High-fat diet and obesity exacerbate muscle wasting in experimental cancer cachexia
Andrea Bonetto1, Chan Ho Lam2, Rui Zhan1, Felipe E. Pedroso1, Leonidas G. Koniaris*, Teresa A. Zimmers1,2
1Department of Cancer Biology, 2Department of Surgery, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA, USA;
3Department of Surgery, University of Miami Miller School of Medicine, Miami, FL, USA

Obesity and high-fat diet (HFD) are risk factors for multiple types of cancer. Compelling evidence indicates that obesity and diet also play a role after the diagnosis of cancer, influencing treatment, tumor progression, overall well-being and survival. Previously we reported that obesity is associated with increased overall survival in lung cancer patients. Others have shown that HFD was protective in murine MAC16 cachexia. Based on those data and similar obesity risk paradox observations in other diseases, we posited that obesity or HFD might provide increased physiological reserve and slow cachexia in cancer. Here we sought to determine whether diet induced obesity (DIO) or HFD were protective in the Lewis Lung carcinoma mouse model of cancer cachexia. DIO obese and C57Bl/6J lean mice were fed a HFD (60 %kcal from fat), while another lean group was fed normal chow (LFD) (10 %kcal from fat). Mice were inoculated with tumor cells and euthanized 17 and 23 days later. Both obese and lean tumor-bearing mice fed HFD showed increased loss of total body mass, skeletal muscle and fat mass compared with tumor-bearing mice fed LFD. This increased muscle wasting in DIO and HFD mice was associated with greatly reduced plasma insulin and adiponectin levels and increased levels of pro-inflammatory cytokines IL-6 and LIF versus LFD tumor-bearing controls. In DIO mice, plasma growth factor/cytokine changes corresponded to decreased muscle pAKT and pFOXO3a, and increased pSTAT3 levels, while pSMAD2 and NF-kB levels were unchanged. Taken together, these changes would inhibit anabolism, promote catabolism and drive the inflammatory phenotype, thus contributing to enhanced muscle wasting. In conclusion, our data suggest that both obesity, as a pre-existing condition, and high-fat diet consumption result in worsening of muscle wasting induced by tumor. Furthermore, neither increased body mass nor HFD were protective in experimental cancer cachexia.

Objectives: Although cachexia has been defined as >5 % weight loss, limited data exists on prevention and treatment of muscle wasting prior to becoming cachectic. Cancer-induced muscle wasting begins early resulting in decline in physical function and other detrimental consequences.

Methods: We conducted a randomized, double-blind, placebo-controlled study to evaluate enobosarm’s effect on physical function and muscle wasting. Subjects (n=159) received enobosarm or placebo for 16 weeks. Subjects were males >45 y and postmenopausal females, with ≥2 % weight loss in the past 6 months and NSCLC, CRC, CLL, non-Hodgkin lymphoma or breast cancer. We report on changes in physical function with weight loss of <5 % in the 6 months prior to randomization.

Results: 103 subjects (MITT) had physical function (stair climb) assessed at baseline and week 16 with 24 % losing <5 % weight in previous 6 months. Distribution of weight loss was similar across genders, however subjects with <5 % weight loss were more likely ECOG = 0 (<5 %: 46.2 %; ≥5 %: 35.8 %). Subjects with ≥5 % weight loss had worse physical function at baseline compared to those with <5 % loss. Significant improvement in physical function was observed in enobosarm subjects regardless of baseline weight loss (<5 %, P=0.002, ≥5 %, P<0.001) while placebo subjects failed to improve.

Conclusions: Enobosarm was well tolerated and showed statistically significant improvement in physical function regardless of baseline weight loss. This provides evidence that enobosarm may play an important role in the management of cancer patients by treating and preventing decline in physical function and muscle wasting before a patient becomes cachectic.

Hypothalamic gene expression of appetite regulators in a hyperphagic cancer cachectic mouse model
Jvalini T. Dwarkasing1, Miriam van Dijk2, Francina J. Dijk2, Mark V. Boekschoten4, A. Visscher1, Josep M. Argilés4, Alessandro Laviano5, Renger F. Witkamp1, Klaske van Norren1,2
1Nutrition and Pharmacology Group, Division of Human Nutrition, Wageningen University, 2Nutricia Advanced Medical Nutrition, Danone Research, Centre for Specialised Nutrition, 3Metabolism and Genomics Group, Division of Human Nutrition, Wageningen University, Wageningen, Netherlands, 4Cancer Research Group, Departamento de Biopatología e Biología Molecular, University of Barcelona, Barcelona, Spain, 5Department of Clinical Medicine, Sapienza University, Rome, Italy

Appetite regulation is frequently disturbed in cancer patients, often leading to anorexia. It is difficult to understand processes important in
the development of anorexia, because anorexia is often accompanied by cachexia. In this study, we report on hypothalamic gene expression profile of a cancer cachectic model with increased food intake. In this model, appetite regulating systems, that fail in anorexia, are able to adapt properly to changes in energy balance. By comparing these findings to changes in anorectic cachetic mice, we can distinguish between common mediators involved in cachexia and processes specifically important for cancer-induced eating disorders

**Methods:** 6w old male CDF1 mice were inoculated subcutaneously with C26 colon adenocarcinoma cells and included 2 groups: C (n=6): sham and TB (n=9): 1×10⁶ cells. Food intake and body weight were measured 3 times/week. Day 20, hypothalamus was dissected and RNA was isolated. For genomic analysis Affymetrix chips were used. Data were analysed using IPA (Ingenuity® Systems)

**Results:** Carcass and muscle weight decreased in tumour-bearing (TB) mice (p<0.05). Food intake increased by 40 % in TB mice after day 15. Hypothalamic serotonin (5HT) signalling pathway was changed in TB (p<0.05 after pathway analysis). Expression of genes involved in 5HT synthesis, 5HT degradation, and 5HT synaptic secretion and re-uptake were down-regulated, suggesting that serotonin levels in brain are decreased in TB mice with increased food intake. In contrast, plasma TRP/BCAA ratio was increased by 144 % in TB. Expression of the orexigenic genes NPY and Agrp increased, whereas expression of anorexigenic CCK and GLP1 decreased in TB mice. PomC expression was not changed. Top 20 of up-regulated genes consisted of inflammatory genes such as lipocalin2, leucin-rich2-glycoprotein1 and oncostatin M receptor and food intake mediators such as growth hormone, oxytocin and Agrp.

**Conclusion:** Genomic analysis of the hypothalamus of C26-TB mice with increased food intake showed changes in NPY, Agrp and serotonin signaling. These changes in appetite-regulating systems are likely to explain compensatory eating behaviour of mice bearing C-26 tumour. Targeting these systems is a promising strategy to avoid the development of cancer-induced anorexia.

**Effect of ghrelin agonists on muscle mass and function: synergism with exercise**

*D. Fuoco, R. Kilgour, Antonio Vigano*

**McGill Nutrition and Performance Laboratory, McGill University Health Centre, Montreal, Canada**

Following a search of the existing patents on the market, we identified 16 pro-anabolic drugs including ghrelin agonists. We have reviewed the central and peripheral mechanisms of ghrelin and we are proposing a possible synergistic coupling with exercise, which could potentially enhance and maximize the physiological effects of these agonists. The initial physiological mechanism that controls muscle anabolism following the activation of the ghrelin receptor in the CNS involves the release of growth hormone/insulin-like growth factor-1 (GH/IGF-1). IGF-1 is an important anabolic hormone that has a specific receptor on the muscle membrane responsible for cell growth. However, clinical experience suggests that IGF-1 alone is not sufficient to induce significant muscle hypertrophy, but that the permissive effect of regular physical exercise can potentiate the anabolic effect of this protein. The stimulus of dynamic muscle contraction induces the secretion of muscle-restricted IGF-1 (mIGF-1) that enhances protein synthesis, increases lean body mass, and eventually leads to the improvement of muscle strength. Physical exercise also exerts a positive feedback on the hypothalamus-hippocampus circuit. More specifically, the effects of ghrelin agonists have been shown to create a crosstalk between the activated growth hormone secretagogue receptor type 1a (GHSR-1a) membrane receptor in pituitary neurons and the regulation of Neuropeptide Y (NPY) gene expression. NPY is considered to be among the most important hormones in the central regulation of energy balance. NPY is secreted by the hypothalamus and, in addition to increasing food intake, it increases the proportion of energy stored as fat and blocks nociceptive signals to the brain. NPY acts as an antagonist of the corticosterone effect in the hippocampus, promoting central adaptation to an environmental stress. The synergy between ghrelin agonists and physical exercise may act together to potentiate the release of endorphins and endocannabinoids, both of which tend to decrease the sensation of fatigue.

**Enteral nutrition improves micronutrient status but fails to improve protein intake in patients with cancers of the head and neck**

*Kaitlin Giles1, Catherine Kubrak2, Vickie Baracos1,3, Karin Olson4, Vera Mazaruk1*

1Alberta Institute for Human Nutrition, Faculty of Agriculture, Life and Environmental Sciences, University of Alberta, Edmonton, Alberta; 2Department of Surgery (Thoracics), Alberta Health Services, Edmonton, Alberta; 3Dept. Oncology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta; 4Faculty of Nursing, University of Alberta, Edmonton, Alberta

**Background:** Patients with cancers of the head and neck (HNC) are at high risk for malnutrition and may be deficient in several nutrients simultaneously.

**Objective:** To compare intakes of folate, zinc, vitamins D and E, and protein in HNC patients consuming self-selected foods. Intakes at various points in the disease trajectory were compared to ESPEN guidelines (ESPEN-g) for cancer patients.

**Methods:** HNC (n=38) patients undergoing RT had weight, BMI, protein, folate, zinc, vitamins D and E prospectively evaluated at diagnosis, after 6 weeks of RT (treatment), and 2.5 months post-RT (follow-up). Intakes of folate, zinc, vitamins D and E, and protein were compared to ESPEN-g. Nutrient intakes in patients consuming ≥15 % daily caloric intake from enteral nutrition (EN) (n=30) were compared to those not consuming EN (n=8).

**Results:** At baseline, >80 % of all patients consumed below ESPEN-g for folate, and vitamins D and E, 50 % of patients were below ESPEN-g for zinc, and 46 % of all patients consumed protein below ESPEN-g. Protein intake decreased from a mean of 1.3 g/kg/day at baseline to 0.92 g/kg/day during treatment but was restored to 1.51 g/kg/day post-treatment. Throughout the study, folate and vitamin D intakes were considerably low, with over 60 % of all patients well below ESPEN-g. During treatment, patients consuming ≥15 % energy from EN increased their intake of folate (4.3 %), vitamin D (30.1 %), vitamin E (88.4 %), biotin (308.5 %), and zinc (2.2 %) from baseline. Despite improving micronutrient intake during treatment, patients on EN consumed 34 % less protein (0.82 g/kg/day) compared to patients not taking EN (1.24 g/kg/day).

**Conclusion:** Dietary interventions in HNC patients must consider the complete range of deficiencies observed throughout treatment. EN may not be adequate in meeting the nutritional needs of this population, most notably in protein intake.

**Protein and energy intake at recommended levels does not prevent weight loss in head and neck cancer patients**

*Kaitlin Giles1, Catherine Kubrak2, Vickie Baracos1,3, Karin Olson4, Vera Mazaruk1*

1Alberta Institute for Human Nutrition, Faculty of Agriculture, Life and Environmental Sciences, University of Alberta, Edmonton, Alberta, Canada; 2Department of Surgery (Thoracics), Alberta Health Services, Edmonton, Alberta, Canada; 3Dept. Oncology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta, Canada; 4Faculty of Nursing, University of Alberta, Edmonton, Alberta, Canada

**Objective:** To compare intakes of folate, zinc, vitamins D and E, and protein in HNC patients consuming self-selected foods. Intakes at various points in the disease trajectory were compared to ESPEN guidelines (ESPEN-g) for cancer patients.

**Methods:** HNC (n=38) patients undergoing RT had weight, BMI, protein, folate, zinc, vitamins D and E prospectively evaluated at diagnosis, after 6 weeks of RT (treatment), and 2.5 months post-RT (follow-up). Intakes of folate, zinc, vitamins D and E, and protein were compared to ESPEN-g. Nutrient intakes in patients consuming ≥15 % daily caloric intake from enteral nutrition (EN) (n=30) were compared to those not consuming EN (n=8).

**Results:** At baseline, >80 % of all patients consumed below ESPEN-g for folate, and vitamins D and E, 50 % of patients were below ESPEN-g for zinc, and 46 % of all patients consumed protein below ESPEN-g. Protein intake decreased from a mean of 1.3 g/kg/day at baseline to 0.92 g/kg/day during treatment but was restored to 1.51 g/kg/day post-treatment. Throughout the study, folate and vitamin D intakes were considerably low, with over 60 % of all patients well below ESPEN-g. During treatment, patients consuming ≥15 % energy from EN increased their intake of folate (4.3 %), vitamin D (30.1 %), vitamin E (88.4 %), biotin (308.5 %), and zinc (2.2 %) from baseline. Despite improving micronutrient intake during treatment, patients on EN consumed 34 % less protein (0.82 g/kg/day) compared to patients not taking EN (1.24 g/kg/day).

**Conclusion:** Dietary interventions in HNC patients must consider the complete range of deficiencies observed throughout treatment. EN may not be adequate in meeting the nutritional needs of this population, most notably in protein intake.
Background: Patients with cancers of the head and neck (HNC) are at high risk for malnutrition. Information regarding the extent to which these patients meet protein and energy recommendations at various stages of the cancer trajectory is limited.

Objective: To relate energy and protein intakes at diagnosis, during and after radiotherapy treatment (RT) to weight loss in orally-fed HNC patients.

Methods: HNC patients (n=38) undergoing RT were prospectively evaluated and completed 3-day food records at diagnosis, after 6 weeks of RT, and at post-RT (follow-up). At each time point, body weight and BMI were recorded; energy and protein intakes were calculated and compared to the ESPEN guidelines of 30–35 kcal/kg/day and 1.2–2 g protein/kg/day.

