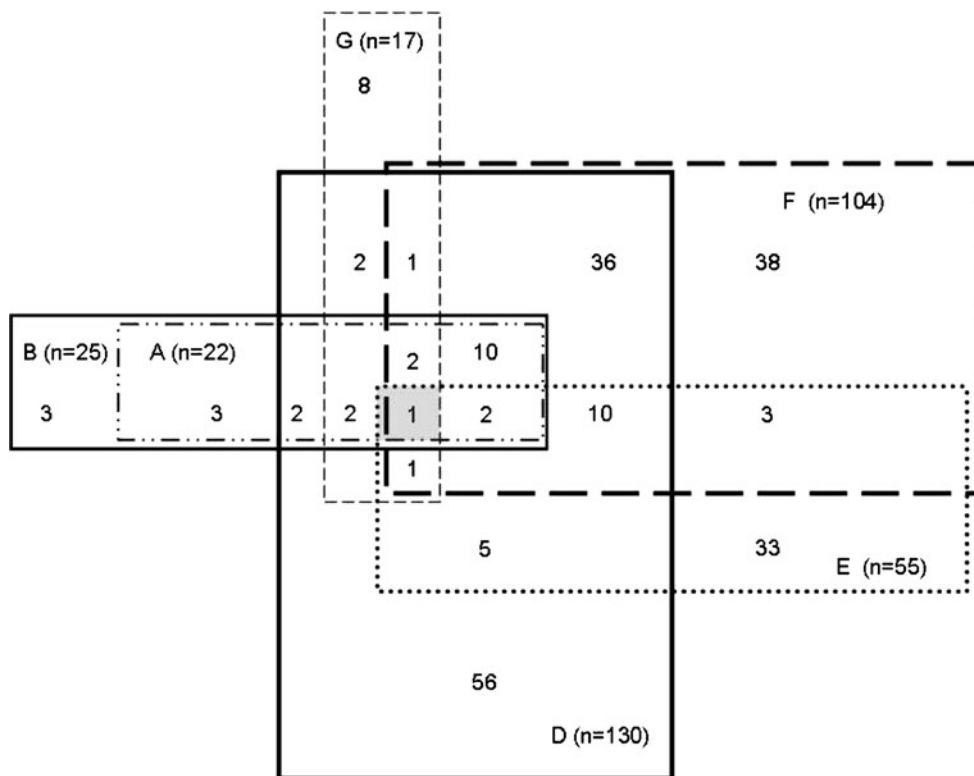
Defining sarcopenia: the impact of different diagnostic criteria on the prevalence of sarcopenia in a large middle-aged cohort

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Sarcopenia, low muscle mass, is an increasing problem in our aging society. The prevalence of sarcopenia varies extremely between elderly cohorts ranging from 7% to over 50%. Without consensus on the definition of sarcopenia, a variety of diagnostic criteria are being used. We assessed the degree of agreement between seven different diagnostic criteria for sarcopenia based on muscle mass and handgrip strength, described in literature. In this cross-sectional study, we included men ($n=325$) and women ($n=329$) with complete measurements of handgrip strength and body composition values as measured by bioimpedance analysis within the Leiden Longevity Study. Prevalence of sarcopenia was stratified by gender and age. In men (mean age, 64.5 years), the prevalence of sarcopenia with the different diagnostic criteria ranged from 0% to 20.8% in the lowest age category (below 60 years), from 0% to 31.2% in the middle (60 to 69 years), and from 0% to 45.2% in the highest age category (above 70 years). In women (mean age 61.8 years), the prevalence of sarcopenia ranged from 0% to 15.6%, 0% to 21.8%, and 0% to 25.8% in the lowest, middle, and highest age category respectively. One participant (0.3%) was identified having sarcopenia according to all diagnostic criteria that marked prevalence above 0%. We conclude that the prevalence of sarcopenia is highly dependent on the applied diagnostic criteria. It is necessary to reach a consensus on the definition of sarcopenia in order to make studies comparable and for implementation in clinical care.

Figure: Number of participants identified as having sarcopenia according to various definitions, represented by letter codes



5-03

Muscle age-related expression of two transcriptional repressors, BHLHB2 and BHLHB3, reveals that BHLHB2 is increased in the oldest healthy subjects

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Background: Sarcopenia is a muscle aging process, characterized by a loss of skeletal muscle mass, quality and performance. This physiological process affects 13–25% of the over 60 years population, and can become pathological when it is exacerbated.

Aim: The aim of this study is to follow in human muscle from different age subjects, BHLHB2 and BHLHB3 expressions, two transcriptional repressors involved in muscle atrophy mechanisms.

Method: Muscle samples (Gluteus Maximus) from 41 subjects were divided into three groups (less than 60 years, 60–75 years and more than 75 years). We measured mRNA and protein levels of BHLHB2 and BHLHB3, and two of their targets genes MyoD and Myogenin. We also studied two atrogens involved in muscle atrophy, MuRF1 and Atrogin1.

Results: We observed an increase in the level of BHLHB2 protein in the muscle from the oldest group compared to the youngest, while the expression of MuRF1, and Atrogin1 remains constant. However, this increase in BHLHB2 seems to have no consequence on the MRF expression, particularly MyoD.

Conclusion: These first results on the expression of BHLHB2 and BHLHB3 factors in the muscle of a healthy population are reference data essential to a further study of a sarcopenic aged population.

5-04

Frailty in Mexican community-dwelling elderly

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Objectives: The study aims to determine the prevalence of frailty syndrome in Mexican elderly people, as well as describing socio-demographic features and the health status implicated with it.

Design: This is a cross-sectional study involving 60 years and older beneficiaries Mexican Social Security (IMSS) of Mexico City.

Measurements: Socio-demographic features, geriatric rating scales (Katz Index, Lawton and Brody Index, Mini-Mental State Examination, CES-D scale, body mass index, Charlson Index), and usage of the health service in the last 6 months were utilized. We used the modified frailty phenotype of Fried et al.

Results: Prevalence of frail was 15.7%, pre-frail of 33.3%, and non-frail of 51.0%. The factors associated with pre-frail elder were: women (OR=0.64), 60–64 years old (OR=0.51), and 65–74 years old (OR=0.56), 1–6 years of education (OR=0.91), 7–12 years of education (OR=1.14), singles (OR=1.03), living alone (OR=1.27), limitations in basic activities of daily living (OR=1.36), and instrumental activities of daily living (OR=1.70), cognitive impairment (OR=1.13), depression (OR=3.44), underweight/malnutrition (OR=1.46), and overweight/obesity (OR=0.75), moderate

comorbidity (OR=1.33), and serious co-morbidity (OR=1.46). The factors associated with frail elderly were: 60–64 years old (OR=0.17), and 65–74 years old (OR=0.20), none year of education (OR=1.52), singles (OR=1.08), living alone (OR=0.92), limitations in basic activities of daily living (OR=2.35), and instrumental activities of daily living (OR=3.77), cognitive impairment (OR=1.84), depression (OR=8.95), overweight/obesity (OR=0.43), moderate co-morbidity (OR=2.17), serious co-morbidity (OR=1.11), and using health services (OR=1.83).

Conclusion: The socio-demographic features and changes in the health status have a stronger force of association with frail elderly rather than with pre-frail elderly compared with non-frail elderly.

5-05

Oxidative parameters as biomarkers of frailty

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Background: Frailty has been defined as a physiological state of vulnerability to increased morbidity and mortality; hence, it is regularly studied in older adults. Oxidative stress may contribute to frailty in part through inflammation and aerobic metabolic changes. Intracellular oxidative stress promotes the formation of oxidized cellular components.

Methods: In the current study, we evaluated the enzymatic response to oxidative stress in a cohort composed by 157 participants of 60 years and older as oxidative parameters for frailty. Frailty status was determined from Fried's criteria. Hence, the cohort was determined as follow: fails (F); 25.8% (23) pre-fails (PF); 56.2% (50) non-frails (NF). M: 13.2% (9) frails; 30.9% (21) pre-Frails; 55.9% (38) NF.

Results: Our findings showed an increase in lipid and protein oxidation in F and PF elderly as follows: 145% more lipoperoxidation (LPX) in frail elderly and 84% more LPX in pre-fail when compared with NF elderly. As well as an increase of 153% and 131% of oxidized proteins in frail and pre-frail population respectively, when compared with NF elderly. GSH levels also decreased in the same groups (75% F and 69% PF). The above was confirmed by a decrease in the expression of the enzyme γ -GCS, which has been described to regulate the levels of GSH. However, catalase activity showed no significant changes among the different groups analyzed.

Conclusion: Our findings regards the oxidative parameters may be related with frailty as a part of a chronic inflammatory state or as part of the decline in physiological processes associated with aerobic metabolism. The mechanism to explain how the oxidative stresses increase during frailty has not been fully clarified. However, it would be interesting to explore the possible relationship between the inflammatory processes and the oxidative stress through the involvement of pro-inflammatory molecules such as NF- κ B.

5-06

Mean corpuscular haemoglobin concentration (MCHC): a marker for metabolic stress and immune activation in immobilisation and chronic fatigue?

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Background: Chronic fatigue syndrome (CFS) is a disabling condition characterised by severe fatigue, exhaustion, and myalgia that results in very low physical activity levels. Unduly immobilisation may affect metabolic balance and muscle functional capacity but this has not been studied in detail. The aetiology of CFS is unknown but immune activation is discussed to be involved in the pathophysiology. Increased mean corpuscular haemoglobin concentration (MCHC) has been suggested as a global marker of metabolic stress secondary to strenuous energy demanding work load. Elevated MCHC in relation to exercise may indicate impaired intracellular glucose availability, disturbed osmotic regulation and cellular swelling. The aim of this study was to investigate the characteristics of MCHC as a global metabolic marker in relation to cellular immune activation in patients presenting with chronic fatigue.

Methods: We evaluated full blood count and immunological profile (CD4+, CD8+, CD11a+, CD28+, CD57+, HLADR+ T-cells) of 190 patients (mean age±SD: 44±12, 64% female) presenting with fatigue symptomatic in our outpatient clinic. The T-cell cytokine profile of inflammatory activation (IL-2, IL-4, IL-5, IL-10, IFN- γ , TNF- α) was assessed in the supernatant of ConA-stimulated blood cells.

Results: MCHC of the assessed population was 34.14±1.0 g/dl. Forty-seven patients (25%) had an elevated MCHC value above the upper normal. Patients showed upregulated T-cells activity (CD57+CD8+ T-cells). Elevated MCHC was positively associated both with chronic T-cell activation (CD57+; $r=0.17$, $p=0.035$) and a type 2 IL-5high profile ($r=0.22$, $p=0.028$).

Conclusions: Increased MCHC is highly prevalent among patients with chronic fatigue indicating metabolic stress. As these patients usually have low activity levels, chronic immune activation is likely to contribute to this metabolic imbalance. It needs to be studied whether these imbalances account for abnormal muscle catabolism in patients with chronic fatigue.

5-07

Adiposity to muscle ratio predicts incident physical limitation in a cohort of 3,153 older adults—an alternative measurement of sarcopenia and sarcopenic obesity

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Background and aims: Conventionally sarcopenia is defined by muscle mass alone. We hypothesized that the disability caused by sarcopenia and sarcopenic obesity (SO) was related to the amount of adiposity or body weight bearing on a unit of muscle mass, or the adiposity to muscle ratio. We therefore examined whether this ratio could predict physical limitation.

Methods: We have recruited 3,153 community-dwelling adults aged >65 years and their body composition were measured by dual-energy X-ray absorptiometry (DXA). Assessment of physical limitation was undertaken at baseline and 4 years later. The relationship between baseline adiposity to muscle ratio and incident physical limitation was examined by logistic regression.

Results: In men having BMI<23, muscle mass could predict physical limitation before ($p<0.01$) but not after ($p=0.82$) adjustment. When the BMI was >23, the adiposity to muscle ratios, namely body fat to lower limb muscle mass, body fat to fat-free mass (FFM) and body weight to FFM, were predictive of physical limitation before and after adjustment (all p values <0.001). In women, throughout the entire BMI range, all three adiposity to muscle ratios were associated with physical limitation 4 years later both before and after adjustment (all p values <0.05). In women

having BMI less than 21, muscle mass alone could not predict physical limitation both before ($p=0.4$) and after ($p=0.5$) adjustment.

Conclusions: Sarcopenia and sarcopenic obesity as measured by either the body weight or fat mass bearing on a unit of muscle mass (the adiposity to muscle ratio), is a valid predictor of incident or worsening physical limitation in older women throughout the entire BMI range and in older men having BMI>23. This ratio can serve as a measurement of both sarcopenia and SO in women and SO in men.

5-08

Accomplishing and maintaining locomotor gains in a group of elderly for a follow-up time of 7–10 years after implementation of a home-based exercise program

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Background: Ageing is associated with progressive decline in muscle mass and muscle function. Regular exercise training is thought to counteract these processes. We sought to establish the effects of a clearly defined daily exercise program (www.fuenfesslinger.de) on usual gait speed, maximal gait speed, chair-rise test, and force plate jump test in a cohort of adults aged 58 years or above.

Methods: We conducted a prospective single-center cohort study at the outpatient department of a geriatric clinic. Sixty-one healthy elderly subjects were enrolled in 2000 and instructed to adhere to a home-based exercise program. Subjects were free of chronic disabling diseases and physical disabilities. Follow-up visits were performed monthly over the first 4 months, bi-annually until 2 years of follow-up, and annually thereafter up to 10 years. Assessments at each visit included usual gait velocity (UGV) and maximal gait velocity (MGV), chair rising test (CRT) performed by rising five times from sitting at a height of 45 cm as quickly as possible, and total power output on counter jumping, measured on the force plate Leonardo mechanography system (FPJ).

Results: Forty-eight subjects (mean age 66.4±5.1 years, body mass index 25.3±5.1 kg/m², 35 female) completed the follow-up visits up to at least 7 years. Forty of these 48 subjects completed more than 80% of follow-up visits. Whilst significant improvements were maintained over 10 years for CRT, there was a significant decrease in FPJ at 7 years (repeated measures analysis of variance, all $p<0.0001$). UGV at baseline was 1.43±0.26 m/s, then increased to 1.56±0.22 at 4.5 months ($p<0.0001$ vs. baseline) to decrease again to 1.41±0.025 at 7 years, and 1.37±0.27 m/s at 10 years ($p=0.06$). The respective values for MGV were 1.94±0.32, 2.13±0.33 ($p<0.0001$), 1.93±0.35, and 1.87±0.42 m/s ($p=0.06$). Time required in CRT decreased from 7.40±1.73 s at baseline to 5.96±1.33 at 4 months, 6.22±1.45 at 7 years to 6.26±1.72 s at 10 years (all $p<0.001$ vs. baseline). FPJ increased from 30.4±6 W/kg body weight at baseline to 31.6±5.7 at 4.5 months to decrease again to 27.7±5.6 at 7 years and 26.3±5.7 W/kg at 10 years (all $p<0.002$ vs. baseline).

Discussion: A home-based daily exercise training was effective in improving UGV, MGV, CRT and FPJ after 4.5 months. The improvements in CRT could be maintained over 10 years of follow-up in motivated healthy elderly subjects. After 7 years of follow-up, UGV and MGV were still at baseline level. FPJ was found significantly decreased after 7 years. It appears likely that the decline in neuromuscular function, measured by CRT or gait velocity, is counteracted by this program. Limitations of our study include its uncontrolled design. Further studies in larger cohorts of patients are required to confirm these findings.

5-09

Is body mass index or weight loss more important in survival? A 9-year prospective study in 1,816 nursing home residents

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Background and aims: Weight loss has been considered predictive of early mortality in nursing home residents. Lower body mass index, irrespective of weight loss, has also been considered detrimental for survival in community-dwelling older persons. We examined which of the two is more important for survival in nursing home residents.