Results: The majority of patients lost >10 % of total body weight (range 0.5–25 %) from diagnosis to follow-up. At diagnosis, patients consumed an average of 1.3 g protein/kg/day, declining to 0.9 g protein/kg during treatment and improving at follow-up to 1.5 g protein/kg. Mean energy intakes fell from 30 kcal/kg/day at diagnosis to 23 kcal/kg/day (3.7–84.2 kcal/kg/day) during treatment, and increasing to 30 kcal/kg/day at follow-up. Mean weight loss of patients with protein intakes ≤1.2 g protein/kg/day at baseline (45 %) through treatment was 11 kg (range 0.6–24.5 kg). Although 32 % of patients met or exceeded energy intakes during treatment, 75 % of these patients experienced a mean weight loss of 10 kg (0.6–24.5 kg). Ninety percent of patients who met or exceeded the minimum 1.2 g/kg/day recommended protein intake during treatment (32 %) lost weight. Despite a restoration of protein and energy intake following treatment, patients continued to lose weight.

Conclusion: Consumption of calories and protein at recommended levels does not prevent weight loss in HNC. Evaluation of guidelines for nutritional support in HNC would be valuable.

Cancer cachexia: Evaluation of body composition using CT scans and identification of genetic markers

Neil Johns1, Benjamin H. L. Tan1, Nathan A. Stephens1, Tora S. Solheim2, Sambasivarao Damara1,3, James A. Ross1, Stein Kaasa1, Vickie E. Baracos4, Frank Skorpen1, Kenneth C. H. Fearon1 European Palliative Care Research Collaborative

1University of Edinburgh, Clinical and Surgical Sciences (Surgery), Royal Infirmary, Edinburgh, UK; 2Faculty of Medicine, Clinical Department of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology (NTNU), Trondheim, Norway; 3Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta, Canada; 4Department of Oncology (Division of Palliative Care Medicine), University of Alberta, Edmonton, Alberta, Canada; 5Faculty of Medicine, Department of Laboratory Medicine, Children’s and Women’s Health, Norwegian University of Science and Technology (NTNU), Trondheim, Norway

Aim: To utilise a candidate gene approach to study the potential association between host genome and the presence of cachexia defined by sarcopenia and weight loss in cancer patients.

Methods: Based on a systematic review, 129 SNPs in 80 genes were analysed for an association with cachexia in 222 patients recruited at first presentation to a surgical or oncology clinic. Diagnostic CT scans were used to define sarcopenia by using a thoracic (T4) or lumbar (L3) vertebral marking to derive a skeletal muscle index. Percentage weight loss was calculated from weight measured at recruitment and pre-morbid weight recalled. Association testing was adjusted for age, sex, tumour type, BMI and stage. Unconditional logistic regression was used to calculate odds ratios (OR) and their 95 % confidence intervals (95 % CI) for the minor allele of individual SNPs and its association with the proposed cachexia phenotype. The study had 80 % power to detect an odds ratio of 1.5 in SNPs with a minor allele frequency (MAF) of >20 %.

Results: To account for multiple testing, permutation testing was performed. 5 SNPs had significant associations with the defined cachexia phenotype (table 1). 3 SNPs provided a reduced risk of acquiring the cachexia phenotype, 2 SNPs were associated with an increased risk (table 1).

Conclusions: This preliminary study provides some significant SNP associations with a robust phenotype of cancer cachexia based on CT analysis. Of the two most significant SNPs found firstly, rs4280262 in the LITAF gene is a missense mutation which may lead to a change in function in regulating transcription of specific genes involved in cancer cachexia. Secondly, the C allele of the SNP rs10636 in the MT2A gene is associated with alteration in the homeostasis of intracellular zinc, a critical component of an anti-oxidant system against cellular damage which may lead to a predisposition to develop cancer cachexia.

Table 1

| CHR | Gene | SNP     | Risk allele | Population frequency | Odds ratio | 95 % CI   | Permutated P value |
|-----|------|---------|-------------|----------------------|------------|----------|-------------------|
| 16  | LITAF| rs4280262| G           | 82 %                 | 0.4028     | 0.3946–   | 0.003787          |
| 16  | MT2A | rs10636  | C           | 25 %                 | 2.019      | 1.98–     | 0.01982           |
| 1   | VCAM1| rs3176860| G           | 43 %                 | 1.813      | 1.783–    | 0.02778           |
| 18  | LPIN2| rs3745012| T           | 64 %                 | 0.5013     | 0.4917–   | 0.4040            |
| 4   | TLR2 | rs3804099| C           | 43 %                 | 0.5693     | 0.5592–   | 0.04372           |

Ubiquitin-proteasome system is not enhanced in skeletal muscle of advanced non-small cell lung cancer patients with cachexia

Andrew J. Murton1, Matthew Maddocks2, Kanagaraj Marimuthu1, R. England1, Andrew Wilcock2

1The School of Biomedical Sciences, The University of Nottingham, Queen’s Medical Centre, Nottingham, NG7 2UH, United Kingdom; 2Department of Palliative Medicine, The University of Nottingham, Nottingham University Hospitals NHS Trust, Nottingham, NG5 1PB, United Kingdom

Introduction: The unintended loss of skeletal muscle is common in patients with advanced non-small cell lung cancer (NSCLC), and this contributes to the high mortality and morbidity of this group. Experimental models of cancer cachexia suggest that enhanced ubiquitin-proteasome (UP) mediated protein breakdown is predominantly responsible for the loss of muscle mass. This has yet to be observed in NSCLC patients, but only those in a pre-cachectic state have been included in studies performed to date. Thus, UP-mediated proteolysis may still be important in later stages once cachexia is evident.

Methods: To test this, 4 NSCLC patients with locally advanced or metastatic disease meeting recommended criteria for cancer cachexia were recruited along with 4 age, sex and smoking-history matched control subjects. Following an overnight fast, a biopsy was taken from the vastus lateralis muscle to assess TNFα, IL-6, MAFbx and MuRF1 mRNA levels by qPCR; protein levels of MAFbx, MuRF1 and proteasome subunits α1-3, 5-7 (PSMA1-3, 5-7) by western blot and chymotrypsin-like activity of the proteasome. A blood sample was obtained for assessment of TNFα and IL-6 by ELISA.

Results: For the NSCLC patients, mean weight loss in the prior 6 months was 9±2 % and lumbar muscle cross-sectional area was 36±3 cm²/m² (assessed by CT at L3), meeting the criteria for sarcopenia. Plasma circulating levels and muscle mRNA levels for IL-6 were significantly higher in NSCLC patients (9-fold and 2.4-fold compared to controls respectively; P<0.05) along with a trend towards increased TNFα plasma levels (1.8-fold; P=0.07). No differences were observed between subject groups for MAFbx and MuRF1 mRNA, MuRF1 and PSMA1-3, 5-7 protein levels, and proteasome activity, while a 3-fold lower MAFbx protein level was observed in NSCLC patients (P<0.05).
Conclusion: UP-mediated proteolysis is not enhanced in cachetic patients with NSCLC in the fasted (post-absorptive) state.

The effect of exercise training on anabolic resistance in IL-6-induced cancer cachexia
Melissa J. Puppa, James P. White, Song Gao, and James A. Carson
Integrative Muscle Biology Laboratory, Exercise Science Department, University of South Carolina, Columbia SC, USA

Skeletal muscle catabolic signaling has a well-established function in the disruption of protein turnover during cancer cachexia; while the regulation of anabolic suppression warrants further investigation. ApcMin/+ mice undergo IL-6 dependent cachexia, and systemic IL-6r antibody administration ameliorates cachexia without improving muscle protein synthesis. Treadmill exercise can prevent IL-6 induced cachexia in ApcMin/+ mice, but it is not certain if skeletal muscle anabolic signaling is enhanced. The purpose of this study was to determine if cachectic skeletal muscle maintained anabolic plasticity related to mTOR signaling in response to insulin activation and how IL-6/STAT3 signaling would affect this plasticity. We also determined if treadmill exercise training would improve muscle mTOR signaling in ApcMin/+ mice. ApcMin/+ mouse muscle mTOR signaling was examined in response to either glucose administration or varying levels of systemic IL-6 over-expression. IL-6 induced STAT3 signaling was examined C2C12 myotubes with or without insulin stimulation. The effect of treadmill exercise (EX;18 m/min, 1 h, 6 days/week, 5 % grade) on IL-6 induced suppression of muscle mTOR signaling was examined in ApcMin/+ mice. Cachexia reduced both basal mTOR signaling, and mTOR responsiveness to glucose administration. ApcMin/+ mice systematically over-expressing IL-6 showed a dose dependent suppression of mTOR signaling, that was rescued by treadmill exercise training independent of muscle STAT3 activation. IL-6 treated myotubes demonstrated suppressed mTOR signaling and increased catabolic signaling, which included FoxO3a and AMPK activation. The IL-6 induced suppression of mTOR and activation of AMPK in myotubes was independent of STAT3 signaling. Insulin stimulated mTOR activity in myotubes was not affected by IL-6 treatment. Besides a dose dependent suppression of muscle mTOR activity by IL-6, our data demonstrates that cachetic muscle has a reduction in mTOR responsiveness to glucose stimulation. Although IL-6 can directly inhibit mTOR signaling in myotubes, STAT3 activation does not appear necessary for this inhibition. Lastly, treadmill exercise training can block IL-6 induced suppression of muscle mTOR signaling ApcMin/+ mice.

The psychological and social consequences of cachexia in patients with advanced cancer: a systematic review
Joanne Reid, Olinda Santin, Sam Porter
School of Nursing and Midwifery, Queen’s University Belfast, Northern Ireland

Introduction: Cachexia affects up to 80 % of terminally ill cancer patients. Cachectic patients struggle with poor appetite and severe weight loss and this has a holistic negative impact across biological, psychological and social domains.

Aim: The aim of this systematic literature review is to describe the psychological and social consequences of cachexia for patients with advanced cancer.

Methods: Databases including Medline, Pub Med and Psych info were searched systematically from inception to identify studies that have examined the psychosocial impact of cancer cachexia.

Findings: From 103 abstracts, 11 studies were eligible for review. A number of key themes were identified that were described as having a psychological or social impact on cachexic patients. The psychological impact of cancer cachexia included distress, anxiety, depression and body image concerns. The main social themes identified were social exclusion and conflict over food. The inability to eat and the associated weight loss often causes conflicts within relationships which can result in the patient socially excluding themselves. As a result of this conflict patients feel forced to eat or feel that they must force themselves to eat. Research conducted which alludes to the psychosocial implications of cancer cachexia, highlights that patients want more appropriate supportive health care interventions in relation to this syndrome and its impact.

Conclusions: Cancer cachexia has severe psychosocial implications for patients. There appears to be a mismatch between the issues reported by patients and the available supports to meet these needs. There is a need for appropriate supportive health care interventions to be developed to help patients manage their psychosocial concerns.

Prevalence and Impact of Hypogonadism in Cancer Patients with Muscle Wasting in a Phase Ib Enobosarm Trial
Mitchell S. Steiner; Mary A. Johnston; Michael L. Hancock; James T. Dalton
GTx, Inc.

Objectives: Hypogonadism is associated with weight loss and poor outcomes in cancer patients. Up to 50 % of males with advanced cancer are hypogonadal at presentation or during treatment. Wasting in cancer patients is also associated with decline in physical function and performance status. We conducted a randomized, double-blind, placebo-controlled study to evaluate enobosarm’s effect on muscle wasting and physical function.

Methods: Patients (n=159) were randomized to enobosarm or placebo for 16 weeks. Patients were males >45 y and postmenopausal females, had ≥2 % weight loss in previous 6 months and NSCLC, CRC, CLL, non-Hodgkin’s lymphoma, or breast cancer. We report on the incidence and impact of hypogonadism (T<300 ng/dL).

Results: Baseline testosterone levels were available for 93/103 men. 60 % of males were hypogonadal at randomization. Distribution of hypogonadism was similar across cancers; however hypogonadal men were less likely to complete the study. Baseline T was correlated with weight loss (r=0.32, P=0.002) with hypogonadal men demonstrating greater loss in previous 6 months (median, −9.5 %). Baseline physical function (stair climb power) was higher among eugonadal versus hypogonadal males (174 W vs. 147 W; P=0.02). Enobosarm significantly improved physical function regardless of baseline gonadal status (hypogonadal:17 %, P=0.006; eugonadal:12 %, P=0.044).

Conclusions: Hypogonadism is common in male cancer patients and is correlated with weight loss and diminished physical function. In this trial, enobosarm improved physical function in hypogonadal and eugonadal men despite poorer baseline physical function in hypogonadal patients. This provides evidence that enobosarm may play an important role in the management of cancer-related muscle wasting.

Peripheral skeletal muscle: characterization of its regenerative potential in patients with chronic obstructive pulmonary disease
Marie-Eve Thériault1, Bruno B. Lemire1, Marte-Eve Paré1, François Maltais1, Richard Debégère1
1 Centre de recherche Institut universitaire de cardiologie et de pneumologie de Québec, Université Laval, GIV 4G5, Canada

Rationales: Chronic Obstructive Pulmonary Disease (COPD) is often associated with limb muscle atrophy, which decreases quality of life and functional capacity in affected individuals. Impaired satellite cells activation, proliferation and differentiation affecting skeletal muscle regeneration could contribute to the progression of muscle atrophy in patients with COPD.
Methods: Biopsies of the vastus lateralis were obtained from patients with COPD (mid-thigh muscle cross-sectional area (MTCSA) >70 cm², n=11; MTCSA <70 cm², n=6) and from healthy subjects (n=7). Accumulation of Notch and Wnt-related proteins were quantified using whole muscle extracts. Satellite cell number and muscle regenerative events were counted on cryosections using nuclear immunostaining. Primary muscle cell cultures were performed from muscle biopsy specimens to measure satellite cell proliferation rates and to assess commitment to differentiation.

Results: The switch between Notch (proliferation) and Wnt (differentiation) signaling pathway appears to be dysfunctional leading to a pro-proliferative state suggested by the increased expression of MyoD, Myf5, GSKα/β and phospho-Numb in patients with COPD (MTCSA <70 cm²). Satellite cell numbers were similar between groups. The number of central nuclei per 100 fibers was increased in patients with COPD (MTCSA >70 cm²) compared to patients with COPD (MTCSA <70 cm²) and controls. In COPD, a decreased in the proliferation of satellite cells was initially observed in vitro at 48 h while their number was increased at 96 h compared to controls. During myogenization, an altered pattern of MRFs (Pax7, Myf5 and Myogenin) accumulation was observed between patient with COPD and patient with normal lung function. Finally, accumulation of the myosin heavy chain protein was reduced during myogenization.

Conclusions: Based on these results, the transition between the Notch and the Wnt pathway seems to be defective maintaining satellite cells into a proliferative state. Deficiencies in their activation and their myogenic program could contribute to the maintenance or the development of muscle atrophy in this population.

Molecular mechanisms underlying reduced knee extensor function and walking endurance in cancer patients: diminished myosin-actin cross-bridge kinetics

Michael J. Toth1,2, Mark S. Miller1, Damien M. Callahan1, Andrew P. Sweeney1, Kimberly Ward1, Joan Braddock2, Marion E. Couch1, Hirak Der-Torossian1, Steven M. Grunberg1, Kim Dittus1.

Departments of Medicine1 and Molecular Physiology and Biophysics2, University of Vermont, College of Medicine, Burlington, VT, USA

Cancer patients often experience reduced physical functional capacity, which substantially reduces their quality of life. Most research has focused on aerobic fitness/muscle oxidative capacity to uncover the physiological determinants of cancer-related physical disability. In contrast, the effect of cancer on the fundamental contractile properties of skeletal muscle has received little attention. Thus, we evaluated skeletal muscle structure and contractile function at the molecular, cellular, whole muscle and whole body level in 11 cancer patients (5 cachectic, 6 non-cachectic) and 5 non-diseased controls. Cancer patients showed a 17% reduction in knee extensor isometric peak torque and changes in myosin heavy chain isoform distribution were noted that might explain reduced cross-bridge kinetics. Collectively, our results indicate that cancer decreases the intrinsic functionality of skeletal muscle myofilament proteins in humans. Relationships among functional indices at the myosin-actin cross-bridge, whole muscle and whole body levels suggest myofilament adaptations may represent a molecular mechanism contributing to functional disability in cancer patients. The concomitant mitochondrial alterations and association to cross-bridge kinetics suggest that molecular contractile dysfunction may develop secondary to dysregulation of oxidative metabolism.

Comprehensive monitoring of bladder cancer animal model for cachexia

Carl-Jorgen Arun; Yosuke Kodama, Helene Johannessen, Chun-Mei Zhao, Duan Chen.

Dept. of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, Trondheim, Norway; Dept. of Urology, St. Olav’s University Hospital, Trondheim, Norway

Although anorexia represents an important factor in the development of cachexia, it by no means accounts for it. It seems evident that metabolic disturbances present in the cancer patient have a definitive role in the development of cachexia. Advanced human bladder cancer is commonly associated with the development of cachexia, as is the case for rodent models. However, comprehensive metabolic monitoring of human cancer patients is hardly feasible and little data on animal models are available. The purpose of the present study was to establish metabolic profiles of rats with progressive bladder cancer.

The orthotopic bladder cancer model was established by exposing male Fischer-344 rats to 4-hydroxy-butyl(butyl)nitrosamine (BBN) in drinking water for 12 weeks. These animals were followed for approximately 1 year for signs of cachexia (10% weight loss), body composition using dual-energy X-ray absorptiometry (DXA) in 12 animals was analysed at 100% weight and after 10% weight loss. Eating behaviour and metabolic parameters was obtained using comprehensive animal monitoring system (CLAMS).

After the animals were sacrificed, autopsy revealed no extravesical tumour invasion, hydronephrosis, lunge or liver metastasis. The animals attained a mean weight of 247 g ±3.1 and at the time of sacrifice 226 g ±4.5. DXA analysis revealed that mean bone mineral density decreased from 0.19 g/cm² ±0.05 to 0.17 g/cm² ±0.05 (p<0.05) and fat compartment decreased from 29% ±1.3 to 21% ±1.4 (p<0.05). Lean body mass + bone mineral content remained unchanged at 175.9 ±(±2.15) vs. 177.7 ±(±3.71). CLAMS revealed increased energy intake ratio from 118 min/g ±4.8 to 187 min/g ±2.7 and decreased daily food intake from 11.5 g ±0.4 to 9.0 g ±0.9 (p<0.03). The energy expenditure (kcal/l/h/100 g body weight) was not significantly changed (0.53 ±0.01 vs.0.56 ±0.01) and the respiratory exchange ratio remained unchanged (0.99 ±0.01 vs. 1.01 ±0.01).

Cachexia occurs in a rat model for orthotopic bladder cancer. In spite of relatively low tumour load significant changes are noted in both body composition and metabolism. In spite of significant fat compartment reduction, the respiratory exchange ratio was not reduced.

Considering a minimal tumour load, this may suggest a systemically increased TCA cycle flux leading to increased glucose consumption (the so-called “Warburg” effect).
them have gastrointestinal symptoms such as a nausea and anorexia. The aim of this study was to investigate, in gastric cancer patients, the behaviour of some peptides that regulate food intake.

**Methods:** Serum ghrelin, leptin, PYY, NPY, AgRP, CART and MSH levels were measured by ELISA (enzyme linked immuno-sorbent assay). BMI and weight loss, were determined in all subjects studied. The patients had been classified in 3 groups: *cachetic* group (gastric cancer patients in treatment that are considered cachetic according to Fearon, 2010); non- *cachetic* group (these gastric cancer subjects had already done the cancer treatment and are gaining weight, the third group consisted on health controls.

**Results:** among the 127 subjects studied, 19 were in the *cachetic* group (47 % underweight and 21 % overweight); 28 in the non- *cachetic* and 80 as health control. PYY, NPY and CART levels were lower in *cachetic* group followed by non- *cachetic*. Ghrelin levels, in contrast were lower in the health group (*p*<0.001). Leptin, AgRP and MSH did not show any difference between all groups.

**Conclusion:** Leptin, MSH and AgRP concentrations did not show any difference in gastric cancer patients (cachetic and non-cachetic). Despite of gastric surgery ghrelin levels were still higher in cachetic group followed by non-cachetic. The concentrations of PYY and NPY were lower while ghrelin were higher in cachetic group suggesting a possible mechanism of reversing the cachexia process.

**Ghrelin, PYY, NPY and AgRP serum levels in colorectal cancer patients**

Katia Barao¹ Tiago D. Silva¹, G.A. Osorio¹, M.A. Vicente¹, Lila M. Oyama², Nora M. Forones¹

¹Grupo de Oncologia, Departamento de Medicina. Universidade Federal de São Paulo, São Paulo, Brasil. ²Departamento de Fisiologia. Universidade Federal de São Paulo, São Paulo, Brasil

**Background:** Obesity is a significant cause of mortality and diseases like cancer. On the other hand, cachexia, or pathologic weight loss, is a significant problem. Despite their differences, both processes involve neuropeptides and hormones that regulate food intake and energy expend. Alterations in this mechanism can lead to obesity or anorexia. Thus far, these peptides have mainly been studied in animal models but not in cancer people. The present study aimed to assess the behaviour of anorexigenic and orexigenic neuropeptides and peripheral signals (Ghrelin, PYY, NPY and AgRP) in colorectal cancer patients compared to a healthy group.

**Material and methods:** 164 subjects were enrolled into the study: 84 with colorectal cancer and 80 healthy controls. The peptides and the neuropeptides were measured by enzyme linked immuno-sorbent assay (ELISA) method in all subjects.

**Results:** PYY, NPY and AgRP were lower in the Cancer group. In overweight subjects the results were the same but we did not find differences between AgRP levels among the groups (*p*=0.181). Between normal weight and overweight we found differences in all groups with lower levels in Cancer group.

**Conclusions:** Ghrelin levels were higher in colorectal cancer patients despite of BMI. PYY and NPY seem to have the same regulation and not to be influenced by the BMI. AgRP concentration increased significantly only in the underweight cancer group. The most important finding was that ghrelin were always higher in cancer group despite of body mass index.

**Leptin serum levels in colorectal cancer patients**

Katia Barao¹ Tiago D. Silva¹, G.A. Osorio¹, M.A. Vicente¹, Lila M. Oyama², Nora M. Forones¹

¹Grupo de Oncologia, Departamento de Medicina. Universidade Federal de São Paulo, São Paulo, Brasil. ²Departamento de Fisiologia. Universidade Federal de São Paulo, São Paulo, Brasil

**Background:** Leptin, anorexigenic hormone involved in body mass regulation, might play a role in cancer cachexia development. Hormones produced by adipocytes are associated with cancer progression. Elucidating the mechanisms by which obesity may increase cancer risk may lead to the identification of treatment and also prevention targets. We aimed to compare the leptin serum levels in colorectal cancer patients and healthy individuals and also to correlate leptin concentration with body mass index.

**Methods:** Eighty-four patients with colorectal cancer and 80 healthy controls were enrolled and subdivided according to their BMI in underweight, normal weight or overweight. Serum leptin levels were measured as ng/ml by enzyme linked immuno-sorbent assay (ELISA) method in all subjects.

**Results:** There were no differences in gender and age. Serum leptin concentration of underweight cancer group was significantly lower than underweight controls (4.79±3.9 vs. 22.3±21.8) (*p*=0.005). On the other hand, on overweight cancer group were higher compared to overweight control (24.8±29 vs. 10.5±15) (*p*=0.022). Between normal weight cancer group and health individuals there were no differences (*p*=0.550). Comparing cancer group to the control group, without consider the BMI, we did not find any difference (*p*=0.229).

**Conclusion:** Our results showed that leptin may play a role in development and progression of colorectal cancer in obese subjects but in underweight. If leptin is produced by adipocytes, those results suggest that in cancer patients the fat free mass is higher probably due to a fat mass depletion priority.

**Nutritional status of patients with advanced cancer**

Susan Buskermolen¹, Henk M.W. Verheul², M.J.P. Admiraal¹, Jacqueline A.E. Langius¹, Marian A.E. van Bokhorst-de van der Schueren¹

¹Department of Nutrition and Dietetics, VU University Medical Center, Amsterdam, The Netherlands. ²Department of Medical Oncology, VU University Medical Center, Amsterdam, The Netherlands

**Background and aims:** The definitions and cut-off points of different subtypes of malnutrition (e.g. (pre)cachexia, sarcopenia) are currently in the center of attention in international cancer literature. The aim of this study was to assess the prevalence of different markers of poor nutritional status in patients with advanced cancer scheduled for chemotherapy.

**Methods:** In patients with advanced cancer (colon, breast, prostate or lung), we inquired weight loss history (6 month), measured body composition (body mass index, fat-free mass index, phase angle, mid-upper arm (muscle) circumference and hand grip strength) and assessed appetite (VAS, EORTC appetite subscale, FAACT questionnaire) before start of chemotherapy. Data on laboratory values (C-Reactive Protein, Albumin, hemoglobin and plasma creatinin) were obtained from the medical records.

**Results:** In 122 patients (63.3 year±10.5, 56 % male), 54.2 % reported any weight loss before start of chemotherapy, whereas still 50 % had a BMI >25. A poor nutritional status and disturbed biochemistry were prevalent in at least 25 % of the patients (table 1). Individual patients reported inconsistently on different appetite questionnaires.

**Conclusion:** This study reveals a high prevalence of different nutritional disturbances in patients with advanced cancer.

**Features of (pre)cachexia in patients with advanced cancer scheduled for treatment with chemotherapy**

Susanne Buskermolen¹, Henk M.W. Verheul², M.J.P. Admiraal¹, Jacqueline A.E. Langius¹, Marian A.E. van Bokhorst-de van der Schueren¹

¹Department of Nutrition and Dietetics, Internal Medicine, ²Department of Medical Oncology

VU University Medical Center, Amsterdam, The Netherlands
Background and aims The diagnostic framework of cancer cachexia is currently in the center of attention in international cancer literature. Consensus is reached on the component weight loss in combination with Body Mass Index (BMI) and low muscle mass but not on other potential cachexia features, for example inflammation and anorexia. The aim of this study is to assess the prevalence of other potential features of (pre)cachexia in cachectic and non-cachectic patients with advanced cancer scheduled for chemotherapy.

Methods In patients with advanced cancer (colon, breast, prostate or lung), we inquired weight loss (WL) history (1 month and 6 month), measured body composition (BMI, fat-free mass index (FFMI), fat mass, mid-upper arm muscle circumference and hand grip strength) and assessed appetite (VAS, FAACT questionnaire) before start of a new chemotherapy treatment line. Data on laboratory values (C-reactive protein (CRP), albumin, hemoglobin and plasma creatinine), assessed within a week before start of chemotherapy, were obtained from medical records. Patients were categorized as ‘Cachetic’ if they had experienced >5 % WL in 6 month or >2 % WL in 6 month in combination with a BMI <20 kg/m²/FFMI <5th percentile. All other patients were defined as ‘Not cachetic’.

Results Data were obtained for 122 patients (63.3±10.5 y, 56 % male) with cancer: 42 % lung, 25 % colorectal, 20 % prostate and 13 % breast. Sixty-six patients (54 %) reported any weight loss before start of chemotherapy, whereas still 50 % had a BMI >25 kg/m². Forty-one patients (34 %) were cachectic. A low FFMI and anorexia according to the FAACT questionnaire were more prevalent in cachectic patients compared to patients without cachexia (Chi² tests, P<0.01 and P=0.04, respectively, Table 1). But also a number of patients without cachexia had signs of a poor nutritional status, anorexia or inflammation, which could be signs of precachexia. However, only 6 patients were precachectic according to the consensus definition. Moreover, there was no correlation between CRP and anorexia (Pearson r: −0.09, p=0.38 for CRP and FAACT and r: −0.03, p=0.75 for CRP and VAS).