Method: Of the nursing home residents, 1,816 were assessed by the Mini-Dataset (MDS) 2.0 at baseline and mortality status was assessed at 6 months, 1, 2, 4 and 9 years later. Relationship between mortality and significant weight loss ($\geq 5\%$ over 30 days or $\geq 10\%$ over 180 days), and body mass index (BMI), was studied by Cox regression with both variables in the same model, adjusted for age, gender, medical conditions (cancer, renal failure, heart disease, dementia, hip fracture, diabetes mellitus), tube-feeding, left 25% food uneaten, swallowing problem, and the activities of daily living hierarchy scale.

Results: Of the residents, 1,816 residents (67.7% female) with mean age 83.5 \pm 8.4 years and mean BMI 21.7 \pm 4.8 were studied. Mortality rate were 7.4% (6 months), 15.6% (1 year), 28.6% (2 years), 48.6% (4 years), and 78.5% (9 years). Significant weight loss was not associated with higher mortality at all follow-up durations, whereas higher BMI was significantly protective: hazard ratio per standard deviation increment 0.58 (6 months), 0.58 (1 year), 0.66 (2 years), 0.80 (4 years) and 0.74 (9 years), all $p < 0.001$. Having $\geq 25\%$ of food uneaten (50.4% of participants) persisted to have no relationship to survival at all follow-up durations.

Conclusion: Significant weight loss as defined by the MDS 2.0 was not associated with short- or long-term survival in Chinese nursing home residents. BMI however is predictive of short- and long-term survival irrespective of weight loss in this population. Low body mass index, detectable at a single point of time, may be another readily available alternative check-point for possible reversible mortality risk factors.

5-10

Muscle loss in patients with chronic heart failure: results from the SICA-HF study

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Background: The term sarcopenia describes age-related loss of muscle mass and strength. A debate exists whether this term should be used in patients with chronic disease. Using the criteria defined for the diagnosis of sarcopenia, we sought to assess muscle mass and other relevant clinical variables in patients with chronic heart failure (CHF) as part of the Studies Investigating Co-morbidities in Heart Failure (SICA-HF).

Methods: We prospectively enrolled 153 patients with symptomatic, clinically stable CHF (male sex 78%, New York Heart Association

[NYHA] class [mean \pm SD] 2.4 \pm 0.6, age 67 \pm 10 years, creatinine 1.2 \pm 0.4 mg/dl). Appendicular skeletal muscle mass was assessed by dual-energy X-ray absorptiometry (DEXA) and compared with a healthy reference group of young adults aged 18–40 years from the previously published Rosetta study. In addition, we analysed muscle strength by hand grip, and all patients underwent 6-min walk test and spirometry. Muscle wasting was defined as appendicular muscle mass 2 SD below the mean of the young reference group.

Results: Muscle wasting was detected in 29 (19%) subjects. Patients with muscle wasting were older (69.5 \pm 8.0 vs. 66.1 \pm 10.3 years, $p=0.1009$), had a lower body mass index (26.7 \pm 12.4 vs. 29.7 \pm 4.9 kg/m², $p=0.0383$), and a lower left ventricular ejection fraction (33.7 \pm 11.7% vs. 40.4 \pm 13.9%, $p=0.0271$) than patients without. Total fat mass was significantly lower in patients with muscle wasting than in those without (22.1 \pm 11.9 vs. 29.9 \pm 9.5 kg, $p=0.0002$). Furthermore, patients with muscle wasting presented with lower exercise time (7.4 \pm 4.1 vs. 10.4 \pm 3.0 min, $p=0.0004$) and lower total peak oxygen consumption (absolute peak VO₂, 1,163.9 \pm 478.2 vs. 1,622.5 \pm 472.9 ml/min, $p=0.0002$) and had lower hand grip strength than patients without muscle wasting (33.2 \pm 9.0 vs. 38.1 \pm 11.8 kg, $p=0.0453$). In addition, a significant difference was detected for 6-minute (368.5 \pm 140.3 vs. 440.4 \pm 141.2 m, $p=0.0248$) and 4-m (4.6 \pm 2.0 vs. 3.9 \pm 1.0 s, $p=0.0218$) walk distance. No difference was detected for NYHA class, creatinine or bone mineral density ($p > 0.1$).

Conclusions: This single-center experience from the multi-national SICA-HF study shows that muscle wasting using the criteria defined for sarcopenia assessment is a frequent co-morbidity among patients with CHF. The presence of muscle wasting may have serious impact on mobility, quality of life, frequency of falls, and overall morbidity. Our preliminary findings warrant further study in larger cohorts of patients.

5-11

Factors influencing exercise performance in people with thoracic cancer

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Background and aims: Patients with thoracic cancer often complain of a reduced ability to exercise which can impede levels of independence and quality of life. Several factors may be responsible but there is little formal study in this group. We have explored how various physiological and psychological factors relate to performance in a test of exercise capacity.

Methods: Inspiratory muscle strength, peripheral muscle power, lung function and mastery over breathlessness were assessed using sniff nasal inspiratory pressure, leg extensor power, simple spirometry and the mastery domain of the Chronic Respiratory Disease Questionnaire, respectively. Exercise performance was assessed using the Incremental Shuttle walk Test (ISWT) during which patients wore a K4 b2 system allowing continuous measurement of heart rate, minute ventilation (VE) and oxygen uptake (VO₂). Relationships between ISWT distance and independent factors were determined using Pearson's correlation coefficient and β regressions coefficients.

Results: Forty-one patients (21 male, mean (SD) age 64 (8) years) took part. They walked a median [IQR] 320 [250–430] metres and reached 76 (10%), 48 (14%) and 77 (25%) of their predicted maximal heart rate, VE and VO₂, respectively. Exercise performance was significantly associated only with inspiratory muscle strength ($r=0.42$, $P < 0.01$) and peripheral muscle power ($r=0.39$, $P=0.01$). These factors were also significant determinants of exercise performance (β coefficients [95% CI] 1.77 [0.53, 3.01] and 1.22 [0.31, 2.14]).

Conclusion: Both inspiratory and peripheral muscle performance appear to influence exercise performance in people with thoracic cancer.

Interventions aimed at maintaining or slowing down the decline in exercise performance should include components targeting muscular performance.

5-12

Elevated gluconeogenesis and its relationship with protein retention in aging and lung cancer cachexia

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Background: Cancer and aging are both associated with metabolic alterations, including insulin resistance, that may lead to muscle loss. This may result in increased use of amino acids for gluconeogenesis (GNG) via the tricarboxylic acid cycle and phosphoenolpyruvate (PEP).

Methods: We measured the fractional contributions (%) of glycogen, glycerol and GNG from PEP to 17-h fasting endogenous glucose production (EGP), using oral ²H₂O and the positional deuterium enrichment of plasma glucose, by NMR spectroscopy. Whole-body protein and glucose kinetics were measured (¹³C-leucine and ³H₃-glucose) in the postabsorptive state and during a hyperinsulinemic, euglycemic, isoaminoacidemic clamp to determine insulin resistance in: young ($n=11$; 27±3 years old), older ($n=9$; 65±2 years old) and older men with lung cancer (CA; $n=9$; 66±2 years old).

Results: Men with CA had higher serum CRP levels and resting energy expenditure (REE) than young and older groups, and a lesser clamp glucose disposal ($p<0.001$) indicating insulin resistance. EGP was elevated with aging and cancer with greater contribution from GNG_{PEP}: 37±2, 43±2 and 48±4% in young, older and CA (ANOVA $p<0.05$), whereas percentage contribution from glycogen and glycerol did not differ. This resulted in greater GNG flux (EGP×%GNG) in older and CA groups, but no difference between the two. Elevated GNG flux correlated positively with REE ($R=0.61$, $p<0.001$) and negatively with fat-free mass index ($R=-0.41$, $p<0.05$). Surprisingly, postabsorptive protein net balance (synthesis-breakdown) was less negative in older and CA groups (ANOVA, $p<0.001$). However, GNG flux was also related to lesser glucose uptake and protein anabolic response ($R=-0.54$, $p=0.001$; and greater protein oxidation) during the subsequent hyperinsulinemic, isoaminoacidemic clamp.

Conclusions: These findings suggest that greater production of glucose from GNG in the fasting state might predict postprandial responses resulting in lesser protein retention in response to insulin and upon feeding small amounts of protein (isoaminoacidemia). (Supported by the CIHR.)

5-13

In vivo progression of muscular defects throughout the lifespan in *Caenorhabditis elegans*

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Background: The deterioration of muscle mass and function with increasing age is a major challenge to the health services within ageing societies. To date, little is known of the onset and progression of defects in the various muscle subcellular processes, and if a temporal relationship exists between them. Such information may provide important information as to the initiating events that induce muscle defects with age, and thus assist in designing diagnostics and therapeutics.

Methods: Here, we used transgenic *Caenorhabditis elegans* worms to study the progression of defects in ageing muscle. These transgenic

animals allowed us to study onset of protein degradation and impairment of the maintenance of myofibres and mitochondria in vivo.

Results: In ageing animals grown at 20°C, we found loss of mitochondrial maintenance occurred first (at mid-adulthood), followed by disruption of myofibrillar maintenance and, finally, increased cytosolic protein degradation. As *C. elegans* is a poikilotherm its lifespan varies with temperature in a negative linear fashion between 15°C and 25°C. We therefore also examined whether the progression of muscle defects are affected by temperature. The order of onset of defects in muscle maintenance was similar between temperatures. However, at lower temperatures where lifespan is increased there was a delayed onset and decreased severity of muscle defects versus 20°C. Conversely at higher temperatures where lifespan is decreased, muscle defects occurred earlier and with greater severity.

Conclusion: Our data provide in vivo evidence that there may be an ordered decline of muscle with age at least in *C. elegans*.

5-14

Molecular regulation of human skeletal muscle atrophy with 4 days immobilization—effects of ageing

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Background: Important insights concerning the molecular basis of skeletal muscle disuse atrophy and age-related muscle loss have been obtained in cell culture and animal models. However, little is known about the underlying mechanisms in humans.

Purpose: Muscle atrophy was induced by short-term immobilization in healthy young and old human individuals to study the effect of aging on transcriptional regulatory signaling pathways involved in the regulation of muscle cell size in vivo.

Methods: Myofiber atrophy was induced by application of a knee-brace for a period of 4 days in young (Y , ~20 years, $n=11$) and aged (O , ~70 years, $n=11$) individuals. Muscle biopsies of the VL muscle were collected pre and at 1, 2, and 4 days of immobility. Changes in mean muscle fiber area (MFA) and maximal voluntary muscle strength (MVC) was measured pre and after 4 days of immobility. Expression levels of MuRF1, Atrogin-1, MGF, IGF-1Ea, PGC-1 α , and PGC-1 β mRNA were determined using real-time RT-PCR and normalized to the Ribosomal Protein Large P0 (RPLP0) mRNA. Protein levels of phosphorylated Akt (P-Akt) and Akt were measured by western blotting of whole muscle protein isolates.

Results: In both age groups, there was a decline in MFA (Y : 10.6%; O : 9.0%, $p<0.05$) and MVC (Y : 13.0%; O : 14.3%, $p<0.05$) at 4 days, along with an increase in expression levels of Atrogin-1 and MuRF at 1 day and 2 days. In contrast, MGF mRNA was upregulated at 1 and 2 days primarily in O , while western blotting of Akt and phosphorylated Akt showed a decrease in phosphorylated Akt in Y after 2 and 4 days and an increased phosphorylation in O at 4 days. In contrast, expression levels of PGC-1 α mRNA were down regulated at 1 and 2 days primarily in Y and in both Y and O at 4 days. PGC-1 β mRNA was downregulated at 1 day primarily in Y and at 2 and 4 days in both Y and O .

Conclusion: The present data demonstrates parallel activations of the ubiquitin-proteasome and IGF/Akt pathways, respectively, and a diminished activation of PGC-1 α and PGC-1 β pathways during the first 4 days of immobility, indicating that proteolysis plays an important role in the

initiation of human disuse atrophy in both young and old muscle. In contrast, the regulation of protein synthesis and mitochondrial function appears to be more age-dependant.

Supported by Lundbeck Foundation, the Danish National Research Council, the Danish Rheumatology Association, and the Nordea Foundation

5-15

TET inducible myostatin overexpression inhibits muscle growth during postnatal development and induces muscle atrophy in aged mice

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Myostatin is a potent negative regulator of muscle growth during the prenatal and postnatal fast-growth period as deletion of myostatin in adult mice leads to a moderate increase in muscle mass, while developmental knockout causes a double muscle phenotype. Little is known of the effect of myostatin on muscle mass during aging. Here we describe the effect of myostatin overexpression on muscle mass in young, adult and aged mice using a doxycycline-inducible myostatin transgenic mouse model. Myostatin mRNA and protein were significantly higher in skeletal muscle, and serum myostatin levels were increased ~15 fold in young transgenic animals after doxycycline induction. In young growing mice (3-week-old), myostatin overexpression significantly decreased body weight (–24%), lean mass (–21%), quadriceps (–49%), and gastrocnemius (–42%) mass over a 9-week period of doxycycline treatment. Grip strength, *in situ* hind limb and *ex vivo* EDL muscle tetanic force were also significantly reduced by myostatin overexpression ($p < 0.05$). In both mature (7-month-old) and aged (18-month-old) mice, myostatin overexpression also resulted in significant loss of quadriceps (mature: –38%; aged: –18.6%) and gastrocnemius (mature: –33%; aged: –16.8%) over a 2-month period of doxycycline treatment. Interestingly, muscle mass and strength returned to control levels after doxycycline withdrawal. These findings identify a novel role for myostatin in regulation of muscle mass at different ages, and suggest that the effects of myostatin on muscle mass diminish with advancing age.

5-16

Upregulation of new pro-inflammatory cytokine/TAK-1/p38/ActivinA/SMAD3 skeletal muscle signaling pathway in rat sarcopenia

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Pro-inflammatory cytokines have been shown to cause skeletal muscle atrophy, differentiation inhibition and also to be upregulated in age-related muscle wasting, called sarcopenia, a condition with impaired muscle regeneration. However, their mode of action, upstream and downstream of NFκB activation, is not completely understood. In a human skeletal muscle cell system (skMC), we describe here that IL-1α and TNF-α block differentiation, and that these effects are counteracted by ActRII/Alk/Smad2/3 pathway inhibitors. The inhibitory effect of IL-1α and TNF-α on skMC differentiation can be subdivided into immediate and delayed signaling events. IL-1α and TNF-α induce the release of Activin A into supernatants (SN) and subsequently released Activin A inhibits skMC differentiation. TAK-1, p38 and NFκB pathway inhibitors prevent immediate cytokine-induced Activin A release into SN and inhibition of skMC differentiation, whereas blockade of ActRII/Alk/Smad2/3 pathway using siRNA

to Smad2/3 (siSmad2/3) has no influence on Activin A release. Although, inhibition of differentiation is blocked by ActRII/Alk/Smad2/3 inhibition, establishing that activin A released by cytokines inhibits skMC differentiation in an autocrine fashion via ActRII/Alk/Smad2/3. In accordance with this pathway, IL-1α and TNF-α induce phosphorylation of immediate signaling events (TAK-1/p38/NFκB) very rapidly (10 min after stimulation) and these phosphorylation events are all blocked by TAK-1 inhibition. In contrast, IL-1α and TNF-α do not induce phosphorylation of Smad2/3 after 10 min stimulation when no Activin A is detectable in SN, but do so after 24 h stimulation, when Activin A is found in SN. Moreover, delayed IL-1α and TNF-α-induced Smad2/3 phosphorylation is blocked by TAK-1/p38/NFκB inhibition confirming that these pathways are upstream of Activin A release and Smad2/3 signaling. Analysis of this new pathway in a rat sarcopenia model showed that Smad3 as well as TAK-1 and p38 phosphorylation significantly increased from 6 to 24 months. Moreover, expression of TAK-1, Activin A beta and serum TNF-α level also increased with age confirming upregulation of the TNF-α/TAK-1/p38/Activin A/Smad3 pathway during aging.