Table 1: Markers of poor nutritional status for cachetic patients and patients without cachexia (n=122)

|                | No cachexia (n=81) | Cachexia (n=41) | P-value |
|----------------|--------------------|----------------|---------|
| FFMI below norm (10th percentile) | 18 (23) | 21 (51) | <0.01 |
| Phase Angle below norm (10th percentile) | 34 (44)* | 22 (59)* | 0.13 |
| MUAMC below norm (10th percentile) | 20 (26) | 14 (36) | 0.26 |
| Hand grip strength below norm (lowest tertile) | 51 (64) | 29 (71) | 0.44 |
| Elevated CRP (>8 mg/L) | 41 (64) | 25 (71) | 0.46 |
| Low serum albumin (<35 g/L) | 25 (36) | 19 (53) | 0.09 |
| Low hemoglobin (♂ <8.5 mmol/L; ♀ <7.5 mmol/L) | 33 (43) | 21 (58) | 0.14 |
| Low creatinine (♂ <64 μmol/L; ♀ <49 μmol/L) | 9 (13) | 8 (23) | 0.20 |
| Poor appetite | FAACT ≥30 | 11 (15) | 12 (32) | 0.04 |
| VAS appetite ≤50 mm | 20 (26) | 14 (37) | 0.21 |

Abbreviations: FFMI: fat free mass index; MUAMC: mid-upper arm muscle circumference, CRP: C-reactive protein
Reference values: Schutz et al (2002), Bosy-Westphal et al (2006), Frisanoche et al (1981), Bohannon et al (2006).
* no reference values available for patients with a BMI <18.5 (n=1 for no cachexia and n=4 for cachexia)

Conclusions Thirty-four percent of patients with advanced cancer were cachetic before start of chemotherapy. A poor nutritional status was also prevalent in patients without cachexia, but only 6 patients were precachectic. We found no correlation between inflammation and anorexia, which substantiates the complexity of the diagnosis of (pre)cachexia.

References: (1) Fearon et al, Lancet Oncol. 2011, (2) Muscariello et al, Clin Nutr. 2010

The Animal CACHexia SCOrE (ACASCO): a tool for evaluating the cancer cachexia degree in Yoshida AH-130 ascites hepatoma model

Silvia Busquets1, 2, Angelica Betancourt1, Miriam Toledo1, Fabio Penna1, Marta Ponce1, David Massa1, Francisco J. López-Soriano1, 2 and Josep M. Argilés1, 2
1Cancer Research Group, Departament de Bioquimica i Biologia Molecular; Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain. 2Institut de Biomedicina de la Universitat de Barcelona, Barcelona, Spain

The aim of the Animal CACHexia SCOrE (ACASCO) is to overcome the problem of cachexia staging in experimental animals. The score considers five main different factors involved in the pathophysiology of cachexia: body weight and lean body mass loss (BWC), inflammatory, immunological and metabolic disturbances (IMD), anorexia (ANO), physical performance (PHP) and quality of life (QoL). The score’s scale classifies cachexia in 4°: mild, moderate, severe and terminal phase.

In the study, we analyzed the above-mentioned factors in Yoshida AH-130 ascites hepatoma model at different days after the tumour inoculation: 2, 4, 6, 8, 10 and 11. The particular parameters analyzed were the following: 1) BWC component: body and muscle weights; 2) IMD component: plasma levels of IL-6, serum amyloid A, albumin, glucose, lactate, and urea 3) ANO component: food intake; 4) PHP component: grip strength test and total physical activity; 5) QoL component: signs of distress (closed eyes, piloerection, chromodacryorrhea and lack of movement) and the intruder-resistant paradigm and forced swim tests.

The analysis of all these components allow the classification of the cachexia degree in this tumour model: tumour-bearing (TB) animals 2 days after the tumour inoculation have been classified as pre-cachexia, TB animals 4 days after the tumour inoculation: mild cachexia, TB animals 6 days after the tumour inoculation: moderate cachexia, TB animals 8 and 10 days after the tumour inoculation: severe cachexia and TB animals 11 days after the tumour inoculation: terminal cachexia.

The present score facilitates cachexia staging in Yoshida AH-130 ascites hepatoma model. ACASCO could be an useful tool for the evaluation of cachexia in other experimental tumours allowing for a more appropriate measurement of the degree of cancer wasting.

Role of vitamin D in the pathogenesis of cancer-induced muscle wasting
Andrea Camperi1, Fabio Penna1, Domiziana Costamagna1, Francesco M. Baccino1, Maurizio Muscaritoli2, Paola Costelli1
1Dipartimento di Medicina e Oncologia sperimentale, Università di Torino, Italy; 2Dipartimento di Medicina Clinica, ‘Sapienza’, Università di Roma, Italy

Cancer cachexia is a syndrome characterized by loss of skeletal muscle protein, depletion of lipid stores and hormonal perturbations. Vitamin D (VitD) has been recently proposed as a potential regulator of skeletal muscle mass since several studies described a relationship between muscle weakness/wasting and VitD deficiency.
Preliminary data obtained in our laboratory indicated a significant decrease in circulating VitD in rats bearing the AH-130 hepatoma compared to controls. On this line, the aim of the present study was to investigate the involvement of VitD deficiency in the pathogenesis of muscle wasting in the AH-130 hosts. The animals were divided into four experimental groups: controls, AH-130, vitD-treated and AH-130 vitD-treated. 25-OH VitD was given per os daily (40 IU/day/kg initial body weight, dissolved in corn oil). Untreated groups received vehicle alone. Treatment started the day of tumor transplantation and the animals were sacrificed after 7 days. VitD receptor (VDR) expression in skeletal muscle was evaluated by RT-PCR.

Both VitD-treated groups (controls and tumor hosts) showed reduced body weight and decreased gastrocnemius and tibialis mass in comparison to the respective untreated groups. VitD administration also resulted in significant increase of VDR expression in the muscle of both C and AH-130 rats. VDR was significantly upregulated in tumor hosts; such a pattern occurs also in other cachexia models (mice bearing the C26 or the LLC1 tumors).

Although preliminary, these results apparently suggest that VitD and VDR-dependent signalling pathway does not prevent tumor-induced muscle wasting.

Such observations might be consistent with results obtained on C2C12 and L6E9 myocyte cultures, where VitD treatment impaired myoblast proliferation as well as their complete differentiation to myotubes. Further experiments (e.g. gain/loss of function) are needed to clarify the role of VDR in skeletal muscle myogenic program.

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

Elisabetta Ferraro¹, Fabrizio Pin², Andrea Camperi², Libera Berghella³, Anna Maria Giammarioli³, Sara Caldarola³, Paola Costelli³ and Giuseppe Rosano⁴

¹Pathophysiology and Treatment of Muscle Wasting Disorders Unit, IRCCS San Raffaele Pisana, Rome, Italy; ²Department of Experimental Medicine and Oncology, University of Torino, Italy; ³Istituto Superiori di Sanità, Rome, Italy; ⁴Department of Biology, University of Rome ‘Tor Vergata’, Italy

The metabolic modulator Trimetazidine (TMZ) blocks fatty acid β-oxidation and shifts ATP production towards glucose oxidation, resulting in improved cell energy metabolism. TMZ is commonly used to treat angina pectoris and has been found to enhance both the efficiency of myocardium metabolism and patient exercise capacity.

TMZ effects on skeletal muscle cells were investigated in the present study, with particular reference to its potential protective effect against atrophy-inducing stimuli. C2C12 myotube cultures were exposed to serum deprivation or to the proinflammatory cytokine TNFα. The results show that TMZ significantly prevents myotube reduction in size caused by both treatments. In addition TMZ also markedly increased MyHC expression. Such an effect is associated with: a) increased levels of phosphorylated S6-kinase, suggestive of enhanced protein synthesis, and b) activation of the PI3K-AKT-mTORC2 pathway, and reduction of muscle-specific ubiquitin ligase mRNA levels, likely inhibiting proteasome-dependent degradation. Finally, TMZ also induces autophagy in untreated myotubes.

In order to study the effectiveness of TMZ also in vivo, the drug was administered to mice bearing the C26 colon-carcinoma, a well characterized model of cancer cachexia. Treatment of tumor hosts with TMZ does not modify food intake, body weight and muscle mass. By contrast, muscle fiber cross-sectional area and voluntary muscle grip strength are improved by TMZ; the latter also correlates with TMZ-induced hypoglicemia, suggesting that treated animals are effectively using more glucose than the untreated ones.

On the whole these results, although preliminary, suggest that TMZ positively interferes with skeletal muscle cell response to stress both in vitro and in vivo, supporting a possible reappraisal of TMZ in the treatment of diseases characterized by muscle atrophy, among which cancer cachexia.

**The Activation Of Signalling Pathways Involved In Muscle Mass Regulation After An Acute Bout Of Resistance Training Exercise In Patients With Chronic Obstructive Pulmonary Disease**

Annie Dubé, Bruno B. Lemire, Marie-Eve Thériault, Richard Debégé, and François Maltais

Centre de Recherche de l’Institut Universitaire de cardiologie et de pneumologie de Québec, Université Laval, Québec, Canada

Rationale: Muscle atrophy is an important consequence of chronic obstructive pulmonary disease (COPD). Information concerning the use of resistance training to increase muscle mass in patients with COPD is sparse and their response to exercise appears to be suboptimal. A dysregulation in the signaling pathways involved in the regulation of muscle mass could play an important role in this phenomenon.

Objective: To investigate the impact of an acute bout of resistance training on key signaling pathways involved in the regulation of muscle mass in COPD.

Methods: We investigated the phosphorylation status of key quadriceps signaling proteins (AKT, p70, p38) as well as total protein content of Atrogin and MuRF1 before and after an acute bout of resistant training exercises in 11 patients with COPD (FEV1: 39±2 % of predicted) and 10 age- and activity-matched healthy controls. All exercises were done at 80 % of max for 2 sets of 12 repetitions of squat, leg press and leg extension. Biopsies of the quadriceps were obtained before and 2 h post-exercise. The phosphorylated levels of the proteins were analyzed by western blotting and presented as percent change from baseline.

Results: Post-exercise, the levels of phospho-p70 was increased by 307±119 % in CTRL and by only 120±32 % in COPD (p=0.03). The levels of phospho-AKT were increased by 228±23 % in CTRL, but were decreased to 89±15 % in COPD (p=0.02). In addition, phospho-p38 was increased by 130±32 % in CTRL while a reduction to 57±12 % was observed in COPD (p=0.01).

Conclusion: Overall, our data shows that kinases associated with hypertrophy (p70, AKT) were less phosphorylated in COPD, while phosphorylation of p38 was significantly decreased in COPD compared to controls. These results point toward a potential biochemical mechanism contributing to the differential training response in COPD.

**Time course of atrogenes MurF1 and MAFbx mRNA expression during skeletal muscle atrophy in C2C12 cells**

Geyssson Javier Fernandez¹, Sandro Jose Conde², Celia Regina Nogueira¹, Patrícia Pintor dos Reis¹, Maeli Dal Pai¹, Robson Francisco Carvalho³

¹Department of Morphology, IBB, UNESP, Botucatu, SP, Brazil; ²Department of Clinical Medicine, São Paulo State University-UNESP, Botucatu, SP, Brazil; ³Dept. of Surgery and Orthopedics, FMB, São Paulo State University–UNESP, Botucatu, SP, Brazil

Skeletal muscle atrophy is a common event in many chronic systemic conditions or diseases such as a sepsis, chronic heart failure, chronic obstructive pulmonary disease, chronic kidney disease, diabetes, AIDS and cancer. These conditions may be accompanied by a complex metabolic syndrome characterized by muscle wasting, known as cachexia. Molecular pathways responsible for cachexia are not completely understood, however, pro-inflammatory cytokines such as Tumor Necrosis Factor (TNF)-α and Interferon (INF)-γ have a key role in molecular pathways related to loss of muscle mass and function.
Complex mechanisms controlling gene expression in muscle atrophy may include genes with a common mode of regulation with deregulated transcript levels over the time course. We performed a time-series expression analysis of four genes in differentiated C2C12 muscle cells; in these cells, atrophy was induced by treatment with TNF-α (10 ng/ml) and IFN-γ (100 U/ml) at different times (0 h, 6, 12 h, 18 h, 24 h, and 48 h). The dynamic gene expression of MyHC IIa, MyoD, MuRF1, and MAFbx was measured by Reverse Transcription-Quantitative Real-Time PCR (RT-qPCR). Our results demonstrated that TNF-α/IFN-γ treatment decreases MyHC IIa and MyoD gene expression (98 % and 78 %, respectively) from 0 h to 24 h. However, MyoD mRNA decay is faster (90 %) compared to MyHC IIa decay (50 %) at 6 h. In addition, atrogenes MuRF1 and MAFbx show a 2-fold up regulation peak at 18 h. Our data support the hypothesis that altered gene regulation loops occurs within the first 18 h and suggest a common regulatory program for E3 ubiquitin ligases Muf1F1 and MAFbx genes. Results from our study will contribute to the dynamic description and regulation of gene expression involved in skeletal muscle atrophy induced by cytokines.

Systemic inflammation is related with lipid infiltration of muscles in patients with cachexia-related tumors

Ioannis Gioulbasanis1, Chr. Vassiliou1, Dionysia Kakalou1, Eleni I. Perdikouri1, St. Tzani2, Marianna Vlychou2, Gerogios Patapasakis2, Michalitsa Makridou3, V.A. Katsiouli4, E. Chatzidaki1, Ath. Zafeiriou1, Ioannis Gioulbasanis1, V. A. Katsiouli4, E. Chatzidaki1, Ath. Zafeiriou1, Greece; 2Dept of Radiology, University Hospital of Larissa, Thessaly, Greece; 3 Dept of Hematology, University Hospital of Larissa, Thessaly, Greece; 4 School of Medicine, University of Thessaloniki, Greece

Background: Cancer cachexia is characterized both by metabolic and structural alterations which lead to physical disability and adverse clinical outcomes. In the present study we evaluated the interrelation between systemic inflammation and lipid infiltration of muscles (LIM) which may represent an early indication of muscular degradation and functional deterioration.

Methods: Sixty three patients [52 (82.5 %) males, median age (range) 66 (40–81)] with metastatic primaries of the lung [48 (71.2 %)] and of the upper gastrointestinal (GI) track [15 (23.8 %)] were accrued before the onset of systemic chemotherapy. Median body mass index (BMI) (range) was 24 (17–33). The majority of patients (66.7 %) had an ECOG performance status of 0–1. C-reactive protein (CRP) was used as an indicator of systemic inflammation and was calculated in plasma by standard methods (measured in mg/L). A CT image at the level of the 3rd lumbar vertebra (L3) was analyzed for each patient with the use of Slic-O-Matic software V4.3 (Tomovision, Montreal, Canada) and LIM was calculated (measured in cm2). Muscle and fat (visceral and subcutaneous) mass was also assessed.

The relations of the aforementioned variables were subsequently evaluated.

Results: CRP plasma levels were correlated with LIM ($r = 0.248, p = 0.05$). In addition, the following correlations were observed: CRP with age ($r = 0.245, p = 0.05$), LIM with BMI ($r = 0.547, p < 0.001$), LIM with visceral fat ($r = 0.331, p = 0.02$) and LIM with subcutaneous fat ($r = 0.500, p < 0.001$).

Conclusions: CRP is associated with LIM in patients with metastatic cancers of the lung and the upper GI. This could partly explain the functional decline which accompanies systemic inflammation.

Which nutritional assessment tool is related with musculature and functional status in patients with cachexia-related tumors?

Ioannis Gioulbasanis1, Dionysia Kakalou1, Chr. Vassiliou1, St. Tzani2, Eleni I. Perdikouri1, Michalitsa Makridou3, D. Nasi1, Konstantinos Kampilasorias1, Chr. Bacavou1, Panagiotsis J. Vlahostergios1, Marianna Vlychou2, Christor N. Papandreou1

1Dept. of Medical Oncology, University Hospital of Larissa, Thessaly, Greece; 2Dept of Radiology, University Hospital of Larissa, Thessaly, Greece; 3Dept of Hematology, University Hospital of Larissa, Thessaly, Greece; 4School of Medicine, University of Thessaloniki, Greece

Background: A reliable assessment of nutritional risk in patients with advanced cancer, although subjective, is the cornerstone of an effective intervention. Sarcoaenia is an objective measurement and the major feature of cachexia which adversely affects patients' quality of life and survival. Our aim was to evaluate the correlation of the two most frequently nutritional assessment tools used in oncology, Mini Nutritional Assessment (MNA) and Patients Generated Subjective Global Assessment (PG-SGA), with Lumbar Skeletal-Muscle index (LSMI) and with Fat Free Mass (FFM).

Methods: Totally, 129 patients [97 (75.2 %) males, median age (range) 67 (39–81)] with stage IV primaries of the lung and the upper GI were eligible. Demographics and other baseline characteristics were recorded. MNA and PG-SGA were completed before the onset of systemic therapy. Slice-O-Matic software V4.3 (Tomovision, Montreal, Canada) was used to analyzed CT images at the level of the 3rd lumbar vertebra (L3) as previously described by other investigators. Cross sectional area of muscles (measured in cm2) at that level was calculated and LSMI (measured in cm2/m2) was produced after normalizing this value for stature. Total body FFM (measured in kg) was also estimated from the muscle cross-sectional area at L3.

Results: Mean (±SD) MNA and PG-SGA scores were 18.8 (±4.6) and 12.6 (±6.8), respectively. There was a significant correlation between the two protocols (p < 0.001, r = -0.576). MNA was also correlated with ECOG’s PS (p = 0.001, r = -0.296), BMI (p = 0.001, r = 0.482), albumin (p = 0.015, r = 0.222), while PG-SGA was correlated with BMI (p = 0.015, r = -0.242) and albumin (p = 0.039, r = -0.213). LSMI and FFM were correlated with MNA (p = 0.003, r = 0.350 and p = 0.018, r = 0.277, respectively), while the same correlations for PG-SGA were not significant.

Conclusions: Based on its association with musculature and functionality, MNA may be the screening tool of choice for the nutritional evaluation of patients with advanced malignancies.

Non-invasive Imaging of Muscle Wasting and Tumor Burden in APCmin + Mice

1Lingyun Hu, 1Howard Mak, 2Jason Gilbert, 2John Eash, 2Chikwenden Ibebujo, 3Vania Kenanova, 4Erica Henning

1Global Imaging Group, 2The Musculoskeletal Disease Area, Novartis Institutes for Biomedical Research

Background: Gastrointestinal (GI) cancer patients are extremely susceptible to cachexia, which negatively affects quality of life and survival. The APCmin + mouse is a valuable model to study spontaneous development of intestinal/colorectal cancer and cachexia, yet it is complicated by heterogeneity in tumor onset/growth, as well as difficulties in quantifying muscle wasting and GI tumor burden in the live animal. In this study, we have developed combined magnetic resonance imaging (MRI) and [F-18] fluorodeoxyglucose (FDG) positron-emission tomography (PET) to non-invasively monitor the progression of muscle wasting related to GI tumor burden in the APCmin + mouse.

Methods: Male APCmin + (N=12) and littermate WT controls (N=10) were studied. MRI and FDG-PET static scans were performed at 2, 3, 4, and 5 months of age, to monitor muscle volume and tumor metabolism, respectively. Ex-vivo histopathology was performed in age-matched APCmin + and WT controls from the same breeding cohort. Statistical comparisons were performed using linear mixed-model (LMM) analysis and post-hoc testing with Fisher’s LSD. P = 0.05 was considered significant.

Results: No differences were observed at 2 months. MRI leg muscle volume was significantly lower for APCmin + versus WT at 3 months (−7 %, P = 0.030), 4 months (−11 %, P = 0.010), and 5 months (−23 %,
C26 cells in 50:50 matrigel:PBS vehicle or an equal volume of the

Conclusions: Combined MRI/PET is a sensitive, reliable method for monitoring the relationship between muscle wasting and GI tumor burden in the APCmin + cachexia model. These methods will enable investigators to: 1) randomize animals to treatment groups by presence and severity of disease; 2) evaluate the efficacy of pharmacological targets on both cachexia and GI tumor burden; and 3) perform longitudinal evaluations in each animal, thus reducing animal use.

Sub-classification of patients with cachexia using targeted metabolomics
Tobias Janowitiz, Baljit Ubhi, Susan C. Connor, Julian L. Griffin, and Duncan Jodrell
University of Cambridge, Cambridge Research Institute, Cancer Research UK

Cachexia is known to be one of the final common clinical processes in end-stage malignancies, malabsorption diseases, some chronic infections such as HIV and some end organ diseases such as chronic obstructive pulmonary disease (COPD). Death or increased morbidity can occur in up to 50 % of patients with advanced cancer who experience cachexia. No reliable molecular marker for cachexia is currently described or established in clinical practice.

We used quantitative liquid chromatography-tandem mass spectrometry (LC-MS/MS) to measure 34 modified amino acids in the serum of 12 patients with pancreatic adenocarcinoma (PAC) (5 patients without cachexia and 7 patients with cachexia). Amino acids were modified using the EZ:Fast amino acid analysis procedure consisting of a solid phase extraction step followed by derivatisation to chloroformates. The results were compared to amino acid concentrations in the serum of patients with cachexia and chronic obstructive airway disease (COPD). Several amino acid concentrations were significantly altered in patients with COPD and cachexia but not patients with pancreatic cancer (serine, sarcosine, tryptophan, BCAAs and 3-methylhistidine; data not shown). Increased γ-aminobutyrate (GABA) levels were specific to cachexia in patients with pancreatic cancer. Metabolic biomarkers could be exploited as a way of monitoring treatment efficacy, tumour recurrence, and suitability for intervention in patients with pancreatic cancer experiencing cachexia.

A comparison of Colon-26 adenocarcinoma induced cachexia in adult male Balb/c vs. CDF1 mice
Nicole Londraville, Brian Clarke, Tracee Kendall, Jason Gilbert, Chikwendu Ibebunjo, David Glass
Musculoskeletal Diseases, Novartis Institutes for Biomedical Research, Cambridge, MA, USA

Cancer cachexia is the loss of body weight (both lean and fat mass) that occurs in about 50 % of cancer patients despite adequate alimentation, and leads to reduced tolerance to cancer treatment and increase morbidity and mortality. The molecular mechanisms of cancer cachexia have not been fully elucidated and there are no approved drugs that effectively prevent or reverse cachexia. Both of these gaps would be facilitated by appropriate mouse models of cancer-induced cachexia. Since the response of the mouse to the tumor (cachexia) and interventions vary between mouse strains, the purpose of this study was to compare the degree of cachexia induced by the colon-26 (C26) adenocarcinoma in adult male Balb/c and CDF1 mice in the absence or presence of the anabolic agent formoterol. Adult Balb/c and CDF1 mice (14 weeks old) were inoculated subcutaneously with either 5 × 10^6 C26 cells in 50:50 matrigel:PBS vehicle or an equal volume of the vehicle alone, concurrent with treatment with or without formoterol at 2 mg/kg/day in drinking water. Body weight, food intake and tumor growth were monitored 3× weekly and after 21 days, the mice were euthanized and skeletal muscles and visceral organs dissected out and weighed. In Balb/c and CDF1 mice, muscle weights declined by 22.9 % and 15.60 %, respectively, compared to PBS control. Furthermore, more treatment with formoterol ameliorated C26-induced muscle atrophy by 37.81 % in Balb/c compared with 28.97 % in CDF1 mice. These findings suggest that Balb/c mice are the better strain for C26-induced cancer cachexia research as they offer a more suitable window for therapeutic intervention.

Efficacy and safety of a two drug-combination regimen for cancer-related cachexia in the clinical practice
Clelia Madeddu, Mariele Dessì, Filomena Panzone, Roberto Serpe, Giorgia Antoni, Giovanni Mantovani
Department of Medical Oncology, University of Cagliari, Cagliari, Italy

Background and aims: To test the safety and efficacy of a two-drug combination (including nutraceuticals, i.e. antioxidants with carnitine + celecoxib for the treatment of cancer-related anorexia/cachexia syndrome (CACS) in the clinical practice. Primary endpoints: safety, increase of lean body mass (LBM) and improvement of quality of life. Secondary endpoints: increase of physical performance (tested by grip strength and 6-min walk test, 6MWT) and decrease of inflammation (assessed by serum levels of IL-6 and Glasgow prognostic score, GPS).

Patients and Methods: Outpatients with advanced cancer at different sites with CACS (i.e. loss of body weight >5 % of the pre-illness or ideal weight in the last 3 months) were eligible to receive: L-carnitine 4 g/day + Celecoxib 300 mg/day. All patients received as basic treatment polyphenols 300 mg/day, lipoic acid 300 mg/day, carboxysteine 2.7 g/day, Vitamin E, A, C, all orally. Treatment duration was 4 months.

Results: From June 2011 to April 2012, 50 patients with advanced cancer (all stage IV) at different sites were enrolled: 40 completed the treatment and were evaluable (mean age 63.8 ±9.6, range 32–81 years). Results showed a significant increase of LBM (by dual-energy X-ray absorptiometry and by L3 computed tomography) from baseline as well as physical performance assessed by 6MWT. Quality of life (assessed by EORTC-QLQ-C30 and EQ-5D) also improved significantly. EOG PS and GPS decreased significantly. The treatment was safe, no grade 3–4 toxicities occurred and no patient had to discontinue the treatment due to severe adverse events.

Conclusions: The results of the present study confirm the efficacy and safety of the two-drug combination regimen previously shown in a randomized clinical trial (Madeddu et al, Clinical Nutrition 31:176–182, 2012). Therefore, this simple, feasible, effective, safe, with favorable cost-benefit profile, two-drug approach could be suggested in the clinical practice as a treatment for CACS.

Relationships between inflammatory cytokines, muscle loss and fish oil in lung cancer patients
Vera Mazurak1, Rachel Murphy1, Taylor Bureyko1, Marina Mourtzakis2, Quincy Chu1
1Alberta Institute for Human Nutrition, Faculty of Agriculture, Life and Environmental Sciences, University of Alberta, Edmonton, Alberta, Canada; 2Dept. Kinesiology, Faculty of Applied Health Sciences, University of Waterloo, Waterloo, Ontario, Canada; 3Dept. Oncology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta, Canada

Introduction: The role of cytokines in evoking muscle loss in cancer cachexia is often highlighted, and interference with inflammatory
pathways cited as mechanism for the benefits of EPA on muscle mass, however evidence from human studies is limited.

**Objective:** To relate change in muscle mass to plasma levels and change in cytokines (IFN-γ, IL-10, IL-12p70, IL-1β, IL-6, IL-8 and TNF-α) measured at baseline and at the end of chemotherapy treatment in non-small cell lung cancer patients who were supplemented or not supplemented with fish oil.

**Methods:** Patients were followed from time of diagnosis until completion of platinum based doublet therapy (~3 months). Patients received 4 capsules of fish oil per day (2.2 g EPA + 240 mg DHA; n=16) or no intervention (n=24; standard of care). Skeletal muscle cross-sectional area was evaluated using lumbar computed tomography (CT) images taken for diagnostic purposes. Change in muscle was expressed as % change from the initial CT scan and divided by the number of days elapsed between the 2 CT images. Plasma cytokines were quantified at baseline and the last day of chemotherapy using commercially available ELISA kits.

**Results:** Baseline demographics were similar in both groups, pooled characteristics: 53 % male, age 64±8.7 years, BMI 26.9±5.2, 6 month weight history 4.9±.6.5 %. No relationships between change in muscle and IFN-γ, IL-10, IL-6, IL-1β or IL-12p70 were observed in either group. Change in IL-8 and TNF-α during chemotherapy were modestly correlated with muscle rate of change (r=−0.40 and −0.36, respectively, p=0.02). Plasma cytokine levels did not change significantly over the 3 month time period in the fish oil or standard of care group.

**Conclusions:** Cytokines may not be a major driver of muscle loss and alternate mechanisms apart from reducing pro-inflammatory cytokines may be responsible for the observed effect of fish oil on preservation of muscle mass.