5-17

The mitochondrial and neuromuscular systems are severely dysregulated in a rat sarcopenia model

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Background and aims: Age-related loss of skeletal muscle mass and strength (termed sarcopenia) occurs in most mammalian species and adversely affects quality of life and survival. The etiology of sarcopenia is believed to be multifactorial but the specific initiating molecular mechanism(s) has not been elucidated, in part, because of dearth of non-invasive methods for such studies in humans and of suitable rodent models. Here, we describe a rat model and applied it to elucidate the genomic and proteomic changes associated with sarcopenia.

Methods: Male Harlan Sprague–Dawley (SD) rats aged 6, 12, 18, 21, 24, and 27 months were characterized for clinical phenotype, skeletal muscle weight and histo-morphometry; evoked muscle strength was assessed in a separate group of 6-, 12-, 18- and 24-month-old rats. Muscle samples were subjected to microarray and proteomic analysis to identify systems and pathways perturbed during sarcopenia as distinct from aging.

Results: Male Harlan SD rats exhibited sarcopenia as determined by progressive loss of muscle mass after 18 months of age. Sarcopenia was associated with atrophy of types 1, 2A and 2B muscle fibers and a decline in fiber oxidative enzyme activity. Gene set enrichment analysis of microarray data revealed that mitochondrial energy metabolism pathways (tricarboxylic acid cycle and oxidative phosphorylation) were the most down-regulated while genes associated with the neuromuscular (NM) junction were the most up-regulated. Pathways involved with protein synthesis and translation were also enriched. Proteomic analysis also revealed depletion of proteins associated with mitochondrial energy metabolism and enrichment of proteins associated with translation of both cytosolic and mitochondrial proteins.

Conclusions: The phenotypic, genomic and proteomic features of sarcopenia in these rats are similar to those of human sarcopenia and suggest that these rats may be a suitable model for mechanistic studies of sarcopenia. That the mitochondrial and NM were the most perturbed systems suggest that interventions that rescue these systems may be beneficial for preventing or treating sarcopenia.

5-18

Loss of ex vivo muscle function with preserved muscle mass may be indicative of sarcopenia onset in aging mice

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Background and aims: Sarcopenia is the decline of muscle mass and function with ageing. The relationship between loss of muscle mass and strength is not fully clear, since part of the decline in muscle strength is independent of muscle mass decline. The aim of this study was to characterize these aspects in mice of increasing age.

Methods: Male C57BL/6 J mice of 7, 10, and 13 months of age (m7, m10, m13; $n=5$ /group) were characterized for their body composition by DEXA. Extensor digitorum longus muscle (mEDL) was weighted and its function determined according to an ex vivo force–frequency stimulation protocol (10–167 Hz). Statistics were performed using a one-way ANOVA with LSD post-hoc testing.

Results: Total and lean body weight of m7 was lower ($p<0.05$; one-way ANOVA) than of m10 and reached a plateau thereafter. Muscle function declined in m13 (157 ± 18 mN*) compared to m7 (210 ± 31 mN) and m10 (234 ± 21 mN), as characterized by a lower maximal force ($p<0.05$; one-way ANOVA). Simultaneously, maximal contraction and relaxation velocity decreased. mEDL mass, however, was not reduced in m13 versus m10.

Conclusions: In 13-month-old mice, a decline in muscle function precedes age-related changes in muscle mass. This coincides with a decrease in bone mineral density. The data suggest that loss of muscle function in an ex vivo protocol is a potential indicator for loss of muscle quality and the onset of sarcopenia in this mice model. These observations offer potential to explore the effect of interventions targeting sarcopenia in the future.

5-19

Lack of apoptotic myonuclei during the initial phase of atrophy in young as well as old human skeletal muscle

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Several animal models have demonstrated that muscle atrophy is initiated by apoptotic loss of myonuclei.

Purpose: To investigate apoptotic signaling in myonuclei during the initial phase of muscle atrophy in healthy young as well as old human individuals.

Methods: Myofiber atrophy was induced by application of a knee-brace for a period of 4 days in young (Y , ~20 years, $n=11$) and aged (O , ~70 years, $n=11$) individuals. Muscle biopsies of the VL muscle were collected pre and at 1, 2, and 4 days of immobility. Changes in gene expression levels of BAX and p53 mRNA was determined using real-time RT-PCR and normalized to the Ribosomal Protein Large P0 (RPLP0) mRNA. Fragmented DNA was analyzed by TUNEL labeling on histological sections.

Results: Muscle atrophy was demonstrated by a decline in (MFA) (Y : 10.6%, O : 9.0%, $p<0.05$) and MVC (Y : 13.0%, O : 14.3%, $p<0.05$) at 4 days. Gene expression analysis revealed an age-specific (old subjects only) upregulation of the pro-apoptotic markers Bax and p53 after only 2 days of immobility, with further increasing levels after 4 days in both young and old muscle. These data were supported by a concomitant increase in TUNEL+ nuclei ($p<$

0.05). No TUNEL positive myonuclei were, however, observed in neither young nor old muscle. TUNEL positive nuclei were localized in the interstitial space between muscle cells. Double immunohistochemical staining for TUNEL and the endothelial marker CD-31, the macrophage marker CD-68 or the satellite cell marker Pax7 did not reveal any TUNEL positive endothelial cells, macrophages or satellite cells.

Conclusion: The present data indicates that disuse atrophy, in young as well as old human skeletal muscle, is not initiated by a degenerative process involving loss of myonuclei.

Supported by Lundbeck Foundation, the Danish National Research Council, the Danish Rheumatology Association, and the Nordea Foundation.

5-20

Assessment of sarcopenia in geriatric dogs

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Background and aims: Sarcopenia is a common problem in aging people but it has not been well-described in dogs. The aim of this study was to evaluate clinically applicable methods of assessing lean body mass in dogs, and to compare muscle mass, inflammatory markers, and insulin-like growth factor (IGF-1) in healthy young and geriatric dogs.

Methods: Healthy young (1–5 years old) and geriatric (>8 years old) Labrador Retrievers of optimal body weight were eligible for the study. Lumbar muscle measurements at the level of T13-L1 were performed by radiography, ultrasound, and computed tomography (CT). Temporal (CT and ultrasound) and quadriceps muscles (ultrasound) also were measured. Serum C-reactive protein (CRP), tumor necrosis factor- α (TNF), and IGF-1 were measured, and dogs' activity for 14 days was assessed using an activity monitor.

Results: Nine young dogs (five female, four male; mean age= 2.4 ± 1.0 years) and 11 geriatric dogs (nine female, two male; mean age= 9.4 ± 1.7 years) were enrolled. Body weight was not different between groups nor was any measures of body size (e.g., length, height) or calorie and protein intake. Muscle condition scores indicated significantly more muscle loss in the geriatric group ($p<0.001$). The mean area of the lumbar muscles was significantly smaller in geriatric compared to young dogs when measured by ultrasound ($p=0.005$) and by CT ($p=0.02$). Differences between groups were not detected for quadriceps or temporal muscles by either technique. Activity was lower in the older dogs ($p=0.02$) but muscle area was more significantly related to age than to activity. TNF, IGF-1, and CRP concentrations were not different between groups.

Conclusions: Muscle loss occurs in both aging dogs and humans. Additional studies evaluating functional changes in this canine model are warranted, as are studies to evaluate potential benefits of exercise and nutritional modifications.

5-21

A molecular predictor of the human ageing biological program

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The ability to monitor the progression of the ageing process requires the identification of mechanisms which also take into account confounding environmental factors. To discover the gene networks that define ageing, we hypothesised that it would be critical to incorporate detailed physiological phenotyping into the analysis, in order to rule out false associations. First, we produced a molecular classifier able to discriminate between healthy young (<30 years, $n=30$) and healthy old (>60 years of age, $n=30$) human skeletal muscle; one that is unrelated to aerobic, strength, metabolic fitness or exercise status. The ~40 gene expression programme is ~90% accurate at classifying age in multiple independent human and murine muscle data sets, including human kidney and human superior frontal gyrus. The ageing

classifier included 10 genes with molecular links to ageing biology, human longevity or pathways that alter longevity in multiple species. The remaining genes represent novel associations, including proteins involved in WNT signalling and RNA binding proteins. Cause-effect analysis using a drug connectivity map identified ~40 candidate drugs that regulated the ageing expression programme in human cells. We found that Rapamycin (mTOR inhibitor) and LY-294002 (PI3K inhibitor) induce the ageing gene network and both drugs inhibit genes which extend lifespan. Finally, we applied the age classifier to 150 middle-aged subjects (40–59 years). As expected only 5% of subjects were defined as having a ‘youthful’ middle-aged profile. While both groups had the same chronological age (~50 years) and cardiovascular and metabolic fitness, remarkably the ‘youthful’ middle-aged human subjects had a significantly lower resting heart-rate, a long-established correlate of longevity in the animal kingdom. The ageing gene network therefore represents not only the first accurate predictor of muscle ageing, but an excellent resource to develop small-molecule drugs and an excellent toxicology biomarker for future drug-safety screening.

6-01

Nutritional Status of the hospitalized dementia elderly people and it's relation with calf circumference

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Background and aims: Dementia is a neurological disease mainly characterized by clinically significant cognitive deficit which includes memory. Behavioral changes may occur and lead to an autonomy loss and a decreased capacity to perform daily professional and social activities. Disease-related undernutrition leads to an increased number of clinical complications as with a negative outcome in the physical and psychological status. Hospitalization can worsen the status. The Mini-Nutritional Assessment (MNA) is the golden standard for nutritional status evaluation and it is validated for Alzheimer's disease. This study aims to evaluate the Nutritional Status of the hospitalized elderly people in the Geriatric Ward of the Hospital de Magalhães Lemos–Porto and its relation with calf circumference.

Methods: This analytical cross-sectional study was made between the periods of February to May of 2011. The Mini Nutritional was applied. Furthermore, the patients were subjected to weight, height, calf circumference and mid-arm circumference measurements and the body mass index was calculated.

Results: This study was built with a sample of 22 elderly dementia people, of which 31.8% were male and 68.2% female. The Mini Nutritional Assessment revealed that 45.5% of the elderly dementia people were at risk for undernutrition, 4.5% did not show nutritional risk and 50% was undernourished. The calf circumference equal or superior to 31 cm showed to be more representative with 54.5%, while inferior to 31 cm showed 45.5%.

Conclusions: This study revealed the major importance of assessing the nutritional status and the need to use the calf circumference as a parameter for that evaluation.

6-02

Accuracy of food intake assessment is influenced by body fatness

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Background and aims: The accurate measurement of energy intake is essential in patients with cachexia and other diseases causing wasting,

which are highly prevalent among older persons. However, there are no accurate methods for the assessment of food intake in older populations, underreporting of intake being highly prevalent. There are reports that obesity is associated with higher prevalence of underreporting of energy intake, but few studies have measured body fatness itself. It is possible that older people with lower body fat underreport less, so that food intake inquiries would be more precise in patients with cachexia and other conditions that promote loss of fat mass. This research aimed to assess the correlation between underreporting of energy intake and different percentages of body fat in independent older people.

Methods: Forty-one volunteers (21 female) were evaluated, representing the four quartiles of fat percentage of a representative sample of 100 volunteers from the community. Resting energy expenditure was measured by indirect calorimetry, total energy expenditure (TEE) was measured by the doubly labeled water method (DLW) and energy intake (EI) was assessed by three 24-h recalls and a food frequency questionnaire (FFQ). Regression and analysis of variance were employed to compare TEE and energy intake values, energy intake-to-TEE ratios and energy intake-TEE values between dietary assessment methods. Bland and Altman analysis were performed for each method.

Results: TEE, as measured by DLW was 2,220±601 kcal, while the EI, measured respectively by the 24 h recall and FFQ, was 1,919±602 and 2,119±670 kcal. Under-reporting was less frequent in subjects with lower percentage of body fat and men ($p<0.05$). In conclusion, underreporting was less frequent among older persons with lower percentage of body fat.

Conclusion: It is possible, therefore, that undernourished patients report more precisely their energy intake. This finding needs confirmation in further studies including patients with cachexia.

6-03

Mechanisms underlying anorexia in adjuvant arthritis: Focus on IL-1beta and orexigenic/anorexigenic neuropeptide mRNA expressions in hypothalamic nuclei

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Background and aims: Adjuvant arthritis (AA), a rat model of rheumatoid arthritis induces anorexia–cachexia syndrome. Pro-inflammatory cytokine interleukin (IL)-1beta serves as a potent anorexigenic agent that interacts with hypothalamic neuronal circuits regulating appetite. In this study, we tested whether the loss of appetite and body weight during AA is associated with alterations in the expressions of IL-1beta and anorexigenic/orexigenic neuropeptides in the hypothalamic arcuate nucleus (ARC) or paraventricular nucleus (PVN).

Methods: AA was induced in rats by complete Freund's adjuvant injection. On day 18 of AA, mRNA expressions of neuropeptide Y (NPY), agouti-related protein (AgRP), cocaine and amphetamine regulated transcript (CART), pro-opiomelanocortin (POMC), melanocortin-4 receptor (MC4R), and IL-1beta in ARC or PVN were determined by quantitative TaqMan PCR. Plasma levels of leptin, insulin, ghrelin and corticosterone were determined by immunoassays.

Results: Arthritic rats displayed a 50% reduction in food intake and a 26% reduction in body weight compared to controls at the end of the experiment. Plasma levels of anorexigenic leptin were lower, insulin unchanged, and orexigenic ghrelin and corticosterone higher in arthritic rats than in controls. This was associated with increased ARC mRNA expressions of orexigenic NPY and AgRP in arthritic rats. The ARC mRNA expressions for anorexigenic CART were decreased, for POMC unchanged, and for IL-1beta increased in arthritic rats. In PVN, mRNA

expressions of IL-1beta and MC4R were also enhanced in the arthritic group.

Conclusions: The anorexia–cachexia syndrome in AA is not mediated by increased ARC mRNA expressions of anorexigenic neuropeptides or suppressed expressions of orexigenic ones. Reduced leptin and increased ghrelin and corticosterone levels indicate normal physiological response to starvation during AA. The failure of this response to increase food intake in arthritic rats can be ascribed to the anorexigenic effect of IL-1beta which over-expresses in the ARC and PVN.

Supported by: MSM 0021620816

6-04

Association of the surrogate measures of dietary protein intake and survival in 100,088 chronic hemodialysis patients

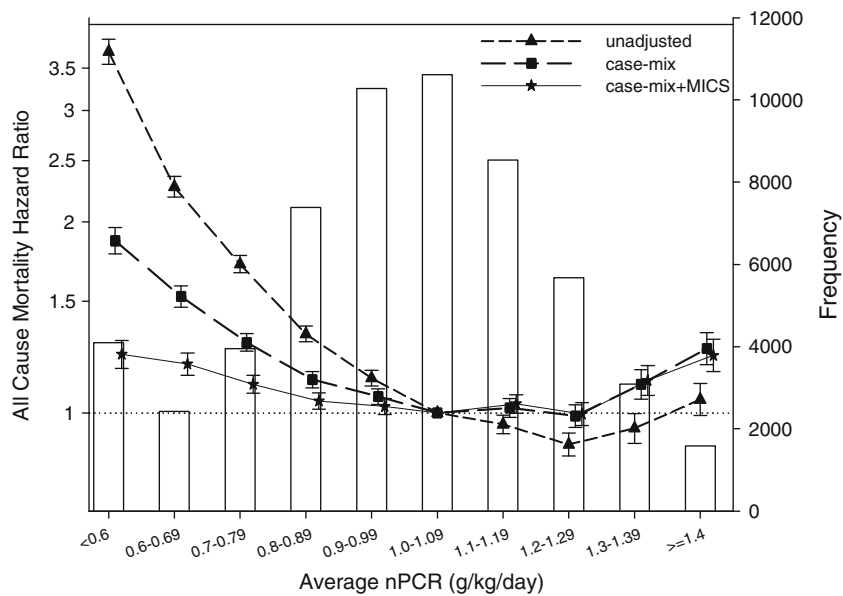
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Background: Decreased dietary protein intake may be associated with increased mortality risk in individuals with kidney failure undergoing maintenance hemodialysis (MHD).