**A role of autophagy in cancer cachexia**

**Kristine Pettersen**1,2, Sonja Andersen1, Ken Fearon2, Florian Strasser1, Anders Molven2, Geir Bjorkøy1

1Sør-Trøndelag University College, Trondheim, Norway; 2Norwegian University of Science and Technology, Trondheim, Norway; 3University of Edinburgh, UK. 4Cantonal Hospital, St. Gallen, Switzerland. 5Haukeland University Hospital, Bergen, Norway

Cachexia is a common feature of many patients at a terminal stage of cancer. The condition is believed, at least in part, to be caused by improper regulation of catabolic processes. Lysosomal degradation via autophagy is the principal catabolic processes employed by cells to clear damaged proteins and organelles, as well as to mobilize metabolites during low-nutrient conditions. Despite the importance of autophagy in cellular degradation, a defined role for the process has not yet been established in cachectic patients. We explore whether cancer cachexia can be caused by systemically accelerated autophagy and hunt for the potential mechanism(s) causing increased autophagy flux. We have established a cellular autophagy flux bioassay where the process can be quantified in non-cancerous cells using flow cytometry. Using this bioassay, we find that cancer cells known to induce cachexia in mice accelerate autophagy in the reporter cells. Induced autophagy in the reporter cells is observed both in co-cultures and by exposing the reporter cells for conditioned media from the cancer cells. Importantly, by screening serum samples from cancer patients, we find that more than 25 % of the serum samples are able to induce autophagy in the reporter cells. Together, our findings support the notion that tumor-derived signaling substance(s) may accelerate autophagy in normal cells and may contribute to increased catabolism in cancer cachexia.

**Effects of a MEK inhibitor–selumetinib on skeletal muscle anabolism**

**Carla M.M. Prado**1, Taniaos Bekati-Saab2, L.A. Doyle3, Sachin Shrestha1, Sunita Ghosh1, Vickie E. Baracos1, Michael B. Sawyer1

1Department of Oncology, University of Alberta. Cross Cancer Institute, Edmonton, AB, Canada, 2Department of Internal Medicine & Department of Pharmacology, The Ohio State University Comprehensive Cancer Center, Columbus, OH, USA, 3Division of Cancer Treatment and Diagnosis, NCI, Bethesda, MD, USA

**Background:** To investigate the potential of a MEK inhibitor–selumetinib to promote skeletal muscle anabolism in patients with cholangiocarcinoma.

**Methods:** Skeletal muscle was assessed by quantitative analysis of computed tomography (CT) images selumetinib (AZD6244; ARRY-142886) in a Phase II study, compared with a separate standard therapy group. Selumetinib is an inhibitor of mitogen-activated protein/extra-cellular signal-regulated kinase and interleukin-6 secretion, a putative mediator of muscle wasting.

**Results:** Muscle gain was observed in 84.2 % of patients after initiating selumetinib; mean overall gain of total lumbar muscle cross sectional area was 13.6 cm²/100 days (~2.3 kg on a whole body basis). Cholangiocarcinoma patients who began standard treatment were markedly catabolic, with overall muscle loss of ~7.3 cm²/100 days (~1.2 kg) and by contrast only 16.7 % of these patients gained muscle.

**Conclusion:** Our findings suggest the potential of MEK and putatively interleukin-6 as a target for cachexia therapy.

A comparative study of gene expression changes in skeletal and cardiac muscle from the colon 26 carcinoma mouse model of cachexia: identification of common molecular targets for future intervention strategies

**Angie M. Y. Shum**1, Timothy C. Tan1,2 and Patsie Polly1

1Inflammation and Infectious Research Centre, Department of Pathology, School of Medical Sciences, Faculty of Medicine, University of New South Wales, NSW, Australia, 2052

2Cardiac Ultrasound Laboratory, Department of Cardiology, Massachusetts General Hospital, Boston, USA

Cancer cachexia is a highly debilitating paraneoplastic disease observed in more than 50 % of patients with advanced cancers and directly contributes to 20 % of cancer deaths. While loss of skeletal muscle is a defining characteristic of patients with cancer cachexia; pathology in heart has only recently been demonstrated in animal models of cachexia. In this study, we have compared the gene expression patterns governing sarcomeric structural degradation and substrate metabolism in skeletal and cardiac muscle. Common gene expression patterns were also seen for skeletal and cardiac muscle due to cancer cachexia. Of particular interest are certain genes involved in substrate metabolism including proliferative activated receptor, gamma, coactivator 1 (PGC1) β and the genes involved in autophagy-lysosomal degradation. However, there were also distinctive gene expression patterns seen in each tissue including the E3 ligases of the ubiquitin-proteasome system suggesting different cellular breakdown responses secondary to the chronic inflammatory stimulation during cancer cachexia. We propose that gene expression profiling may be a useful, initial step in the identification of potential molecular targets common to both types of muscle which may serve as therapeutic targets to prevent muscle breakdown in these tissue.

**Mitochondrial-targeted antioxidants protect against doxorubicin-induced skeletal muscle toxicity**

**Ashley J. Smuder**1, Kurt J. Sollanek1, Kisuk Min1, Michael P. Wiggs1, Hazel H. Szeto2 and Scott K. Powers1

1Department of Applied Physiology and Kinesiology, University of Florida and 2Department of Pharmacology, Weill Cornell Medical College, USA
Doxorubicin (DOX) is a highly effective antitumor agent widely used in the treatment of solid tumors and hematologic malignancies. However, DOX has been shown to induce deleterious effects in skeletal muscle. Numerous studies have attempted to identify molecular mechanisms responsible for DOX myotoxicity. Nevertheless, a complete understanding of DOX-mediated muscle toxicity remains elusive. The principal mechanism believed to cause DOX-induced toxicity is increased mitochondrial reactive oxygen species (ROS) production leading to the induction of proteolysis and apoptosis. Thus, its clinical use is limited due to drug-induced cellular toxicity. Hence, developing a countermeasure to prevent DOX’s cytotoxicity is important. Therefore, we tested the hypothesis that treatment with a mitochondrial targeted antioxidant would protect skeletal muscle from DOX-induced dysfunction. Cause and effect was determined by preventing DOX-induced mitochondrial ROS emission in rats using a novel mitochondrial targeted antioxidant (SS-31). Importantly, treatment with SS-31 resulted in a significant attenuation in the DOX-induced accumulation of 4-HNE modified proteins. In addition, compared to untreated DOX animals, DOX animals treated with SS-31 showed a significant sparing of muscle cross-sectional area. Finally, prevention of mitochondrial ROS emission in DOX administered animals was also sufficient to inhibit DOX-induced increases in apoptosis. These data confirm that the mitochondria are the dominant source of DOX-induced ROS production in skeletal muscle and that inhibiting mitochondrial ROS emission is sufficient to protect the skeletal muscle from DOX-induced muscle atrophy and apoptosis.

**A mitochondria-targeted antioxidant protects against activation of autophagy and the proteasome system during disuse atrophy**

**Erin E. Talbert**, Ashley J. Smuder, Kisuk Min, Oh Sung Kwon, Hazel H. Szeo, and Scott K. Powers

1University of Florida, Gainesville, FL. 2Weill Cornell Medical College, New York, NY, USA

Increased production of mitochondrial reactive oxygen species (mROS) promotes disuse skeletal muscle atrophy. Indeed, our laboratory has demonstrated that a mitochondria-targeted antioxidant is sufficient to prevent skeletal muscle atrophy induced by hindlimb immobilization. Four proteolytic systems contribute to muscle wasting during prolonged disuse. Both the calpain and caspase-3 systems are activated in immobilized skeletal muscle by increased mitochondrial ROS emission. It remains unknown if increased mROS emission is required to activate autophagy and the ubiquitin-proteasome system in skeletal muscle exposed to prolonged periods of inactivity. These experiments tested the hypothesis that increased mROS emission is required for activation of both autophagy and the ubiquitin-proteasome system in skeletal muscle during 7 days of immobilization. As expected, SS-31 treatment prevented the casting-induced increase in ROS emission from permeabilized soleus and plantaris muscle fibers. Similar to previous reports, SS-31 treatment prevented the casting-induced atrophy of both the soleus and plantaris muscles. Importantly, treatment of ambulatory control animals with SS-31 did not alter muscle fiber size. Casting activated the proteasome system, as evidenced by increases in mRNA expression of MuRF-1 and atrogin-1 in the soleus muscle and atrogin-1 in the plantaris muscle. Treatment of animals with SS-31 prevented inactivity-induced increases in E3 ligase expression in both the soleus and plantaris muscles. The autophagy system was also activated in both muscles during casting as indicated by increases in the ratio of LC3 II/I. Further, cathepsin L mRNA expression increased in both muscles following 7 days of casting and SS-31 treatment prevented these increases in autophagy biomarkers. In conclusion, these results indicate that the proteasome system and autophagy system are sensitive to mROS emission during disuse atrophy and confirm that mROS are important signaling molecules in skeletal muscles exposed to prolonged periods of inactivity.

**Limb and respiratory muscle dysfunction in a murine model of cancer cachexia**

**Jeffrey Widrick**, EunHi Choi, Kadir Cruthers, Nathan Thomas, Li Zhang, Ricardo Battaglino, Leslie Morse

1Dept. of Physical Medicine and Rehabilitation, Spaulding Rehabilitation Hospital and Harvard Medical School, Boston, MA, USA; 2Dept. of Physical Medicine and Rehabilitation, Hallym University College of Medicine, Gangwon-do, South Korea; 3Forsyth Institute, Cambridge, MA, USA

Approximately 50 % of cancer fatalities are associated with cachexia and half of these deaths are attributed to muscle dysfunction secondary to excessive skeletal muscle wasting. While it is tempting to assume that muscle function declines in direct proportion to the loss of muscle mass, reports of a selective loss of the motor protein myosin and extensive sarcomeric disarrangement in muscles of cachexia animals suggest otherwise. We tested the hypothesis that force deficits in cancer cachexia cannot be attributed solely to a loss of skeletal muscle tissue. Lewis lung carcinoma (LLC) cells were injected into the left thigh of C57BL/6 mice (0.75×10⁶ cells/animal). Control mice received an equal volume injection of growth media. Tumors were observed in all LLC-treated animals at 21 and 25 days post-inoculation. Functional properties of extensor digitorum longus (EDL) and soleus muscles (isolated from the right, non-injected hindlimb), and muscle strips dissected from the diaphragm, were determined ex vivo. EDL muscles showed an earlier and a quantitatively greater loss in mass, physiological cross-sectional area (pCSA), and peak tetanic force compared to soleus muscles. At 25 days post-inoculation, EDL muscles showed a significant reduction in force normalized to muscle pCSA. This reduction in specific force was not trivial, accounting for ~40 % of the total force deficit of the muscle. LLC did not affect the twitch force to tetanic force ratio, suggesting normal Ca²⁺-release and -activation. Similar results, i.e. a reduction in specific force and a normal twitch to tetanic ratio, were observed for diaphragm muscle strips of LLC treated mice. These findings indicate that a mass-independent component can make a substantial contribution to the muscle dysfunction that characterizes cancer cachexia. This intracellular component appears to lie downstream of Ca²⁺ release, most likely at the level of the actomyosin cross-bridges.

**Nutritional Assessment in Acute Care Palliative Medicine**

**Declan Walsh**, Aynur Aktas, Marianne Fischer, Barbara Hullien, Ellen Schleckman, Chanell Upshaw

1Cleveland Clinic Taussig Cancer Institute, Harry R Horvitz Center for Palliative Medicine, 2Cleveland Clinic Digestive Institute, Center for Human Nutrition

**Introduction:** Malnutrition is common but under-diagnosed in cancer. We evaluated the nutritional status of inpatients in an acute care palliative medicine unit on admission.

**Methods:** Nutrition therapy assessments (NTA) by a registered dietitian (RD) and subsequent physician notes from electronic medical records (EMR) for 2011 were reviewed. Eligibility criteria: newly admitted inpatients, initial consult by a single RD. RD used Cleveland Clinic standard assessment tool with 6 criteria to assess nutritional status: 1) unintentional weight loss (WL) 2) nutritional intake 3) body mass index (BMI) 4) muscle wasting 5) wounds 6) lab values.
Results: 150 consecutive NTAs were eligible from 2011; 50 were reported. Median age 58 years (range 26–85); 52 % male; 76 % Caucasian, 20 % African-American; 98 % cancer diagnosis. 86 % had metastatic disease. RD identified malnutrition in 24 (48 %). 42 % were moderate/severe. Malnutrition was usually identified by unintentional WL (48 %), low serum albumin (44 %), and low nutrient intake (28 %). WL was severe in 68 %. 92 % of physician notes lacked formal nutrition status. Anorexia (50 %), nausea (28 %), vomiting (22 %), dysphagia (18 %), and cachexia (14 %) were reported by the physicians. Median (range) value for BMI (kg/m²) and albumin (mg/dL) for the entire study population were 25 (14–38) and 3 (2–5), respectively.

Conclusions: (1) Moderate to severe malnutrition was prevalent (42 %); (2) WL, albumin level, and nutrient intake were the most common malnutrition criteria; (3) Most physician notes did not formally document nutrition status. Various GI symptoms and cachexia.

The Cancer Weight Loss Trajectory: An Analysis of a 6,800 Patient Database
Declan Walsh¹, Aymur Aktas¹, Aditya Nair¹
¹Cleveland Clinic Taussig Cancer Institute, Harry R Horvitz Center for Palliative Medicine

Introduction: Large cancer databases can provide valuable information about weight loss prevalence and severity. We examined weight loss (WL) trajectory in adult out-patients with solid tumors.

Methods: Electronic medical records (EMR) (N=6800) from a tertiary academic center were retrospectively reviewed. Data from first and last ambulatory (face-to-face) encounters were analyzed. Percent weight changes were calculated against the pre-illness weight. All results were summarized with percentages and median (range 25th, 75th quartiles).

Results: Age: 60 (52–70 years); 57 % male. Most common cancers: prostate (17 %), lung (13 %), breast (12 %). 82 % had metastatic disease. Previous antitumor treatments included chemotherapy (73 %), radiotherapy (43 %), and hormonal therapy (16 %). Body weight at first and last encounter was 81 (68, 95) and 79 (66, 93) kg, respectively. Number of visits which included weight measures was 14 (7–25). Abnormal WL was reported by the physician as a separate diagnosis (ICD 9 code) in 3 %. From first to last encounter, weight change percent was −0.6 (−5, 2) in the entire group. N=2624 (56 %) had any degree of weight loss; 31 % <5 %, 12 % 5–10 %, 13 % had >10 %. Resting energy expenditure (REE) and albumin level remained same; 1555 kcaLS (1354–1791) and 4.2 (3.7–4.5) mg/dL respectively. Among individuals who had weight loss, the median BMI value remained within the normal range.

Conclusions: (1) 25 % of cancer out-patients had >5 % weight loss; (2) BMI, REE, and albumin were not associated with WL severity; (3) Documentation of weight loss diagnosis was deficient.

Cause of death in cancer cachexia—the impact of CV drugs
Andrew J Stewart Coats
Norwich Research Park, UK

The mechanisms of death in advanced cancer are frequently unknown. Estimates of cardiovascular contributions to death in cancer sufferers have varied from 10 % to 50 %. End-stage cancer patients may not receive detailed cardiovascular evaluation and abnormalities in CV function or structure may be put down to coincident risk factors or the adverse effects of chemotherapeutic agents. Recent research has discovered specific cardiac abnormalities and dysfunction in animal models of cancer and reports of cardiac abnormalities being common in human cancer sufferers. With unknown cause of death or sudden death being common in cancer the possibility of heart related mortality and morbidity is likely.

This may lead us to consider the role cardiovascular drugs may play in treating cancer patients. Recent retrospective reports have showed the use of beta-blockers in cancer (for non-cancer related conditions) has been associated with reduced cancer progression or metastases and reduced mortality. The precise mechanisms remain unclear but the inhibition of metastatic spread and protecting a heart damaged by the consequences of cancer and protecting against cancer related cachexia are all possible mechanisms.

Therapeutically, a phase 3 trial of ACE inhibitor Imidapril showed a significant reduction in the rate of weight loss in both non-small cell lung and colorectal cancer patients but not in pancreatic cancer. During the trial patients receiving Imidapril lost a further 1.91 lbs (0.868 k) on average while those on placebo lost 2.68 lbs (1.218 k). The trial would have been positive in the first two cancer types but the lack of efficacy in pancreatic cancer reduced the average result and led to the trial just missing its primary endpoint. One of the problems may have been that in the 6 months prior to starting the trial the average patient had lost 15 % or an average of 24 lbs (10.9 k) of bodyweight, so that these patients may have been too advanced before treatment was started. A confirmatory phase 3 trial omitting pancreatic cancer was commenced, but subsequently put on hold as the sponsor concentrated on its non-cachexia portfolio.

The fourth generation beta blocker, MT-102, has been shown to improve survival in a cancer model and to reduce cachexia. In addition in an ageing rat sarcopenia model it led to significantly improved food intake and weight gain, particularly lean mass combined with loss of body fat. In addition it improved anabolic and catabolic protein signalling. The ACT-ONE trial is testing the effect of the fourth generation beta blocker (10 mg bd dose) MT-102 in comparison to placebo on the rate of weight change over a 16 week period in patients with cachexia related to underlying stage III and stage IV colorectal or non-small cell lung cancer. It has recruited more than 50 % of its target and is estimated to complete in June 2013, aiming for the study of 132 patients from Europe, India and Malaysia.

Oral Serum-Derived Immunoglobulin as a Potential Therapy for Cachexia: Improved Growth, Nutritional Status, and Reduction of GI Inflammation in Animals and Humans
Gerald L. Klein, MD¹; Eric Weaver, PhD¹; Jeffrey Cohn, PhD²; Audrey Shaw, PhD¹
¹Enterica Health, Cary, NC 27511; ²Tab Clinical Trials, Cary, NC 27511, USA

Numerous non-clinical studies of serum-derived bovine and porcine immunoglobulin (Ig) isolates (SBI) consistently demonstrate positive effects across multiple species on growth, food intake, and nutritional status in animal populations with inflammation. This suggests a role for SBI as a medical food to augment traditional medications. Its underlying mechanism of action suggests SBI may prove beneficial versus disease or treatment-related cachexia. In environmentally stressful or diseased states, increased cytokine production can promote an increase in enteric epithelial tight junction permeability with resultant antigenic penetration of the gut barrier. These effects may be ameliorated through SBI’s prevention of pro-inflammatory cytokine expression, including TNF-α, INF-γ, and interleukins (e.g., IL-1, IL-6), thus facilitating restoration of normal GI function and improved nutritional utilization of accompanying SBI proteins.
SBI dietary supplementation must be non-digestible and able to reach the GI tract to benefit an immunocompromised or otherwise dysfunctional GI mucosa. While the exact mechanism of action remains unknown, non-clinical and clinical studies demonstrate that Ig survives initial digestive processes, providing improvement in GI function and morphology. This includes increased absorption, reduced permeability and mitotic activity, increased villus heights, as well as improved weight gain and bone density among animals treated with Ig supplements. Further, early studies in patients with malnutrition or HIV enteropathy found statistically significant improvements in nutrient retention and GI symptoms. These data suggest a potential role for SBI in the treatment of patients with cachexia.

Safety and efficacy data from animals, as well as adult and pediatric patients for up to 8 months support a role for SBI dietary supplementation to inhibit cytokine activity, thus reducing gut inflammation with resultant improvements in absorption, more efficient nutrient and protein utilization, increased lean weight gain, muscle mass and bone density. The implications for cachexia patients suggest strongly the need for additional research.

Identification of inflammatory markers associated with weight loss and poor prognosis in cancer patients

**Methods:** We measured body weight change, appetite and a panel of plasma inflammatory markers in 62 males with cancer-cachexia (CC), 72 non-cachectic cancer subjects (CNC) and 64 non-cancer controls (Co) matched by age, gender and pre-illness body weight. In a subset of patients we also measured grip strength (HGS), appendicular lean body mass (aLBM), ECOG and KPS.

**Results:** GDF-15, IL-6 and IL-8 were increased in CC vs. other groups. Activin and G-CSF were significantly upregulated in CC vs. Co. Subset analyses showed that GDF-15, Activin A and IL-8 are increased in CC vs. CNC in lung cancer patients and that GDF-15, IL-6 and IL-8 are increased in CC patients treated with platinum-based chemotherapy.

GDF15, IL-6 and IL-8 levels significantly correlate with 6-month weight loss and with IL-1ra, IL-2, IL-4, IL-9, IL-10, IFN, MCP-10, MIP-1a, MIP-1b, TNF-a, VGEF and Activin A in cancer patients. Analysis in a subset of patients showed that CC had lower grip strength, aLBM, and fat mass; and that ECOG and KPS were lower in CC and CNC compared to controls. GDF-15 and IL-8 correlated negatively with aLBM, HGS and fat mass. Activin correlated negatively with aLBM.

**Conclusion:** The inflammatory cytokines Activin A, GDF15, IL-6 and IL-8 are associated with weight loss, decreased muscle mass and strength and poor survival in cancer patients. These cytokines may serve as prognostic indicators in cancer patients and present novel therapeutic targets for treatment of cancer cachexia.

**Identification of inflammatory markers associated with weight loss and poor prognosis in cancer patients**

Lorena Lerner PhD1, Teresa Hayes, MD PhD2, Nianjun Tao1, Brian Krieger2, Bin Feng PhD3, Richard Nicolletti1, M. Isabel Chiu PhD1, Jeno Gryus PhD1, Jose M Garcia, MD, PhD2

1AVEO Pharmaceuticals, 2Michael E DeBakey VAMC and Baylor College of Medicine

**Background:** Cachexia is associated with increased inflammatory markers and decreased survival in cancer. A number of such inflammatory cytokines have been associated with poor prognosis in several cancer types but their role in cachexia is not well-understood.

**Methods:** We measured body weight change, appetite and a panel of plasma inflammatory markers in 62 males with cancer-cachexia (CC), 72 non-cachectic cancer subjects (CNC) and 64 non-cancer controls (Co) matched by age, gender and pre-illness body weight. In a subset of patients we also measured grip strength (HGS), appendicular lean body mass (aLBM), ECOG and KPS.

**Results:** GDF-15, IL-6 and IL-8 were increased in CC vs. other groups. Activin and G-CSF were significantly upregulated in CC vs. Co. Subset analyses showed that GDF-15, Activin A and IL-8 are increased in CC vs. CNC in lung cancer patients and that GDF-15, IL-6 and IL-8 are increased in CC patients treated with platinum-based chemotherapy.

GDF15, IL-6 and IL-8 levels significantly correlate with 6-month weight loss and with IL-1ra, IL-2, IL-4, IL-9, IL-10, IFN, MCP-10, MIP-1a, MIP-1b, TNF-a, VGEF and Activin A in cancer patients. Analysis in a subset of patients showed that CC had lower grip strength, aLBM, and fat mass; and that ECOG and KPS were lower in CC and CNC compared to controls. GDF-15 and IL-8 correlated negatively with aLBM, HGS and fat mass. Activin correlated negatively with aLBM.

**Conclusion:** The inflammatory cytokines Activin A, GDF15, IL-6 and IL-8 are associated with weight loss, decreased muscle mass and strength and poor survival in cancer patients. These cytokines may serve as prognostic indicators in cancer patients and present novel therapeutic targets for treatment of cancer cachexia.

Identification of inflammatory markers associated with weight loss and poor prognosis in cancer patients

**Methods:** We measured body weight change, appetite and a panel of plasma inflammatory markers in 62 males with cancer-cachexia (CC), 72 non-cachectic cancer subjects (CNC) and 64 non-cancer controls (Co) matched by age, gender and pre-illness body weight. In a subset of patients we also measured grip strength (HGS), appendicular lean body mass (aLBM), ECOG and KPS.

**Results:** GDF-15, IL-6 and IL-8 were increased in CC vs. other groups. Activin and G-CSF were significantly upregulated in CC vs. Co. Subset analyses showed that GDF-15, Activin A and IL-8 are increased in CC vs. CNC in lung cancer patients and that GDF-15, IL-6 and IL-8 are increased in CC patients treated with platinum-based chemotherapy.

GDF15, IL-6 and IL-8 levels significantly correlate with 6-month weight loss and with IL-1ra, IL-2, IL-4, IL-9, IL-10, IFN, MCP-10, MIP-1a, MIP-1b, TNF-a, VGEF and Activin A in cancer patients. Analysis in a subset of patients showed that CC had lower grip strength, aLBM, and fat mass; and that ECOG and KPS were lower in CC and CNC compared to controls. GDF-15 and IL-8 correlated negatively with aLBM, HGS and fat mass. Activin correlated negatively with aLBM.

**Conclusion:** The inflammatory cytokines Activin A, GDF15, IL-6 and IL-8 are associated with weight loss, decreased muscle mass and strength and poor survival in cancer patients. These cytokines may serve as prognostic indicators in cancer patients and present novel therapeutic targets for treatment of cancer cachexia.
NF-κB signaling proteins and transcription factors required for cancer-induced muscle wasting
Susan Kandarian, Evangelie Cornwell, Chia-Ling Wu, Azadeh Mirbod, Robert Jackman
Department of Health Sciences, Boston University, Boston, MA, USA

Existing data show that NF-kappaB signaling is a key regulator of cancer-induced skeletal muscle wasting. However, the identification of the components of this signaling pathway and of the NF-κB transcription factors that regulate wasting is far from complete. In muscles of C26 tumor bearing mice, overexpression of d.n. IKKβ blocked muscle wasting by 68%, the IκBα-super repressor blocked wasting by 41%, and d.n. p65 blocked wasting by 20%. In contrast, overexpression of d.n. IKKα or d.n. NIK did not block C26-induced wasting. Genome-wide mRNA expression arrays showed upregulation of many genes previously implicated in muscle atrophy. To test if these upregulated genes were direct targets of NF-κB transcription factors, we compared genome-wide p65 or p50 binding to DNA in control and cachectic muscle using ChIP-sequencing. Bioinformatic analysis of ChIP-seq data comparing chromatin isolated from control and C26 muscles showed increased p65 and p50 binding to some regulatory genes and to structural genes, many of which are involved in skeletal muscle development. However, there was no increase in the binding of p65 or p50 to putative atrophy genes. The p65 and p50 ChIP-seq data are consistent with our finding only a small increase in protein binding to an NF-κB oligo in a gel shift assay and a minimal change in binding to an NF-κB oligo in a transcription factor plate assay. The multiplex transcription factor assay did however, show marked increases in C26 muscle nuclear protein binding to SMAD, C/EBP, GR, CREB, and ATF2 (CREB2) oligos. Taken together, these data support the idea that although genetic inhibition of IKKβ and IκBα blocks cancer-induced wasting, the downstream NF-κB transcription factors may not play a major role. Other transcription factors we found showing increased binding in C26 cachectic muscle may be important regulators of wasting, possibly induced by IKKβ. These data are consistent with the growing body of literature showing many NF-κB-independent substrates of IKKβ and IκBα, and we propose that these substrates are some of the mediators of cancer-induced muscle wasting.

Signaling mechanisms in cancer cachexia: role of TRAF6
Ashok Kumar, Pradyut K. Paul, and Sajedah M. Hindi
Department of Anatomical Sciences and Neurobiology, University of Louisville School of Medicine, Louisville, KY 40202, USA

Skeletal muscle atrophy is a major cause for morbidity and mortality in many conditions including cancer, starvation, and disuse. The ubiquitin-proteasome and autophagy-lysosomal systems are the two major proteolytic systems involved in regulation of both physiological and pathological muscle wasting. However, the signaling mechanisms leading to the activation of these proteolytic systems have just begun to be elucidated. Tumor necrosis factor receptor (TNFR)-associated factor 6 (TRAF6) is an important adaptor protein involved in receptor-mediated activation of various signaling pathways in response to cytokines and tumor products. TRAF6 also possesses E3 ubiquitin ligase activity causing lysine-63-linked poly-ubiquitination of target proteins. Our recent studies have uncovered a novel role of TRAF6 in activation of various proteolytic systems and eventual loss of skeletal muscle mass in cachectic states. Muscle-wasting stimuli such as cancer growth, starvation, or starvation augment the expression as well as the auto-ubiquitination of TRAF6 leading to downstream activation of major catabolic pathways in skeletal muscle. Muscle-specific depletion of TRAF6 preserves skeletal muscle mass in a mouse model of cancer cachexia, starvation, and denervation. Inhibition of TRAF6 also blocks the expression of the components of the ubiquitin-proteasome system (UPS) and autophagosome formation in atrophying skeletal muscle. In addition, our study provides the first evidence that TRAF6 mediates the activation of endoplasmic reticulum stress and unfolded protein response pathways during starvation-induced muscle atrophy. Finally, our experiments suggest that lysine 63-linked autoubiquitination of TRAF6 is essential for its regulatory role in muscle wasting. Collectively, our study provides strong evidence that blocking TRAF6 activity can be used as a therapeutic approach to preserve skeletal muscle mass and function in different catabolic conditions.