Objectives: We examined 8-year (7/2001-6/2009) all-cause mortality in 100,088 MHD patients from DaVita dialysis clinics across the nation. We hypothesized that survival would improve across increased levels of normalized protein nitrogen appearance (nPNA), also known as protein catabolic rate (nPCR) and that this trend would be consistent even in hypoalbuminemic patients and in Blacks, Caucasians, and Hispanics.

Methods: Time-averaged Cox models were used to estimate death hazard ratios for quarterly averaged nPCR categories controlled for case-mix, comorbidity, dialysis dose (Kt/V), and available markers of malnutrition-inflammation-complex syndrome (MICS).

Results: In all patients, the best survival was associated with an nPCR between 1.2 and 1.3 g/kg/day, while nPCR <1.0 or ≥1.4 g/kg/day was associated with higher mortality. Adjustment for MICS attenuated the associations considerably, indicating that protein intake may be related to survival via the MICS axis as intermediary. Subgroup analyses revealed similar trends for hypoalbuminemic patients and in Blacks, Caucasians, and Hispanics, with Black and Hispanic subjects experiencing higher mortality rates than Caucasians at low nPCR levels.



Conclusions: A low daily protein intake is associated with increased risk of death in MHD patients, esp. in Blacks and Hispanics, with likely mechanism of modulating the MICS axis. Trials of dietary intervention to improve survival in MHD patients are indicated.

6-05

Nutritional status in patients with chronic obstructive pulmonary disease (COPD)

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Background and aims: COPD is chronic inflammatory disease with systemic symptoms. The most often extrapulmonary manifestation is loss of lean body mass named cachexia. Study aim was: (1) Evaluation of body

composition and frequency of cachexia in COPD patients in comparison with healthy subjects. (2) Assessment the relation between some parameters of cachexia and COPD stage, as well as subtype of the disease.

Methods: Fifty-five COPD patients—43 males, 12 females (mean age 62.31±11.08) and 32 healthy controls (mean age 57.43±8.79) was enrolled to the study. Body composition was measured using analyser based on bioimpedance. Percentage of ideal body weight (PIBW), body mass index (BMI), fat free mass index (FFMI), and fat mass index were assessed. Spirometry with evaluation of: forced vital capacity (FVC), forced expiratory volume in the first second (FEV1), FEV1/FVC (Tiffeneau ratio) was performed in each COPD subjects. Subtype of disease: emphysematous or chronic bronchitis was assessed on the base on clinical symptoms and parameters of emphysema evaluated in bodyplectysmography or computer tomography.

Results: PIBW, BMI, and FFMI indicated malnutrition were confirmed in 5.45%, 3.64%, 18.18% of COPD patients and 3.12%, 0%, 3.12% of control group, respectively. Average BMI did not differ between groups. FFMI was significant

lower among COPD patients (19.05 ± 3.44 vs. 20.55 ± 3.19 kg/m²). FFMI, but not BMI, correlated with stage of disease. In COPD patients with predominant emphysema, FFMI was lower than in patients with chronic bronchitis.

Conclusions: (1) Cachexia is frequent problem of COPD patients—it concerns about 20% of subjects. (2) Analysis of body composition with FFMI assessment is essential for evaluation of nutritional status—BMI is not sufficient indicator. (3) Cachexia appears more often in advance stages of COPD and in phenotype with emphysema

6-06

Mini nutritional assessment score predicts rehospitalisations in patients with chronic obstructive pulmonary disease

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Background: Limited information about nutritional status and risk of malnutrition is available in patients with chronic obstructive pulmonary disease (COPD). We have conceived this analysis to study the associations between malnutrition, body composition, and rehospitalisations in patients with COPD.

Methods: This prospective study recruited COPD patients during hospitalisation for acute exacerbation. In addition to routine assessment, we performed pulmonary function testing, nutritional assessment with Mini Nutritional Assessment (MNA) questionnaire, body composition measurement, and dyspnoea evaluation. We recorded hospitalisations during 6-month follow-up. Same investigations were performed in control group.

Results: We recruited 22 healthy controls (71 ± 5 years, 59% men) and 108 COPD patients (71 ± 10 years, 75% men, 85% severe or very severe COPD). Control subjects had significantly higher MNA score than COPD patients (27.0 ± 1.7 vs. 21.2 ± 4.9 , $p < 0.001$). MNA score indicated malnutrition in 14% of patients, and further 55% were at risk of malnutrition. With increasing severity of COPD per GOLD stage the MNA score decreased ($p = 0.02$). Body mass index but not body composition parameters was higher in control subjects when compared to COPD patients (29.1 ± 3.8 vs. 27.0 ± 6.3 , $p = 0.041$). A positive correlation between MNA score, body fat content ($p = 0.001$), and lean body mass ($p < 0.001$) was observed. During 6-month follow-up, 45 (41%) patients were rehospitalised. Malnourished patients had higher risk of rehospitalisation (Figure), which was maintained after adjustment for age, gender, GOLD stage, and body composition parameters (HR 2.93, 95% CI 1.05–7.32).

Conclusions: Malnutrition and risk of malnutrition is frequent in patients with COPD. Malnutrition predicted rehospitalisations at 6 months independently of body composition parameters.

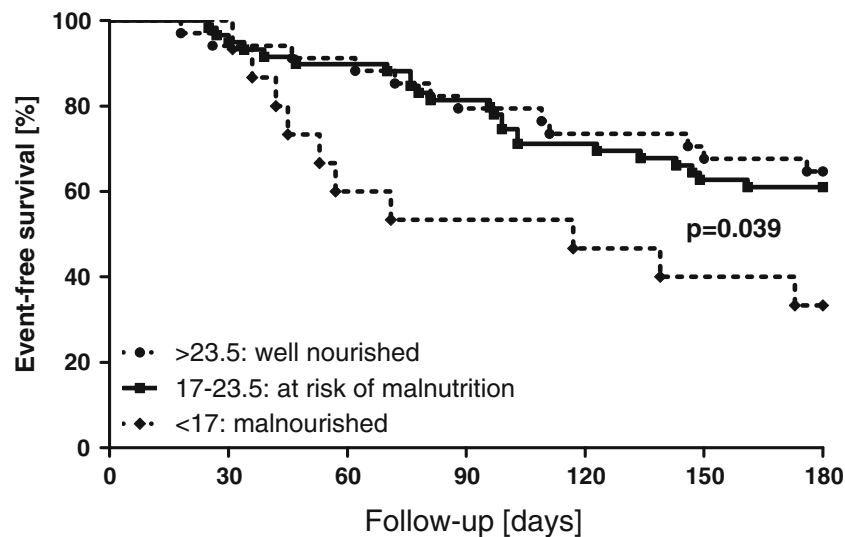


Figure.

6-07

Application of bioelectrical impedance analysis in nutritional assessment of COPD patients

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Background and aim: Chronic obstructive pulmonary disease (COPD) is characterized by a range of pathophysiologic changes contributing to a progressive loss of skeletal muscle mass. Body composition can be analysed by bioelectrical impedance analysis (BIA), a noninvasive, inexpensive and portable method. Phase angle (PA), a ratio obtained by

BIA, has been interpreted as an indicator of membrane integrity and function and reflects body cell mass. Low values of PA could be interpreted as malnutrition, defined as decreased body cell mass. We investigated whether PA could be a useful indicator of nutritional status in COPD patients and whether it would reflect disease severity.

Methods: One hundred thirty-two stable COPD patients were evaluated for BMI, parameters measured by BIA, airway obstruction (FEV1, FEV1/FVC), exercise capacity (6-min walkdistance), chronic dyspnea using Medical Research Council (MRC) scale, blood gases (pO₂, pCO₂), biochemical blood tests (albumin) and for all patients we calculated BODE index.

Results: PA shows a good significant correlation with FEV1 ($r = 0.521$, $p < 0.01$) while BMI and FFM are weakly but significantly correlated with FEV1 (respectively, $r = 0.329$ and $r = 0.262$). PA is statistically different among patients in the four stages of COPD. PA values are higher in first and second stage characterized by a smaller airflow limitation and

obstruction. PA is significantly associated with BODE index too: PA values are higher in patient with lower BODE score.

Conclusion: PA seems to be representative of body cell mass and therefore it could be used as a nutritional marker in COPD patients in which the BIA assumptions are not fully valid. The quite strong correlation between PA and FEV1 allow us to speculate that the decrease of PA reflects the progression of disease. In our population the good association found between PA and BODE confirm the possibility that PA should be evaluated as a prognostic indicator.

6-08

Parenteral nutrition in patients with cancer cachexia and bowel obstruction: International survey on indications, outcomes and practises

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Background: Patients having cancer cachexia and mild to severe bowel obstruction sometimes might benefit from parenteral nutrition. However current guidelines (e.g. ESPEN) do leave many decisions to the treating team, and therefore practices seem to vary substantially. We aim to systematically collect expert opinions and together with results of a systematic literature review provide information for further development of clinical practice and research.

Methods: Based on systematic literature review on parenteral nutrition in advanced incurable cancer patients, having mild to severe bowel obstruction (presented at ESPEN 2011) and expert opinions, a group consisting of physicians (oncologists, palliative medicine specialists, surgeons, others) and nutritionists composed a survey to be distributed to different professional societies (ESPEN, ESMO, EAPC). The survey is provides an introduction to the topic, followed by introductory questions on healthcare providers' view on the indications for parenteral nutrition in the population, describing patient characteristics and views on current oncological palliative management to be able to make decisions for parenteral nutrition, on outcomes for parenteral nutrition in the setting and whether guidelines are used and are helpful for the practice. Then, basic demographics of the healthcare provider are queried (age, gender, profession, working setting). Then inclusion criteria for in-depth survey are requested, defined level of experience with this specific patient population (exposure last years with defined number of patients, defined setting of interdisciplinary decision making). In the following part, three carefully described case reports, detailing all necessary components for decision making, the practice of the responding experts is asked in terms of patient population (important variables to make decisions; opinions about current oncological and palliative management, which need to be available to make decisions for or against parenteral nutrition; outcomes that respondents believe to be important and should be measured in the specific case; opinions about practices in terms of products components used; monitoring for safety and efficacy; conditions for delivery at home etc.). In the final part, generic questions about indications, outcomes and current practice are requested. Analyses will be mostly descriptive, hypothesis might be tested based on professions, countries and reimbursement system.

Results: The Survey will be launched in October 2011 and will be open for 4 weeks, therefore late braking results will be available by end of November 2011.

Discussion: This international survey on indications, outcomes and practices of parenteral nutrition in cancer cachexia patients having bowel obstruction, based on the careful design and the inclusion criteria for experts will provide solid informations to identify priorities and develop clinical trial design for guideline development.

6-09

What do patients eat when living with advanced cancer: findings from an exploratory trial of the Macmillan Approach to Weight loss and Eating (MAWE)

Jane B. Hopkinson¹, Issy Scott² (¹Cardiff University, UK, ²University of Southampton, UK)

Background: Evidence, although limited, suggests that advice on what to eat when living with refractory cancer cachexia syndrome should include (1) take a nutrition dense diet, (2) eat small amounts often, and (3) take nutritional supplements¹. Following this advice, within the confines imposed by disease, is important to minimising the malnutrition component of the syndrome.

Aim: To discuss the potential for nutritional counseling to benefit patients living with involuntary weight loss, poor appetite and advanced cancer.

Methods: In the UK in 2006–2007, 50 patients were recruited into an exploratory trial of a psycho-educational intervention for weight- and eating-related problems in patients with advanced cancer. A baseline interview invited self-report of food and fluid intake both the day before the interview and prior to receiving a cancer diagnosis. Framework analysis enabled evaluation of adherence to optimal food and fluid intake for someone with refractory cachexia.

Results: Forty-six patients reported poor appetite with 27 admitting to food and fluid intake half or less than half of pre-illness. As pre-illness, the majority took a main meal, comprising meat and vegetables ($n=30$), even though many were experiencing taste/smell changes that made this difficult. This led to comments like 'I can't eat much.' Only one person was taking their main meal at the time of day they found easiest to eat. Few were snacking ($n=5$), fortifying foods ($n=5$) or taking a nutritional supplement ($n=13$).

Conclusion: There is potential for nutritional counseling to support improvement in nutritional intake in patients with refractory cachexia. Work is needed to establish how best to support these patients in changing eating behaviours.

1. Hopkinson et al. (2011) What to eat when off treatment and living with involuntary weight loss and cancer: a systematic search and narrative review. *Supportive Care in Cancer*, 19(1): 1–17.

Funding body: Macmillan Cancer Support, UK

6-10

Prognostic role of nutritional factors and C-reactive protein (CRP) in locally advanced head and neck carcinoma (LAHNC) treated with definitive or postoperative chemoradiation (CRT)

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Background: Head and neck cancer patients experience nutrition problems due to the disease itself and/or to the effects of CRT and surgery. Malnutrition can impact on clinical outcome and reduce treatment efficacy. We reviewed the prognostic role of clinical and laboratory nutritional factors and acute-phase protein CRP in a consecutive series of LAHNC patients treated with definitive or postoperative CRT.

Patients and methods: From 2004 to 2010, 305 patients with LAHNC were treated with definitive CRT (82%) or surgery plus postoperative CRT (18%).

Baseline and 3 months after therapy values of body mass index (BMI), haemoglobin (Hb) and lymphocytes (Ly) were collected; CRP was considered only at baseline. Weight loss, the highest CRP and the lowest Hb level reached during CRT were reviewed. All these parameters were analysed as prognostic factors. Survival was calculated according to Cox multivariate analysis.

Results: Median age was 56 years, M/F ratio 231/74 (76%/24%), median ECOG 0. Site of primary cancer was oropharynx (33%), nasopharynx (27%), larynx-hypopharynx (14%), oral cavity (12%), and other (14%); stage IV was most represented (79%). Median follow-up time was 28 months (range, 1–138). Low BMI (<18.5), Hb (<12 g/dL), Ly (<900/mm³) were present respectively in 5%, 18% and 14% of the patients at baseline and in 10%, 43% and 58% 3 months after therapy. High value of CRP (>5 mg/dL) was identified in 27% of the patients at baseline. Thirty-seven percent experienced a high weight loss (>10%) during CRT. A prognostic role was identified for baseline CRP ($p=0.0114$) and for low Ly ($p=0.0026$) 3 months after CRT.

Conclusions: Nutritional factors and acute phase proteins have an impact on LAHNC patients prognosis and could help in stratifying patients for better therapeutic intervention and surveillance.

6-11

Relationship between nutritional assessment, Glasgow Prognostic Score and tumor staging in patients with breast cancer: preliminary report

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Background and aims: Malnutrition in cancer patients is associated with increased morbidity and mortality as well as higher medical expenses and longer hospital stay. Tumor-derived agents and the systemic response to the tumor, such as the presence of pro-inflammatory cytokines and hormones, have been associated with the pathogenesis of malnutrition and cachexia which impact on outcomes and functionality. Therefore, it is important to assess the nutritional and the inflammatory status of these patients.

Methods: Patients with breast cancer were evaluated between September 2010 and August 2011. Subjective Global Assessment (SGA) was used to assess nutritional status. Albumin and C-reactive protein (CRP) were determined for classification of the Glasgow Prognostic Score (GPS). Disease stage was determined according to the TNM classification. Patients were divided according to the stage (1 and 2; 3 and 4) to assess the association between GPS and tumor stage. The data were analyzed by Fisher's exact test.

Results: This study enrolled 49 patients with breast cancer, mean age of 52.8±12.4 years. Patients were divided into malnourished and well nourished according to SGA. The first group comprised patients with moderate malnutrition (16.3%) and the second group encompassed the nourished patients (83.7%). There was no significant relationship between nutritional (SGA) and inflammatory status, classified by GPS (Fisher's exact test, $p>0.05$). There was a significant association between GPS and tumor state (Fisher's exact test, $p<0.05$). No associations were observed between stage and SGA, SGA and albumin and between SGA and CPR.

Conclusions: Higher GPS values are found in patients with advanced breast cancer. GPS is not an indicator of patients' nutritional status.

6-12

Security hunger: an understanding of cancer cachexia-related distress in families

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Background and aims: Weight loss and anorexia are symptoms of cancer cachexia syndrome that can be experienced as distressing by patients and their family members. This study aimed to develop a theoretical understanding of cachexia-related distress that can underpin family focused psychosocial interventions for the problem.

Methods: A novel understanding of cachexia-related distress was generated from a secondary analysis of in-depth, semi-structured interviews conducted in the South of England in 2006–2007 with 31 advanced cancer patient–family carer dyads (a total of 62 interviews). The two stages of analysis comprised a discourse analysis followed by thematic analysis and interpretation, which enabled the identification of interdependency in patient and family carer experience and the construction of a conceptual model. The analysis was critical in that pre-existing theory was then used to explain the model and to generate an understanding of how cachexia-related distress might be alleviated. Emergent concepts, propositions and theory were tested through engagement with the study User Involvement Group comprising healthcare professionals, academics and patients.

Findings: Our food habits are a taken-for-granted behaviour that can communicate connection with and separation from those around us. Interactional patterns between patient and family carer are disrupted by weight loss and changing eating habits. Dyads can either adapt to this change (dual acceptance) or resist (dual resistance or mismatched resistance). Resistance can be understood as an indicator of threat to emotional security and driven by security hunger. Cachexia-related distress in both patients and their family members is symptomatic of unstable connections and difficulty adapting relationships as disease progresses.

Conclusion: This theory challenges researchers and clinicians to seek ways of aiding not only with the physical changes of weight loss and anorexia, but also with adaptation to the inevitable relationship disruption in families affected by cancer cachexia syndrome.

6-13

PTHrP and cachexia

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Cancer cachexia is reported to be a major cause of cancer-related death. Its pathogenesis remains unclear. For that reason, few effective therapies have been established. Myriad tumors produce parathyroid hormone-related protein (PTHrP) and, consequently, plasma level of PTHrP is increased in cancer cachexia syndrome. Peripherally administered PTHrP induced negative energy balance by decreasing food intake and gastric emptying, while not producing conditioned taste aversion. The mechanism involved activation of hypothalamic urocortins 2 and 3 through vagal afferent pathways, and the suppression of gastroduodenal motor activity. Continuous administration of PTHrP reduced food intake and body weight gain with a concomitant decrease in fat and skeletal muscle. These findings suggest that PTHrP influences food intake and body weight and may be a therapeutic target for cancer cachexia syndrome. We will summarize the relationship between PTHrP and cachexia.

6-14

Providing nutritional support to patients with thoracic cancer: findings of a dedicated rehabilitation service

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Background and aims: UK guidelines recommend screening patients with thoracic cancer to identify those requiring nutritional support.

To help quantify this area of need, the associated workload and explore its impact, we report findings from a dedicated rehabilitation service.

Methods: Patients were screened soon after diagnosis to determine the prevalence of malnutrition and various aspects compared between malnourished and not malnourished groups. A nutritional care plan was instigated and all contacts recorded, together with follow-up body weight.

Results: Of 243 patients seen, 84 (35%) were malnourished which was associated with advanced disease ($P=0.02$) and a reduced survival (median 52 days less, $P<0.01$). About one-third of these were being considered for, or receiving treatment with curative intent. Overall, about two thirds were failing to meet their daily recommended energy intake. The dietitian provided over 870 episodes of care, with a median [IQR] of 3 [2–5] contacts per patient. More of the malnourished group received oral nutritional supplements, but also experienced problems tolerating them. After one month, weight was stable or increased in 69% and fell in 31%. Neither the pattern nor magnitude of the change in weight differed between groups.

Conclusion: All patients with thoracic cancer require a dietetic assessment if those who are malnourished, or at risk of malnutrition, are to be reliably identified and receive appropriate nutritional support. Our data provide a pragmatic insight into the implications of following UK national guidance on nutritional screening and support in this patient group.

6-15

Nutritional screening in obese patients with metastatic lung cancer. Does it make any sense?

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Background and aims: Patients with metastatic lung cancer frequently develop anorexia–cachexia syndrome which is characterized by loss of lean and fat body mass. However, in obese patients progressive loss of musculature may be obscured due to the abundance of fat leading to misdiagnosis of the syndrome. In the present study, we evaluated the prevalence and prognostic value of nutritional depletion in obese patients with metastatic lung cancer.

Methods: Newly diagnosed metastatic lung cancer patients accrued in two University Hospitals in Greece were eligible. Obese patients, defined as those with body mass index (BMI) ≥ 30 were analyzed. The Mini Nutritional Assessment (MNA) score was used for the evaluation of nutritional status and patients were classified into three groups: adequately nourished (group A), at nutritional risk (group B) and malnourished (group C). The prognostic significance of MNA was also assessed.

Results: From an original cohort of 421 patients, 75 (17.8%) patients were obese and were subsequently selected for analysis. Mean age (\pm SD) was 64.2 (± 10.6) and 58 (77.3%) patients were males. Mean BMI (\pm SD) was 32.8 (± 3.3). According to MNA, 32 (43.2%) patients belonged to group A, 39 (52.7%) to group B and 3 (4.1%) to group C. Median survival (\pm SD) for the aforementioned groups was 10 (± 10.9), 7 (± 6.7) and 2 (± 1.7) months, respectively, and this difference was statistically significant ($p<0.001$).

Conclusions: Obese cancer patients represent a heterogeneous cohort in terms of nutritional adequacy and this may impact on survival. These patients should not be spared from nutritional evaluation and should be offered specialized counseling and/or nutritional support.

6-16

Nutritional status and immune reactivity in patients with metastatic lung cancer. correlations and clinical implications

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Background and aims: Lung cancer patients frequently develop malnutrition and/or acute phase response in the context of cachexia and may present defects in their immune system. Our aim was to evaluate the association between nutritional status and chemokine-induced response of lipopolysaccharide (LPS)-stimulated, ex vivo cultured peripheral blood monocytes (PBMCs) in metastatic lung cancer patients. The correlations of the recorded parameters with clinical outcomes were also evaluated.

Methods: Patients with metastatic lung cancer were eligible. Demographics and other baseline characteristics were recorded. Mini Nutritional Assessment (MNA) protocol was used for nutritional assessment. PBMCs were isolated and cultured in vitro for 2 h in the presence of autologous serum and LPS. Interleukin (IL)-8 levels were measured in plasma before and after LPS-stimulation, as an indicator of immune reactivity after exposure to a high immunogenic stimulant. MNA was correlated with “folds of IL-8 production” after LPS stimulation. Correlations with clinical outcomes were also evaluated.

Results: Totally, 143 patients (89.5% males) [median age 67 years (range, 32–84)] were enrolled. The distribution of patients across MNA groups A, B and C was: 40 (28%), 67 (46.9%) and 36 (25.1%), respectively, while the median stimulation times for IL-8 (\pm SD) was 27.5 (± 126.8). The correlation between MNA groups and IL-8 stimulation times was significant ($p<0.05$). MNA was further correlated with the development of infections during first-line chemotherapy ($p<0.05$) as well as hospitalizations ($p<0.05$), although this was not the case for IL-8 stimulation times. MNA score and IL-8 stimulation times were further correlated with time to tumor progression after first-line chemotherapy ($\rho=0.318$, $p<0.01$ and $\rho=0.321$, $p<0.01$) and overall survival ($\rho=0.506$, $p<0.01$ and $\rho=0.298$, $p<0.01$), respectively.

Conclusions: Immune reactivity may be impaired in cachectic lung cancer patients and this may impact on their quality of life and survival.

6-17

Endocrine abnormalities in advanced cancer patients with anorexia

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Background: We investigated the frequency of endocrine abnormalities in patients with advanced cancer with poor appetite.

Methods: Retrospective review of 80 consecutive patients with an appetite score of ≥ 4 referred to a supportive care clinic. We screened patients for abnormalities in the following abnormal serum levels: 25(OH) vitamin D < 20 ng/ml, cortisol < 4 mcg/dl, vitamin B12 < 211 pg/ml, thyroid stimulating hormone > 5.5 μ IU/ml and bioavailable testosterone < 50 ng/mL.

Results: Male gender 51 (64%), white 53 (66%) with a median age of 60 (range, 27–91). Gastrointestinal 24 (30%) and lung 16 (20%) were predominant. Average appetite score was 6.26 (SD 2.0). Sixteen patients (20%) had a body mass index ≤ 20 . While 67/80 patients (84%) had a history of $> 5\%$ weight loss, of which 26/37 patients (70.3%) were hypogonadic and 29/63 patients (46%) were vitamin D deficient. No patients with anorexia had

deficiency of vitamin B12, only 1/73 patients (1.4%), not on steroids, had low cortisol, while 9/73 patients (12.3%) had abnormal thyroid studies.

Discussion: The majority of advanced cancer patients with an appetite score ≥ 4 had a significant history of weight loss. Vitamin D deficiency and hypogonadism, in males, were highly prevalent among advanced cancer patients with cachexia, while thyroid dysfunction, adrenal insufficiency, and low vitamin B12 were uncommon.

6-18

Nutritional impact symptoms and management in patients with cancer cachexia

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Background: Nutrition impact symptoms (NIS) such as nausea may contribute to weight loss in patients with cancer by decreasing nutrient intake. We determined the frequency and type of contributors to poor appetite and weight loss, and the effect of their management on outcomes such as weight and symptoms.

Methods: The study is a review of 150 cancer patients referred to an outpatient supportive care clinic for involuntary weight loss. All patients received dietary counseling and exercise recommendations. Assessments included weight, body mass index (BMI), assessment of nutritional impact symptoms, and resting energy expenditure by indirect calorimetry.

Results: Median weight loss in the 100 days before referral was 9% (4–13%) and median BMI at presentation was 20.8. Median number of NIS was 3 (2–4). NIS were most commonly treated with metoclopramide (early satiety in 62% of patients and nausea in 44%), laxatives (constipation in 52%), antidepressants (depression in 42%), zinc sulphate (dysgeusia in 28%), speech therapy or endoscopy evaluation (dysphagia in 14%) artificial saliva (dry mouth in 9%), and opioids (mucositis in 7%). Poor appetite and weight loss before referral ($r=0.18$, $p=0.036$) were associated with increased NIS ($r=0.22$, $p=0.008$). Forty-one percent (24/59) of patients were hypermetabolic appetite improved ($p<0.001$) and 31/92 (34%) of patients returning for a second visit gained weight.

Conclusions: Patients had a high frequency of multiple NIS, and hypermetabolism. A combination of inexpensive pharmacological and nonpharmacological interventions improved appetite significantly, and increased weight in one third of patients who were able to return for follow-up.

7-01

Comparison of electroporation and lipofection for invitro transfer of plasmid PEGFP-N1 into human myoblasts

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One of main goals of in vitro research is to develop efficient methods for drug delivery. Effective delivery of genetic material into cells is crucial for application in clinical environment for gene therapy or genetic vaccination. The tissue of choice for gene therapy or DNA vaccination are skeletal muscles, representing 40% of the body mass and which are known to actively participate in the immune response. Here we compared the efficiency of two different methods for transfer of plasmid DNA: electroporation, where electric pulses are used to permeabilize cell membrane; and lipofection which uses liposomes as carriers. Electroporation is promising for clinical application while lipofection is auspicious for developing strategies for gene therapy. Plasmid pEGFP-N1 coding for green fluorescent protein was transfected into primary human myoblasts with lipofection (Lipofectamine 2000) or by applying high-voltage pulses. Our results demonstrate that efficient transfection of human myoblasts with DNA in vitro

can be obtained both methods. The highest transfection ratios were similar (40.9% with lipofection versus 41.4% with electroporation). However, the main difference was in cytotoxicity of both methods. Less than 40% of cells were viable after the electroporation under conditions that give comparable transfection results with lipofection. On the other hand, by lipofection, almost 80% of myoblast were viable after the treatment. Due to lower cytotoxicity, more suitable method for transfection studies on myoblasts in vitro is lipofection. Electroporation has advantage over lipofection in in vivo environment due to simplicity of protocol and lack of need for using additional chemicals.

7-02

Resistance exercise modulates testosterone levels in tumour bearing rats

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Introduction: Hypogonadism is a frequent symptom of cancer patients and the decrease in testosterone may aggravate muscle mass loss. Exercise is a simple, low-risk intervention and is associated with positive effects on lean mass. Therefore, it could play a relevant role in counteracting the effects of cancer cachexia on muscle wasting.

Aim: We carried out a study to assess the effects of a resistance exercise program on Walker 256 tumour-bearing rats testosterone profile.

Methods: Forty rats were randomly assigned to one of the three experimental groups: control (C), tumour-bearing (TB), resistance training (RT) and tumour-bearing RT (RTTB). During the 8 weeks of resistance training, climbing sessions were performed once every 3 days. Training sessions consisted of three ladder climbs with 75%, 90%, and 100% of their maximal carrying capacity, determined in the previous session. At the sixth week of resistance training, tumour cells were inoculated in tumour groups subcutaneously in the right flank (2×10^7 Walker 256 carcinosarcoma cells). Plasma testosterone was determined with a commercial radio assay kit and expressed in nanogram per milliliter.

Results: Testosterone levels were higher 55% ($p<0,042$) in RTTB group when compared with CT group and 63% ($p<0,03$) compared with TB group.

Table 1. Hormone and muscle protein profile

Parameters	Control	TB	RT	RTTB
Testosterone (ng/ml)	254±41.3	221±23.1	734±88.2 ^{a,b}	577±131 ^{a,b}
Gastrocnemius Protein (ug/ul)	4.7±0.1	4.5±0.08	5.9±0.03b	5.1±0.3

a=compared with control

b=compared with TB

Conclusions: The resistance exercise protocol showed a marked effect effects on testosterone profile in the plasma of tumour bearing rats, preserving muscle protein.

7-03

L-Carnitine: an adequate supplement for a multi-targeted anti-wasting therapy in cancer

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Background and aims: Tumour growth is associated with weight loss resulting from both adipose and muscle wasting.

Methods: Administration of L-carnitine (1 g/kg body weight) was given to rats bearing the AH-130 Yoshida ascites hepatoma, a highly cachectic rat tumour.

Results: L-Carnitine treatment resulted in a significant improvement of food intake and in muscle weights (gastrocnemius, extensor digitorum longus (EDL) and soleus). These beneficial effects are directly related with improved physical performance (total physical activity, mean movement velocity and total travelled distance). Administration of L-carnitine decreases proteasome activity and expression of genes related with this activity, such as ubiquitin, C8 proteasome subunit and MuRF-1. Interestingly, L-carnitine treatment also decreases caspase-3 mRNA content therefore suggesting a modulation of apoptosis. Although some of the effects of L-carnitine on muscle tissue could be indirect, addition of a 50 μ M of L-carnitine to isolated EDL muscles results in a significant decrease in the proteolytic rate.

Conclusions: The reported results clearly demonstrate for the first time that L-carnitine supplementation increases food intake and can prevent muscle wasting in animals with cancer cachexia. L-carnitine is a natural compound free from toxicity up to 7 g/day in human subjects and since it is readily excreted, supplement ingestion is well tolerated. Further clinical trials are needed to clarify its efficacy in the cancer patient; however, the results presented here suggest that L-carnitine may be an essential component for a multi-targeted approach of nutritional intervention in cancer cachexia.

7-04

Curcumin supplementation attenuates lean body mass loss during the progression of cancer cachexia in the Lewis lung carcinoma-tumor-bearing mouse

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Background and aims: The interest in understanding and treating cachexia has intensified over the past decade as a result of the high mortality index associated with cancer and other chronic diseases that also involve muscle wasting. Thus, interventions that can improve or maintain muscle mass should provide favorable outcomes for the health of cachectic patients. Nutraceutical compounds, including the natural phenol curcumin, have been the center of significant research recently due to their anti-inflammatory, antioxidant, and anticarcinogenic properties. The purpose of this study was to determine if curcumin supplementation could improve body composition and physical function in the Lewis lung carcinoma (LLC) mouse model of cancer cachexia.

Methods: C57BL6 mice injected with LLC cells were supplemented daily with 10 mg of curcumin (C) ($n=7$) or vehicle (V) ($n=11$) over a 3-week period and C57BL6-V ($n=8$) mice served as controls. Body weight, body composition, strength, and neuromuscular performance were assessed over the course of the experiment.

Results: LLC-V mice lost 7% ($p=0.010$) of their body weight, and DEXA analysis revealed a 6% ($p=0.046$) loss of lean mass, while there was no significant change in fat mass. Curcumin supplementation significantly attenuated body weight loss ($p=0.025$) without affecting tumor growth. Curcumin supplementation significantly increased lean mass compared to LLC-V mice ($p=0.050$). Related to physical function, voluntary grip strength in LLC-V mice was significantly decreased during the study, and curcumin supplementation did not alter this change. RotoRod performance, a measure of neuromuscular function, was not affected by cachexia or curcumin supplementation.

Conclusion: In the LLC model of cachexia, curcumin supplementation can attenuate body weight loss and maintain lean body mass independent of strength loss during the progression from pre-cachexia to cachexia.

7-05

Elevated fatty acid content in muscle is reversed by fish oil in an animal model of colorectal cancer receiving irinotecan/5FU

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Colorectal cancer is associated with significant weight loss characterized by depletion of skeletal muscle and altered lipid metabolism. Using computed tomography (CT) images, our research revealed a high degree of fat in the muscles of patients with advanced cancer. Fat deposition into skeletal muscle is associated with poor function and strength. Irinotecan (CPT-11) combined with 5-fluorouracil (5-FU) are the most commonly used chemotherapy drugs to treat colorectal cancer; however, these drugs are highly toxic and lead to altered lipid metabolism. Clinical studies suggest that fish oil (FO) supplementation increases muscle mass, improves chemotherapy efficacy, however, the effect of neither chemotherapy nor fish oil on intramuscular adipose tissue is not known. Our study aimed to assess the amount and types of lipids in skeletal muscle in the Ward colon tumor rat model undergoing CPT-11 and 5-FU with or without fish oil. Throughout the study, female fisher 344 rats were fed either a control diet (CON; $n=8$) or fish oil diet (FO; $n=9$). Rats either received CPT-11/5-FU treatment (CMO; $n=8$) or no treatment was provided (CON). Rats without tumor served as a reference group (REF; $n=8$). A Folch method was used to extract lipid from muscle tissue. Phospholipid (PL) and triglyceride (TG) were separated by thin layer chromatography. Fatty acids (FAs) were determined by gas liquid chromatography. Rats receive chemotherapy exhibited more fat in muscle tissue ($P=0.02$); however, FO was not different from REF group. CON and CMO had low omega-3 FA in muscle tissue ($P=0.01$) and more saturated FA ($P=0.05$) than REF. Rats fed FO had lower saturated FA ($P=0.06$) and more omega-3 FA than CMO. Our study suggests that omega-3 fatty acids might be useful to decrease fat in muscle which would affect muscle function and quality of life in cancer patients.

7-06

Oxypurinol and eicosapentaenoic acid in the treatment of muscle wasting in cancer cachexia

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Background: Cancer cachexia (CC) is a profound wasting condition, driven by systemic inflammation and oxidative stress. This study investigated oxypurinol (xanthine oxidase (XO) inhibitor) in combination with eicosapentaenoic acid (EPA; anti-inflammatory) as a treatment to attenuate muscle wasting in a mouse model of CC.

Methods: Mice with CC were randomised into four treatment groups (EPA (0.4 g/kg/day), oxypurinol (1 mmol/L ad libitum), combination, or control), and euthanized after 29 days of treatment. Gene expression and enzyme activity analyses were completed on gastrocnemius muscle.

Results: The oxypurinol and combination groups displayed weight loss significant from control for an extended duration (9 and 10 days) compared to EPA (19 days), while oxypurinol indicated a trend toward increased rate-of-decline in condition. Expression of CuZnSOD and MnSOD decreased in the combination compared to oxypurinol, MnSOD increased with oxypurinol compared to control, and combination decreased EcSOD compared to all groups. Catalase expression increased with oxypurinol compared to combination and control, and in EPA compared to control. EPA and oxypurinol showed increased expression of GPx-1 compared to combination and control. Oxypurinol increased XDH expression compared to all groups. EPA and

oxypurinol increased expression of UbB, MURF-1 and MAFbx compared to combination and control. SOD activity increased in oxypurinol compared to all groups. Activity of GPx was reduced by EPA group compared to control and combination. Catalase activity was reduced by combination treatment compared to oxypurinol and control. There was no significant difference in XO activity between groups.

Conclusion: Whilst several antioxidant genes were upregulated by oxypurinol, so too were XDH and proteolytic subunits, and though further study is required, may shed light on the pathways that lead to the exacerbated decline in condition observed. In combination with EPA, there was little significant improvement from control, indicating oxypurinol is unlikely to be a viable candidate for multimodal therapy in CC.

7-07

Peripheral administration of the leptin antagonist BL-5040 ameliorates cachexia and normalizes muscle function in mice with chronic kidney disease

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Background and aim: We have previously shown that elevated circulating levels of cytokines such as leptin may be an important cause of chronic kidney disease (CKD)-associated cachexia (Cheung et al., JCI 2005). BL-5040 is a recently developed pegylatedleptin antagonist, which binds leptin receptor with high affinity but does not activate it. BL-5040 has been previously shown to increase appetite and weight gain in normal mice (Elinav et al., Endocrinology 2009). We tested whether BL-5040 would be effective in attenuating CKD-associated cachexia.

Methods: CKD was induced by 5/6 nephrectomy in 8-week-old c57BL/6 J mice. CKD and Sham (S) mice received either BL-5040 (7 mg/kg, i.p.) or vehicle (V) once daily for 28 days. All mice were fed ad libitum during this period. Metabolic rate was measured by Oxymax, body composition by Echo-MRI and muscle function by rotarodactivity and grip strength.

Results: BL-5040 reverses anorexia in CKD. The food intake of the CKD/BL-5040 mice was significantly increased compared with CKD/V mice (3.7±0.0 vs. 3.1±0.1 g/mouse/day; $p<0.001$). CKD/BL-5040 mice gained more weight than CKD/V mice (15.1±0.4% vs. 3.0±0.3%; $p<0.001$). CKD/BL-5040 mice gained fat mass (gain of 0.4±0.1 g) and lean mass (gain of 0.2±0.1 g) while CKD/V mice continued to lose fat mass (loss of 0.2±0.0 g) and lean mass (loss of 1.3±0.1 g; $p<0.001$). Basal metabolic rate was increased in CKD/V mice (3,845±62 mg/kg/h) compared with S/V mice (3,369±43 mg/ml/h; $p<0.001$) and was normalized in CKD/BL-5040 mice (3,246±79 mg/ml/h). Rotarod activity and grip strength was decreased in CKD/V mice (130.3±5.8 s, 1149.6±49.2/100 g; $p<0.001$) compared with S/V mice (187.0±12.0 s, 1643.3±28.2/100 g) and was normalized in CKD/BL-5040 mice (175.8±3.8 s, 1,568.2±46.0/100 g).

Conclusions: BL-5040, a peripheral leptin antagonist, reverses anorexia, ameliorates lean body mass losses and normalizes muscle function in a mouse model of CKD-associated cachexia.

7-08

Peptidic agonists of ghrelin with a prolonged orexigenic effect

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Ghrelin originates mainly from the stomach where it increases acid secretion and motility. In the adenopituitary, ghrelin acts as a growth hormone secretagogue, and in the hypothalamus as an orexigenic hormone. Ghrelin is the only food intake stimulating peptide born in the periphery and acting in the

brain. Physiological effects of ghrelin are mediated through its growth hormone secretagogue receptor GHS-R1a. Ghrelin, a single peptide of 28 amino acids, has its serin in position 3 (Ser³) acylated with octanoic acid which is crucial for ghrelin activity but prone to hydrolysis by esterases. In this study, ghrelin analogues with octanoic acid coupled to diaminopropionic acid (Dpr) replacing Ser³ were synthesized in order to make ghrelin resistant to esterases. Beyond using Dpr(N-octanoyl) in position 3, ghrelin analogs were stabilized replacing phenylalanine in position 4 (Phe⁴) with noncoded bulky hydrophobic Phe derivatives and/or using sarcosine instead of glycine in position 1. The stabilized derivatives had a similar binding affinity to cell membranes with transfected GHS-R1a as ghrelin, but showed a longer-lasting orexigenic effect in lean mice and a higher stability in the blood compared to ghrelin. Replacement of the ester bond for the amide ones plus using non-coded amino acid(s) instead of natural ones resulted in effective ghrelin agonists with a prolonged stability that could be efficient for a long-term application at cachexia accompanying cancer or chronic inflammatory diseases where ghrelin anti-inflammatory effect could also be beneficial.

This study was supported by grant of Grant Agency of the Czech Republic 303/09/0744 and Z40550506 of the Academy of Sciences of the Czech Republic.

7-09

Dln101 a naturally occurring ghrelin splice variant for the treatment of cachexia

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Unexplained weight loss is a common and vexing problem in many chronic diseases and can lead to the development of cachexia. There are currently no approved medications for cachexia or frailty. Three therapies are approved for treatment of AIDS-related cachexia (megestrol acetate, somatropin, and dronabinol), but adverse effects and questionable efficacy render them poor choices in many cases. Ghrelin is a 28-amino acid hormone secreted by the stomach that has been extensively studied since its discovery in 1999. It is the natural ligand of the growth hormone (GH)–secretagogue receptor in the pituitary gland, and it is also the only known circulating orexigen (appetite stimulant) to date. In humans, endogenous ghrelin secretion rises before eating and falls afterward, and exogenous ghrelin stimulates hunger and food intake, inducing weight gain. Ghrelin has been administered successfully to over 400 men and women, to date, including patients with cancer, pulmonary disease, diabetes, renal failure, and heart failure. Dln101 is a proprietary 24 amino acid, acylated peptide that can be easily manufactured at low cost. Extensive preclinical studies have shown that Dln101 acts similarly to ghrelin in increasing food intake, promoting weight gain, and increasing GH release. These extensive preclinical studies also suggest beneficial effects on lean body mass, cholesterol, and glucose that are specific to Dln101. These positive unique metabolic effects of Dln101 make it a better candidate for the chronic treatment in diabetes, renal failure, heart failure, cancer, and COPD-related cachexia. Dln101 has also successfully completed the requisite preclinical toxicology, pharmacokinetic, and pharmacodynamic testing and has received approval to start phase 1 clinical trials.

7-10

Leveraging the myostatin signaling pathway with small molecules: inhibitors of the activin receptors' kinase activity

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Background and aims: Inhibition of myostatin signaling has the potential to be an effective treatment for muscle wasting and osteoporosis. Knockout of the activin receptors as well as treatment with the soluble extracellular domain protein of ActR2B show muscled phenotypes in the mouse. While biologicals have the potential to treat muscle wasting disorders, small molecules targeting the myostatin signaling pathway may also have potential. **Methods:** Protein and cell-based screening assisted by structure-based drug design were used to identify small molecule kinase inhibitors of the activin receptors ActR2A and B. Compounds were profiled in *in vitro* assays to determine their potency and selectivity and to elucidate their true mode of action. Molecules with suitable pharmacokinetic profiles were investigated *in vivo* for muscling effects.

Results: Two highly selective and potent ActR2B kinase inhibitor series of molecules were developed. Dual ActR2B and 2A kinase inhibitors were also discovered. Despite their promising profiles, both subtype selective and dual ActR2 kinase inhibitors showed only modest muscling effects.

Conclusions: The failure of potent and selective activin receptor kinase inhibitor tools to directly influence myostatin signaling demonstrates a significant gap in the accepted dogma surrounding TGFbeta family member signaling.

7-11

Acute anabolic effects of β -hydroxy- β -methylbutyrate (HMB) in human skeletal muscle

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Background: The leucine metabolite β -hydroxy- β -methylbutyrate (HMB) has shown promise as an anabolic (e.g. enhancing resistance exercise training-induced hypertrophy) and anti-catabolic (e.g. reducing immobilization, AIDS and cancer-associated atrophy) supplement in humans and animal models. However, there have been no investigations into its mechanism(s) of action: viz., the acute anabolic effects of HMB on skeletal muscles of otherwise healthy animals/humans.

Methods: We studied eight healthy young men (18–35 years old) over a 5-h period during which we gathered baseline ‘pre-HMB’ measurements for 2.5 h before subjects’ consumed a single oral HMB bolus (~3 g as free-acid form), such that the subsequent 2.5 h represented the ‘post-HMB’ measurement period. Muscle protein synthesis (MPS) was measured in biopsies of the vastus lateralis by incorporation of 1,2[13 C]-leucine into myofibrillar proteins, plasma HMB by GC-MS, plasma insulin by ELISA, leg [femoral] blood flow (LBF) by Doppler ultrasound and intramuscular signalling proteins regulating MPS by immunoblotting.

Results: Oral HMB was rapidly absorbed with increases in plasma HMB peaking 30 min post-ingestion (basal, $5 \pm 1 \mu\text{mol l}^{-1}$ vs. 30 min: $408 \pm 27 \mu\text{mol l}^{-1}$; $P < 0.001$) and remaining significantly elevated throughout the study ($275 \pm 12 \mu\text{mol l}^{-1}$ 2.5 h post HMB; $P < 0.001$ from basal). HMB robustly stimulated MPS with baseline fractional synthetic rates (FSR) of $0.039 \pm 0.004\% \text{h}^{-1}$ compared to $0.073 \pm 0.01\% \text{h}^{-1}$ ($P < 0.01$) in the period following HMB ingestion. HMB also increased LBF (baseline: 0.55 ± 0.03 vs. post-HMB: $0.73 \pm 0.05 \text{ lmin}^{-1}$; $P < 0.01$). Temporal resolution of intramuscular signalling (sampling 30–120 min post-HMB) revealed that HMB likely exerts its anabolic effects via mammalian target of rapamycin complex 1 (mTORc1). All these changes occurred in the absence of alterations in plasma insulin (mean for study: $5.87 \pm 0.45 \text{ mU l}^{-1}$; $P > 0.05$).

Conclusions: HMB increases MPS and LBF even in the absence of exogenous nutrients or increases in plasma insulin. These findings highlight novel mechanisms of HMB action which likely underlie its efficacy/promise for countering human muscle wasting.

7-12

Metabolic modulators might have a key role in enhancing muscle differentiation and contrasting atrophy: possible application in the treatment of cachexia and sarcopenia

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Background: Metabolic modulators have been found to improve cardiac energy metabolism. Their beneficial effect in patients with ischaemic cardiomyopathy has been well established. Trimetazidine (TMZ), for example, inhibits fatty acid oxidation thereby increasing glucose oxidation, which results in a cytoprotective activity of trimetazidine. It has been recently reported that TMZ might also improve exercise capability in patients suffering from angina, which suggests that TMZ improves muscle performances. We therefore decided to test the effect of trimetazidine on myotube function and morphology and to evaluate its potential protective effect against stressors causing myotube atrophy.

Methods: C2C12 myotubes were treated with TNF and the protective role of TMZ was evaluated. In particular, we analyzed myotube morphology by means of actin and myosin immunofluorescent staining and by transmission electron microscopy (TEM). We also studied the expression of atrophy/hypertrophy/autophagy proteins. Moreover, we wanted to determine the effect of TMZ on myogenic differentiation; for this purpose C2C12 cells were induced to differentiate in presence of TMZ and expression of myogenic markers was evaluated.

Results: We observed, in presence of TMZ, a reduction of TNF-induced atrophy as evidenced by MuRF-1 and Atrogin-1 expression decrease. We also observed that TMZ was capable of inducing cytoprotection tophagy. Static cytometry and TEM analyses also suggested (1) that cytoskeletal integrity and function was preserved by TMZ administration and (2) that TMZ was capable of bolstering protein synthesis and metabolic pathways exerting a powerful ‘trophic’ activity on myotubes. Finally, very strikingly we also found that TMZ increases myogenic differentiation and myoblast fusion.

Conclusions: Our experiments suggest that TMZ protects from muscle atrophy, enhances myogenic differentiation and bolsters cell response to stress. We are also testing these effects on mouse models of cachexia and sarcopenia. Our results clearly suggest a reappraisal of TMZ in the treatment of muscle wasting disorders.

7-13

Muscle mass loss in ageing rats is restricted to hindlimb and can be restored by formoterol

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Ageing is a world wide concern and it is estimated that 25% of the population in 2025 will be constituted of elderly (60 years old or more). The loss of muscle mass and function in the senior, namely sarcopenia, is one of the main causes of the disability, loss of autonomy and increase risk of falls and frailty. The etiology is multi-factorial and includes intrinsic factors such as altered hormonal levels, high level of inflammatory cytokines, neural remodeling (alteration of NMJ number

and function), deficiencies of satellite cells for muscle regeneration, or includes extrinsic factors such as poor nutritional status or physical activity. Actually, there is no clinical treatment for this symptom. Formoterol is a long-acting β_2 -adrenoceptor agonist used extensively in clinic to supplement prophylactic corticosteroid therapy for the treatment of asthma and also known to have muscle anabolic effects when administered at millimolar doses. The objective of the study was to determine whether formoterol could reverse the age-related changes in skeletal muscle in rats assessed longitudinally by MRI. Compound-treated aged Wistar rats (21 months) received a continuous administration by mini-osmotic pump of formoterol (0.03 mg/kg/day) for 8 weeks and were compared to saline-treated rats. From days 1 to 56, saline-treated 9 and 21 months rats exhibited little variation in body mass. Interestingly, muscle mass loss in saline-treated 21-month rats was restricted to hindlimb muscles (−20%) whereas forelimb muscles were not affected (e.g. pectoralis). Muscle mass of Formoterol-treated 21-month rats increases gradually during the experimentation and was comparable or even greater after 8 weeks of treatment to saline-9 Mo treated rats. These findings demonstrate a putative therapeutic potential of formoterol for sarcopenia but further studies are needed in order to determine the optimum design to prevent age-related loss of muscle mass and function.

7-14

The use of formoterol in a multi-targeted anti-wasting therapy in cancer: beneficial effects upon chemotherapy

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Background and aims: Tumour growth in cancer patients is associated with weight loss resulting from both adipose and muscle wasting; this constitutes the cachexia syndrome. Moreover, the contribution of chemotherapeutic agents aimed to reduce tumour growth in the pathogenesis of cachexia remains uncharacterized.

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 and with the chemotherapeutic agent sorafenib—a multi-kinase inhibitor which targets receptor tyrosine and serine/threonine kinases involved in tumor progression and tumor angiogenesis.

Results: The results obtained clearly show that administration of the beta-2 agonist formoterol, significantly contributes to improve the anti-tumoural treatment by decreasing its adverse effects on muscle wasting. The beta-2 agonist contributed to not only improving muscle size but also in increasing physical performance.

Conclusions: Further clinical trials are needed to clarify its efficacy in the cancer patient; however, the results presented here suggest that formoterol should be an essential component for a multi-targeted approach of intervention in cancer cachexia.

7-15

Megestrol acetate treatment influences tissue amino acid uptake and incorporation during cancer cachexia

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Background and aims: Tumour growth is associated with weight loss resulting from both adipose and muscle wasting.

Methods: The aim of the present investigation was to study the effects of megestrol acetate (MA) (100 mg/kg) on the rate of leucine incorporation (¹⁴C-leucine) into muscle tissue and on the uptake of α -amino-³H-isobutyrate (AIB, a non-metabolizable analogue of alanine) in both skeletal muscle and liver tissue in cachectic tumour-bearing rats (Yoshida AH-130 ascites hepatoma).

Results: Administration of MA to tumour-bearing rats resulted in an important reversal of the muscle wasting process, as reflected by individual muscle weights. Treatment with the progestagen resulted in a significant increase in the uptake of AIB in GSN (15%), tibialis (20%) and soleus muscles (25%), together with an increased leucine incorporation into GSN (43%), tibialis (50%) and EDL (23%) muscle protein. MA treatment significantly decreased AIB uptake by the liver (25%).

Conclusions: Altogether, the data clearly show that MA treatment is able to influence tissue interorganic amino acid uptake by increasing these parameters in muscle and decreasing them in the liver, normalizing the pattern of amino acid uptake found in non-tumour bearing rats. The results presented here clearly support those previously described by our research group indicating the mechanisms involved in the anabolic action of the synthetic progestagen drug upon muscle tissue during cachexia.

7-16

Low dose formoterol combined with megestrol: a potential therapeutic use in cancer cachexia

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Background and aims: Options for treatment of cancer cachexia are very limited. Megestrol acetate, though unapproved in most countries, is sometimes used to stimulate appetite but has no significant effect on muscle mass. Formoterol fumarate has been found to limit muscle degeneration in rodent cancer models, but also has the potential detrimental effects of reducing fat mass and increasing heart weight (doses of 0.3 mg/kg/day and above) and decreasing food consumption (doses of 1 mg/kg/day and above). The aim of this study was to examine the effects of lower doses of formoterol alone and in combination with megestrol using an experimental cancer cachexia model.

Methods: Individual muscle weights, adipose weights and organ weights, along with body and carcass weights and food intake were measured in rats bearing the Yoshida AH-130 ascites hepatoma, treated with low doses of formoterol and/or megestrol over a 7 day period.

Results: Formoterol at 1 and 10 μ g/kg/day sc produced significant increases in gastrocnemius muscle weights compared with control animals. These doses of formoterol had no significant effects on heart weight or food consumption. Formoterol at 10 μ g/kg combined with megestrol at 100 mg/kg (intra-gastric administration) produced greater increases in skeletal muscle, carcass and body weights and food intake than either drug given alone. The combination had no significant effect on the carcass weight/heart weight ratio, whereas megestrol alone decreased this ratio, reflecting a relative increase in heart size.

Conclusions: Formoterol given at low, therapeutically relevant doses selectively increases skeletal muscle weight without increasing heart size or reducing food consumption. Low dose formoterol and megestrol when administered in combination have synergistic effects on some key measures of cancer cachexia. These data support the clinical development of formoterol combined with megestrol in the treatment of cancer cachexia.

8-01

Thalidomide for managing cancer cachexia: a systematic review

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Background: Cancer cachexia is an important and common clinical problem. Although reviews have examined different treatment modalities for cancer cachexia, evidence has not identified an optimum treatment. Preliminary studies of thalidomide for cancer cachexia have demonstrated encouraging results. However the safety and efficacy of thalidomide for cancer cachexia has not been systematically reviewed.

Aim: This study aims to evaluate the effectiveness of thalidomide for cancer cachexia and identify and assess any adverse effects.

Method: Detailed search strategies have been developed for electronic databases including MEDLINE, EMBASE, CINAHL, Web of Science and clinical trials registers. Hand searching will also identify additional grey literature. The results from the searches will be downloaded to a reference management database (Reference Manager) and duplicates will be removed. The remaining citations will be read by two review authors and checked for eligibility. Studies that are deemed ineligible for inclusion will have clear reasons for exclusion documented. Non-randomised studies including quasi-experimental, cohort and case control studies will be considered systematically after all eligible RCTs have been identified. Independent data extraction will be performed and all studies which meet the review inclusion criteria will be independently assessed for quality by at least two review authors. A meta-analysis will be conducted on comparative trials of adequate quality and a narrative analysis will be conducted on studies that are not amenable to quantitative synthesis.

Conclusion: This systematic review will provide clinicians and researchers with best available evidence about the use of thalidomide for the management of cancer cachexia.

8-02

Curcuma longa extract is effective in reducing blood levels of reactive oxygen species (ROS) and increasing antioxidant enzyme glutathione peroxidase (GPx) in patients with cancer-related cachexia and oxidative stress

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Background and aims: Curcumin (diferuloylmethane) is the biologically active compound of Turmeric (*Curcuma longa*) and has been used

for a long time in traditional Asian medicine to treat diseases ranging from heartburn to arthritis: it is thought to have antioxidant and antiinflammatory properties. The aim of this study was to assess the antioxidant capacities of a curcumin extract in advanced cancer patients with cachexia.

Methods: In September 2011, 10 patients (M 5/F5; age range, 55–85 years) with cancer at different sites were enrolled, all stage IV and cachectic. Twenty-one age/sex-matched healthy controls were studied. All enrolled patients had oxidative stress (high levels of ROS, low levels of blood antioxidant enzymes GPx and SOD and low levels of total blood antioxidant status, TAS, compared to controls). Patients received 6 tablets (1.2 g)/day curcumin extract (Meriva, Indena, Milan, Italy). Treatment duration was 10 days.

Results: A significant reduction of ROS levels (510±120 vs. 390±186 Fort U) and a significant improvement of circulating levels of GPx (5,630±1,189 vs. 6,590±2,390 U/l) as well as a significant improvement of TAS (0.61±0.23 vs. 0.69±0.4 mmol Trolox eq.) was observed after treatment. No patient was withdrawn from the study and the treatment was safe and well tolerated.

Conclusions: Curcumin extract at a dose of 1.2 g/day added to the normal diet was shown to be able to significantly counteract some of the key parameters of oxidative stress, such as ROS GPx and TAS, found in advanced cancer patients with cachexia. Curcumin antioxidant activity could be useful in a multi-targeted combined pharmaco-nutritional approach to treat cancer-cachexia. Further phase III clinical studies are warranted.

Table 1. Patients’ clinical characteristics

	No.
Patients enrolled	10
Female	5
Male	5
Mean age (mean±SD)	65±21
Stage of disease	
IV	10
Tumor site	
Head and neck	3
Lung	2
Ovary	1
Pancreas	1
Breast	1
Colon	1
Stomach	1
ECOG PS	
0	0
1	2
2	5
3	3

Table 2. Oxidative stress parameters in healthy controls and cancer patients before and after treatment

Parameters	Healthy controls	Cancer patients		
Baseline	After treatment	<i>p</i> Value after treatment vs. baseline		
ROS (U Fort)	332±118	510±120*	390±186	<0.001
GPx (U/l)	8,622±2,314	5,630±1,189*	6,590±2,390	<0.05
SOD (U/ml)	163.78±43.59	101±36*	112±62	n.s.
TAS (mmol Trolox eq.)	0.92±0.28	0.61±0.23*	0.79±0.4	<0.05

**p*<0.05 in comparison to healthy controls. ROS reactive oxygen species, GPx glutathione peroxidase, SOD superoxide dismutase, TAS total antioxidant status

8-03

Immunotherapy for cancer with polysaccharide of Maitake mushroom

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It is well-known that medical mushrooms contain polysaccharide which demonstrated anti-tumor activity. The specific beta-glucan, designates as the MD-Fraction, extracted from Maitake edible mushroom (*Grifola frondosa*) was indicated to have strong anti-cancer effects by activating antigen presenting cells (APCs), such as macrophage and dendritic cells, and also T cell in C3H/Hev mice in which a Th-1 dominant response was established. The study using BALB/C mice in which a Th-2 response was genetically dominant, MD-Fraction reduced the expression of Th-2 cytokine interleukin (IL)-4 but markedly increased the expression of the Th-1 cytokine interferon (IFN)-gamma in CD4+T cells and also increased IL-12p70 production as well as IFN-gamma production by APCs, suggesting that MD-Fraction promotes the differentiation into Th-1 cells of CD4+T cell through enhancement of IL-12 by APCs. Also this increasing IL-12 level could activate the cytotoxicity of Natural Killer (NK) cells. Amounts of cachexia due to cancer-bearing were also decreased. All these facts indicated by MD-Fraction, could challenge to kill the cancer cells, respectively. The clinical trial with MD-Fraction for human suffered with breast, lung, colon and pancreas cancer were done in Japan and USA. Cachexia due to cancer was decreased and also the each cancer was regressed to 40–60% when MD-Fraction was used as complementally drug with chemotherapy treatment. Especially, Memorial Sloan–Kettering Cancer Center (MSKCC, New York) as done the Phase I and II study supported by the USA government for Beat Cancer and result was already published on *Jour. Cancer Res. Clin. Oncol.*, 2009, 135 (9), 1215–1221. Daily dosage of 2 mg/kg MD-Fraction was associated with the greatest increase of CD3+25+ or CD4+CD25+T cell in the peripheral blood, and 6 mg/kg administration was indicated with the most significant increase in IL-1 production by NKT cells. MSKCC institute concluded that oral administration of MD-Fraction was associated with measurable change in peripheral blood. The dose associated with the most significant change varies by immunological parameter. The 6 mg/kg MD-Fraction is selected as the dose in the future studies with clinical endpoint.

8-04

Glutamine-enriched immunonutrition in treatment of patients with inflammatory bowel diseases

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Aims: The study effects of glutamine enriched oral nutritional supplements on system inflammatory response in patients with acute inflammatory bowel diseases.

Methods: Twenty patients with severe ulcerative colitis in acute phase were observed. All patients were treated by corticosteroids and mesalazine. In addition to drug therapy, four patients were received glutamine enriched immunonutrition in dose 10 g glutamine (500 kcal per day) during 2 weeks in addition to usual diet, five patients were received polymeric formulas for enteral nutrition in the same dose and duration, and three patients were received only drugs and usual diet. The level of pro-inflammatory cytokines in the blood was studied before and after 2 weeks of treatment.

Results: After treatment with glutamine, enriched immunonutrition were detected significant decrease of TNF-alpha (up to 25%), IL-6 (up to 50%)

and IFN-gamma (up to 20%) in the blood in comparison with patients received polymeric formulas for enteral nutrition and usual diet.

Conclusions: Glutamine-enriched immunonutrition in addition to drug therapy may be to reduce the system inflammatory response in patients with inflammatory bowel diseases.

8-05

Randomised phase III clinical trial of a combined treatment with carnitine+celecoxib±megestrol acetate for patients with cancer-related anorexia/cachexia syndrome

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Background and aims: A phase III, randomized non-inferiority study was carried out to compare a two-drug combination (including nutraceuticals, i.e. antioxidants) with carnitine+celecoxib±megestrol acetate for the treatment of cancer-related anorexia/cachexia syndrome (CACS): the primary endpoints were increase of lean body mass (LBM) and improvement of total daily physical activity. Secondary endpoint was: increase of physical performance tested by grip strength and 6-min walk test.

Methods: Sixty eligible patients were randomly assigned to: arm 1, L-carnitine 4 g/day+Celecoxib 300 mg/day or arm 2, L-carnitine 4 g/day+celecoxib 300 mg/day+megestrol acetate 320 mg/day, all orally. All patients received as basic treatment polyphenols 300 mg/day, lipoic acid 300 mg/day, carbocysteine 2.7 g/day, vitamin E, A, C. Treatment duration was 4 months. Planned sample size was 60 patients.

Results: The results did not show a significant difference between treatment arms in both primary and secondary endpoints. Analysis of changes from baseline showed that LBM (by dual-energy X-ray absorptiometry and by L3 computed tomography) increased significantly in both arms as well as physical performance assessed by 6-min walk test. Toxicity was quite negligible and comparable between arms.

Conclusions: The results of the present study showed a non-inferiority of arm 1 (two drug combination) vs. arm 2 (two drug combination+megestrol acetate). Therefore, this simple, feasible, effective, safe, low cost with favourable cost-benefit profile, two-drug approach could be suggested in the clinical practice to implement CACS treatment.

8-06

Safety and tolerability of LGD-4033, a novel non-steroidal oral Selective Androgen Receptor Modulator (SARM) in healthy men

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SARMs are tissue-selective androgen receptor (AR) ligands that are being developed to treat muscle wasting associated with cancer, and acute and chronic illness, as well as age-related muscle loss. LGD-4033 is a novel non-steroidal, orally active SARM. In animal models, LGD-4033 demonstrated anabolic activity in muscles, anti-resorptive and anabolic activity in bones, and robust selectivity for muscle versus prostate. In a randomized, double-blind, placebo-controlled phase I study, the safety and tolerability of LGD-4033 was evaluated. Healthy men (aged 21–50 years) were randomized to oral daily doses of 0.1, 0.3, or 1 mg LGD-4033 or placebo over 21 days. Safety,

including lab tests was evaluated during the treatment period and a 5-week observation period. Lean body mass (LBM) was measured using DXA scan, maximal voluntary leg press strength using 1-RM method, and physical performance using stair climb time and power. LGD-4033 was safe and well tolerated at all doses including no effects on liver enzymes, displayed a long elimination half-life of 24–36 h, and linear pharmacokinetics. There were no drug-related serious adverse events and no subject was discontinued due to an adverse event. Adverse events were mild to moderate with no statistical difference between the LGD-4033 and placebo groups. No clinically significant changes in PSA, hematocrit or ECG were seen at any dose. Testosterone, LH, SHBG and HDL levels decreased dose-dependently, consistent with an androgenic effect. LGD-4033 administration was associated with dose-dependent increases in LBM (~1.2 kg increase at 1 mg dose). Positive trends towards an increase in maximal voluntary leg press strength and in physical performance measures (stair climb power and speed) were also seen over the short treatment period. Phase II studies with 12 weeks of treatment are planned to evaluate LGD-4033 in conditions such as muscle wasting associated with cancer, acute illness, and to promote rehabilitation.

8-07

GTx-024, a selective androgen receptor modulator (SARM), improves physical function in non-small cell lung cancer (NSCLC) patients with muscle wasting

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Background: Muscle wasting in cancer patients leads to a decline in physical function. At diagnosis, >50% of lung cancer patients have substantial wasting, increasing to >80% prior to death. Data shows that NSCLC patients with muscle wasting are less likely to tolerate chemotherapy, have worse outcomes and shorter survival. This has detrimental consequences early in a patient's malignancy, underscoring the importance of diagnosing and treating this condition at an early stage. Literature shows that a 10% improvement in physical function is a substantial clinically meaningful benefit. We conducted a randomized, double-blind, placebo controlled, multi-center study to evaluate the effect of GTx-024 on muscle wasting and physical function in patients with cancer cachexia.

Methods: Subjects ($n=159$) were randomized to oral GTx-024 or placebo for 16 weeks. Subjects were males >45 years and postmenopausal females, with $\geq 2\%$ weight loss in 6 months prior to randomization and diagnosed with NSCLC, colorectal cancer, non-Hodgkin's lymphoma, chronic lymphocytic leukemia or breast cancer. The primary endpoint was change in lean body mass with QOL and physical function as secondary endpoints. For this analysis, clinical benefit is defined as 10% improvement in physical function (stair climb).

Results: One hundred three subjects (MITT) had stair climb assessed at baseline and week 16. GTx-024 subjects demonstrated clinical benefit compared to placebo ($P=0.03$). Among NSCLC subjects, 28 were included in the physical function analysis. Seventy-eight percent treated with GTx-024 responded compared to 30% treated with placebo ($P=0.02$). Physical function was positively correlated with QOL as assessed by the FAACT questionnaire further substantiating clinical benefit (Spearman correlation coefficient=0.60, $P=0.001$).

Conclusions: GTx-024 was well tolerated and showed a statistically significant and clinically relevant improvement in physical function in NSCLC subjects. Clinical benefit in stair climb power correlated with a clinically relevant improvement in quality of life. These data provide evidence that GTx-024 may play an important role in the management of patients with NSCLC by improving physical function and quality of life.

8-08

In a phase IIb trial GTx-024 overcomes the negative impact of >8% weight loss on overall survival in non-small cell lung cancer (NSCLC) subjects

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Background: Non-small cell lung cancer (NSCLC) represents >80% of newly diagnosed lung cancer. At diagnosis, >50% of lung cancer patients have substantial weight loss, increasing to >80% prior to death. Much of this weight loss is attributed to muscle wasting leading to a decline in physical function and other detrimental effects. Studies show that NSCLC patients with wasting at diagnosis are less likely to tolerate chemotherapy, have worse outcomes and shorter survival. The negative impact of wasting underscores the importance of preventing and treating this condition early. We conducted a phase IIb, randomized, double-blind, placebo-controlled, multi-center study to evaluate the effect of GTx-024 on muscle wasting and physical function in patients with cancer.

Methods: Subjects ($n=159$) were randomized to oral GTx-024 or placebo for 16 weeks. Subjects were males >45 years and postmenopausal females, had experienced $\geq 2\%$ weight loss in the 6 months prior to randomization, and NSCLC, colorectal cancer, non-Hodgkin's lymphoma, chronic lymphocytic leukemia or breast cancer. The primary endpoint was change in total lean body mass. We report on overall survival in the entire study population and NSCLC cohort based on weight loss of > or $\leq 8\%$ in the 6 months prior to randomization.

Results: In placebo subjects (ITT), overall survival was significantly ($P=0.003$, log rank) reduced in subjects with >8% weight loss compared to subjects with $\leq 8\%$ weight loss. Among NSCLC subjects ($n=61$) placebo subjects with >8% weight loss demonstrated a similar survival disadvantage ($P=0.04$); 4 month Kaplan–Meier estimates 100% vs. 49% \pm 14.8%. In GTx-024-treated subjects in both the ITT and NSCLC groups, baseline weight loss did not negatively affect survival.

Conclusions: Preceding weight loss among NSCLC patients not treated with GTx-024 is predictive of decreased survival. NSCLC subjects randomized to placebo with >8% weight loss at baseline were two times more likely to die than subjects with $\leq 8\%$ weight loss. In the GTx-024 group baseline weight loss was not predictive of survival. These data suggest that GTx-024 may overcome the negative prognostic effect of >8% weight loss. Further research is needed to assess the effect of GTx-024 on overall survival.

8-09

Phase I/II trial of formoterol fumarate combined with megestrol acetate in patients with advanced malignancy

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Background and aims: Cancer cachexia is a debilitating condition with a major impact on quality of life. We tested a novel combination of an anabolic beta2-agonist and an appetite stimulant in cachectic cancer patients.

Methods: Eleven patients to date (M/F, 3:8) with an advanced solid tumour and involuntary weight loss have received oral formoterol fumarate

(FF: 80 µg/day) and megestrol acetate (MA: 480 mg/day) for up to 8 weeks. We measured quadriceps size (MRI) and strength (dynamometry), lower limb extensor power (Nottingham Power Rig) and physical activity (accelerometry). Protocol defined response criteria were ‘non-response’ (any decline); ‘minor response’ (0–2% increase (size) and 0–5% increase (function)); ‘moderate response’ (>2% and <4% (size) and >5% and <10% (function)) and ‘major response’ (≥4% (size) and ≥10% (function)).

Results: Of the 11 patients, six did not reach 8 weeks due to disease progression. Few adverse events were considered probably or possibly related to the study drugs, the commonest being mild tremor (seven reports), peripheral oedema (three), tachycardia (two) and heartburn/indigestion (two). Of the five patients who completed 8 weeks, 5/5 had a muscle size response (four major, one moderate) and 4/5 had a muscle function response (three major, one moderate). Physical activity markedly improved (average daily step count >1,000) after 8 weeks in two patients. In those reaching 8 weeks ($n=5$), mean quadriceps volume increased significantly (R leg 0.94 versus 0.98 l, $p=0.009$; L leg 0.91 versus 0.97 l, $p=0.04$). Increases in mean values for the other variables over the treatment period did not achieve statistical significance.

Conclusions: In this frail cohort with advanced cancer and cachexia, MA and FF in combination are well tolerated and appear to have a beneficial effect on muscle size. Muscle function and physical activity are improved in a proportion of individuals. This warrants further investigation in larger, randomised trials.

Acknowledgements: We thank Claire Lamb and staff of the Clinical Research Facility, Royal Infirmary Edinburgh

8-10

The feasibility and acceptability of Neuromuscular Electrical Stimulation (NMES) in patients with advanced cancer

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Background: The McGill Cancer Nutrition–Rehabilitation Program at the Jewish General Hospital (CNR-JGH) addresses weight loss, nutritional symptoms and impaired functional status in ambulatory patients with advanced cancer. Exercise training is a central component of the intervention plan for many patients. However, regular attendance for hospital-based exercise training is difficult for patients with poor performance status, or those that live far from the hospital. The objective of this study was to assess the feasibility and acceptability of neuromuscular electrical stimulation (NMES) to facilitate improvements in physical functioning in this patient population.

Methods: Patients who were unable to attend regular exercise training were recruited from the CNR-JGH clinic. All assessment, instruction and follow-up were performed by the study physiotherapist. Participants were asked to use NMES at home on both quadricepses daily for 6 weeks (30 min, 200 ms pulses, 50–100 Hz, with individually adjusted stimulation intensity, simultaneous active contraction). Compliance was measured using a patient-completed diary. Acceptability and feasibility were assessed using compliance scores and questionnaire. In addition, physical functioning was assessed at the start and end of the study using performance status (PS), repeated sit-to-stand (STS), 6-min walk test (6MWT) and SOBOE (Borg).

Results: Fifteen participants were recruited (9 (60%) male, mean (SD) age 68 (9) years) of whom 13 (87%) had PS 2–3. 5 (33%) patients did not complete the study, four of these were for medical reasons and only one patient dropped out because of discomfort using NMES. For patients completing treatment, mean compliance was 57%, and NMES was well-received with patients reporting 6 weeks’ treatment acceptable (mean score 3.9/5) and helpful (mean score 4/5). Assessment of physical functioning

also showed a general trend to improvement after NMES: PS (0.4, $p=0.03$), STS (1.2 s), and breathlessness (1/10).

Conclusions: Home NMES is a feasible and acceptable exercise intervention with potential beneficial functional impact even in advanced cancer patients.

8-11

Blocking interleukin-6 signal ameliorates inflammatory manifestations and laboratories of cachexia in a patient with malignant mesothelioma: a case study

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Background: Interleukin-6 (IL-6) is pathologically involved in cancer cachexia especially in IL-6 producing cancers including malignant mesothelioma (MM). Tocilizumab (TCZ) is a humanized anti-human IL-6 receptor (IL-6R) antibody that specifically blocks IL-6 signaling. In this study, we treated a patient with MM utilizing TCZ and changes in the systemic manifestations and laboratories of cachexia were examined.

Methods: A 76-year-old man with recurrent malignant pleural MM refractory to combination chemotherapy and radiation therapy was treated with TCZ (8 mg/kg body weight every 2 weeks) after obtaining approval of ethics committee and patient’s informed consent. Cancer associated manifestations were monitored including Eastern Cooperative Oncology Group performance status (ECOG PS) scale and SF-36. Laboratories including platelet counts (PLT), hemoglobin (Hb), CRP, albumin, rapid turnover proteins such as prealbumin and retinol binding protein (RBP), cholesterol, leptin, ghrelin, and vascular endothelial growth factor (VEGF), as possible IL-6 dependent markers of cachexia, were measured before and after TCZ treatment.

Results: During the TCZ treatment, low grade fever disappeared and ECOG PS scale and patient’s assessment of fatigue using visual analogue scale significantly improved, but SF-36 and face scale did not change. All the laboratories improved within 2 weeks as shown in the table. Furthermore, VEGF, which induces pleural effusions of MM and angiogenesis required for the tumor growth, was also decreased into normal. Although general status was considerably ameliorated, the patient suffered from radiation pneumonitis caused by the previous treatment and TCZ was discontinued, thereafter the tumor grew rapidly.

Parameter	Unit	Baseline	Week 1	Week 2
		1st TCZ	2nd TCZ	2nd TCZ
Hb	g/dL	9.4	10.4	11.1
PLT	$\times 10^4/\mu\text{L}$	38.7	40.4	23.7
CRP	mg/dL	6.43	0.34	0.65
ALB	g/dL	3	3.6	3.6
TCHO	mg/dL	151	214	191
VEGF	pg/mL	108	67	44
Prealbumin	mg/dL	8.9	26.1	21.4
RBP	mg/dL	1.6	3.6	3.2
Leptin	ng/mL	1.7	1.9	1.9
Active ghrelin	fmol/mL	33	21	15
Desacyl ghrelin	fmol/mL	161	114	100

Conclusions: Blocking IL-6 action with TCZ ameliorates the inflammatory manifestations and laboratory markers of cachexia in a patient with IL-6 producing MM, confirming that the important roles of IL-6 in MM associated cachexia. Further studies will be required to know therapy targeting IL-6 indeed can improve cancer cachexia.

8-12

Phase I/II study of IP-1510 a novel interleukin-1 receptor antagonist in the management of cancer-related cachexia

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Background and objective: IP-1510 is a new synthetic peptide interleukin 1 receptor antagonist. Rat toxicity and tumor burden studies in rodents with IP-1510 showed that this peptide had limited toxicity and suggestive effectiveness for cachexia due to cancer. The objective of this study was to determine whether there was any obvious toxicity in advanced cancer patients with IP-1510 and whether any improvements were seen on their appetite depression, performance status, and quality of life.

Methods: Eligible patients received 1 mg of IP-1510 in subcutaneous bidaily injections. Patients underwent biweekly evaluations during the 28-day treatment. Evaluations included full blood examination, physical check, Kamofsky performance status, and Edmonton Symptoms Assessment Scale (ESAS) and grip strength.

Results: Of 26 enrolled patients, 20 completed the course. Eighteen patients chose to continue with IP-1510 treatment for another month. Seventeen patients reduced the dose and frequency of their breakthrough pain medication throughout the month. Subcutaneous administration of IP-1510 at 1 mg bidaily was well tolerated with no serious side effects reported. Weight stabilisation or gain was observed in 17 of the patients. Appetite ($P \leq 0.01$) and depression ($P \leq 0.01$) scores improved on ESAS. Performance scores also improved significantly on Karnofsky scale ($P \leq 0.01$).

Conclusions: IP-1510 was well tolerated and safe in advanced cancer patients. IP-1510 in the dosages reported mediated statistically significant improvements in anorexia, physical performance, and depression in this trial. Further larger trials are to be initiated on cachexia due to cancer and COPD.

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