STAT3 is a promoter of cytokine-induced muscle wasting: implication of posttranscriptional regulation
Jennifer F. Ma*, Derek T. Hall*, Sergio Di Marco and Imed-Eddine Gallouzi
Dept of Biochemistry, Rosalind and Morris Goodman Cancer Centre, McGill University, Canada

Cachexia, characterized by excessive weight loss and skeletal muscle deterioration, is a disorder that often affects individuals with cancer. Cachectic patients experience loss of skeletal muscle mass due to decreased synthesis and enhanced degradation of muscle proteins. Although 20 to 50% of all cancer-related deaths are due to consequences of this condition, no efficient treatment is available at this time. Pro-inflammatory cytokines such as TNFα and IFNγ have been shown to mediate cancer-induced muscle wasting through the activation of the transcription factor NF-κB. One of the principle effectors of NF-κB-mediated muscle wasting is IL-6, a cytokine normally secreted by immune cells to defend against infection. IL-6 has been shown to mediate the loss of muscle by inducing the phosphorylation and thus activation of STAT3. The posttranscriptional regulation of many short-lived mRNAs that encode pro-cachectic cytokines, including IL-6, involves AU-rich elements (AREs) found in the 3’UTR. These AREs mediate the stability, cellular localization and translation of their host messages through association with ARE-Binding proteins, such as HuR. Here we show that STAT3 mRNA is a novel HuR target during IT-induced muscle wasting. We show that HuR associates both in vivo (IP coupled to RT-PCR) and in vitro (gel shift) with the STAT3 mRNA in cytokine-treated muscle fibers. Although cytokines increase STAT3 mRNA and protein levels in muscle cells, the knockdown of HuR decreases the expression of STAT3 protein and mRNA. The importance of these findings is highlighted by our observations showing that cytokine-induced muscle wasting is delayed by inhibiting the activity of STAT3. Our data demonstrate that STAT3 is a key player in cytokine-induced muscle wasting and that this effect is dependent on HuR-mediated stabilization of STAT3 mRNA.

Mechanisms underlying exercise-mediated rescue of cachexia
Viviana Moresi1, Paola Aulino1, Emanuele Berardi1, E. Rossi1, E. Pigna1, Sergio Adamo1, Dario Coletti1,2
1Dept. of Anat., Histol., Forens. & Orthop. Sciences, Sapienza University of Rome, Italy; 2UR4 Ageing, Stress and Inflammation, Pierre et Marie Curie University Paris 6, France

Recent studies showed that physical activity after cancer diagnosis ameliorates the prognosis, although the underlying mechanisms are still poorly understood. Cachexia, experienced by most cancer patients, is a negative prognostic factor, interfering with therapy and worsening quality of life. With the aim to delineate the pathways involved in exercise-mediated rescue of cachexia, we investigate the effects of spontaneous physical activity (wheel running) in colon carcinoma (C26)-bearing mice. All major diagnostic criteria for cachexia are reversed by exercise, including rescue of body weight, muscle atrophy and fatigue, ultimately leading to increased survival. In order to assess whether muscle contraction plays a role
in the exercise-mediated rescue of cachexia, we denervated one limb of (C26)-bearing mice and assessed muscle mass following spontaneous wheel running. Interestingly, muscle innervations and/or contraction are required for positive effects exercise exerts on (C26)-bearing mice muscle mass. At the molecular level, exercise promotes protein synthesis, by mTOR activation, and attenuates protein degradation, by downregulating Atrogin1, in muscle from C26-bearing mice. We propose that exercise counteracts muscle wasting through a systemic effect, by both inhibiting catabolic pathways and favouring satellite cell recruitment into muscle fibers, unveiling a novel mechanism of exercise-mediated beneficial effects on cachexia.

Combination of endurance training and erythropoietin prevents cancer-induced muscle alterations
Fabio Penna1,2, Silvia Busquets2, Miriam Toledo3, Fabrizio Pin1, Francisco J. Lopez-Soriano2, Paola Costelli1, Josep M. Argiles2

Cancer cachexia is a syndrome characterized by loss of skeletal muscle protein, depletion of lipid stores, inflammation, anorexia, weakness, and perturbations of the hormonal homeostasis [1]. In addition to nutritional approaches, exercise training (EX) was proposed as a suitable tool to manage cachexia, in view of recent observations suggesting that decreased physical activity plays a role in the onset of muscle atrophy in cancer patients [2]. Aim of the present work was to verify if endurance training coupled to erythropoietin (EPO) administration could prevent the wasting process in Lewis Lung carcinoma(LLC)-bearing mice. LLC mice were got used to a treadmill for 5 days before tumor injection (106 cells s.c.) and then exercised 5 days/week (45 min,14 m/min). At the end of the experimental protocol (28 days after tumor implantation), tumor-bearing (TB) mice were characterized by a marked body and skeletal muscle weight loss, resulting in impaired muscle strength. Moreover, tumor growth induced a dramatic anemia (50 % hematocrit reduction) and, likely consequently, heart hypertrophy. The combination of EX with EPO (100 U/mouse, i.p., weekly) partially counteracted tumor-induced hematocrit reduction and prevented heart hypertrophy. Although in the EX-EPO group skeletal muscle mass was similar to the sedentary TB mice, grip strength was significantly increased. Ultrastructural analysis of the EDL and soleus muscles of TB mice showed inter-myofibrillar mitochondrial swelling and reduced sub-sarcolemmal glycogen storage; both alterations were got used in EX-EPO mice. Overall, the present data suggest that endurance exercise can be an effective tool to be included in combined therapeutic approaches against cancer cachexia. Further ongoing studies will unravel the molecular mechanisms underlying the reported effects.

References: (1) Fearon K et al., Lancet Oncol 2011; (2) Al-Majid S, Waters H. Biol Res Nurs 2008.

The p97/VCP ATPase is critical in muscle atrophy and for the accelerated degradation of most muscle proteins
Rosanna Piccirillo1 and Alfred L. Goldberg2

1Oncology Department, “Mario Negri” Research Institute, Milan, Italy; 2Cell Biology Department, Harvard Medical School, Boston, MA, USA

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

Clinical, metabolic and biochemical characteristics of the cachexias
Declan Walsh, Shiva Shrotriya, Aynur Aktas, Nabila Bennani-Baiti

Introduction: Cachexia occurs in various chronic diseases like cancer, congestive heart failure (CHF), acquired immunodeficiency syndrome (AIDS), end-stage renal disease (ESRD), rheumatoid arthritis (RA), and chronic obstructive pulmonary disease (COPD). The diagnosis has been based on physical appearance or body weight alone, but may be inadequate. Our objective was to review cachexias among chronic medical disorders by clinical, metabolic and biochemical characteristics.

Methods: The electronic database Medline (via PubMed and Ovid Medline) was searched to identify original research and review articles. The terms “cachexia” or “wasting” or “weight loss” were used, then in combination with any of the associated disorders (AIDS, COPD, CHF, Chronic Kidney Failure, RA, Cancer or Neoplasm). The search limited to English papers (1950–2009).

Results: Of 3344 citations, 585 papers were reviewed, 71 papers included. Different studies used various diagnostic criteria. In literature terminology; sundry terms were used interchangeably to describe cachexia e.g. wasting, malnutrition, and sarcopenia. Metabolic abnormalities like proteolysis and lipolysis similar amongst the cachexias.

Conclusions: (1) Clinical and metabolic characteristics were similar across all cachexias; (2) Anorexia and weight loss were common except in rheumatoid arthritis; (3) Cachexia seemed reversible with dietary intervention.

Systematic Review of C-reactive protein As A Prognostic Indicator in Solid Tumors
Declan Walsh, Shiva Shrotriya, Aynur Aktas, Nabila Bennani-Baiti

Harry R. Horvitz Center for Palliative Medicine, Cleveland Clinic Taussig Cancer Institute

Introduction: Serum C-reactive protein (CRP) has been linked to shorter survival in malignancy. It may help determine treatment response and tumor recurrence. We conducted a systematic literature review to examine the value of CRP to predict prognosis in solid tumors.

Methods: The following MeSH terms were used: [(prognosis OR treatment outcomes OR Survival) AND (C-reactive protein OR CRP) AND (cancer OR Neoplasm)]. The following electronic databases were searched: PubMed, EMBASE, Web of Science, SCOPUS, EBM-
Cochrane Database. A quality assessment scoring system was developed and utilized. Studies with scores <50% were deemed inadequate and excluded.

Results: 92 studies made up the final literature review: 70% were prospective and 24% retrospective. The median quality assessment (QA) score was 60 (range 50–80). Elevated CRP predicted prognosis in 78% by multivariate analysis, and 13% by univariate analysis only. Over half (55%) of studies were either in gastrointestinal malignancies or renal cell carcinoma. High CRP level predicted prognosis in 80% of the studies in these two specific tumor groups. Most studies in other tumors suggested a prognostic role.

Conclusions: (1) CRP seemed valuable as a predictor of prognosis in some cancers; (2) CRP may have a role in determination of treatment response and tumor recurrence; (3) CRP should be more widely used and investigated for this purpose; (4) Better quality large studies with standardized study design should define the role of CRP in prognosis.

The role of C-reactive protein in cancer prognostication
Declan Walsh, Shiva Shrotriya, Ayur Aktas, Nabila Bennani-Baiti
Harry R. Horvitz Center for Palliative Medicine, Cleveland Clinic Taussig Cancer Institute

Introduction: C-reactive protein (CRP), a non-specific marker of inflammation may be used for cancer prognostication. Our objectives were (1) To look if solid tumors are associated with high CRP; (2) To evaluate if clinical predictors correlate with CRP in solid tumors; (3) To see if CRP relates to survival.

Methods: A retrospective review of the Electronic Medical Record (EMR) was conducted. Data included multiple CRP measurements at a tertiary cancer center (2006–2008). Hematological cancer diagnoses were excluded. Survival defined as days from the highest CRP to date of death. CRP value reported in median (25th, 75th percentiles).

Results: N=6809 with solid tumors were identified. The most common were genitourinary (GU) (29%), breast (14%), gastrointestinal (GI) (14%), and lung (7%). ≥1 CRP and survival data was available for 462. 261 (56%) were males; 385 (83%) Caucasian; 67 (15%) African American. CRP was done a median of 2.0 (1–3) times. Highest CRP was 5.1 (1.3, 12.1). Higest CRP for GI, GU, lung, and breast were 7.7 (2.4, 15.2); 5.7 (1.8, 14.8); 3.2 (0.6, 8.1); and 2.1 (0.7, 4.8). Median survival were 13.1 (7.9, 30.1); 18.4 (10.7, 33.2); 15.9 (8.0, 27.2) and 25.3 (14.9, 40.9) months respectively.

Conclusions: (1) Highest CRP within reference range (<10) for GI, GU, and lung cancers; (2) An inverse relationship existed between highest CRP and survival; (3) CRP may have a role in determination of treatment response and tumor recurrence; (3) CRP should be more widely used and investigated for this purpose; (4) Better quality large studies with standardized study design should define the role of CRP in prognosis.

Clinical implication of the autophagy-lysosomal pathway, but not the ubiquitin-proteasome pathway, in cancer cachexia
Nicolas Tardif1, Maria Kludie1, Thomas Gustafsson2, Lars Lundell1, Anders Thorell3 and Olav Rooyackers1
Karolinska University Hospital Huddinge and Karolinska Institutet, 1Dept Anaesthesiology and Intensive Care, 2Dept Clinical Physiology, 3Dept Surgery, Ersta Hospital, Stockholm, Sweden

Animal models have shown that ubiquitin-proteasome pathway may be the effector of muscle loss during cancer cachexia. However, evidence from clinical studies is still needed to understand mechanisms involved in cancer induced muscle catabolism in patients. Patients (n=17, 64±6 years) diagnosed with esophageal cancer were undergoing surgery with intent of resection of the primary tumor. As a control group, weight stable patients undergoing reflux surgery (n=10, 60±7 years) were included. Vastus lateralis muscle biopsies were taken with a Bergstrom needle. Proteasome, caspase 3, calpains and lysosomal enzymatic activities were measured by using specific fluorogenic peptide substrates. Protein expressions were measured by western-blot and coomassie blue staining was used as a loading control. Differences between the groups were tested with a Student’s t-test. Cathepsin L and B activities were respectively 115 % (5.3±0.4 vs. 2.5±0.3 pmol/min/μg; p<0.001) and 85 % (2.4±0.2 vs. 1.3±0.2 pmol/min/μg; p<0.001) higher in the vastus lateralis of cancer patients compared to the controls. Cathepsin B activity was correlated to the self-reported body weight loss of cancer patients (R²=0.41, p=0.03). Proteasome, calpain and caspase3 activities did not differ between the two groups in vastus lateralis. According to the enzymatic activities, no change in expression of Mrf4 (28.8±3.3 vs. 29.1±1.5 A.U) and atrogin-1 (0.88±0.1 vs. 0.64±0.1 A.U) was measured in vastus lateralis of control patients compared to the cancer patients. LC3 expression was significantly increased by 70% (p=0.015) in vastus lateralis of the cancer group (12.6±1.1 A.U) compared to the control group (7.4±1.1 A.U).

In our clinical study, we observed weight-loss related activation of cathepsins and increased expression of LC3, a marker of autophagy flux, in skeletal muscle of cancer patients. These results suggest that autophagy-lysosomal pathway is involved in the development of cancer cachexia and might be an important therapeutic target for fighting cachexia.

Biomarker evaluation and staging in cancer cachexia: are cytokines still relevant diagnostic and treatment aids? Antoio Viganò1, Lorena Lerner2, Nianjun Tao2, Brian Krieger2, Bin Feng2, Richard Nicoletti2, T. Alcindor3, D. Fuoco2, Michel L. Tremblay4, Jeno Gyuris2 and M. Isabel Chiu2
1McGill Nutrition and Performance Laboratory, Montreal, Canada; 2AVEO Pharmaceuticals, Cambridge Massachusetts; 3Medical Oncology Department, McGill University Health Centre; 4 Rosalind and Morris Goodman Cancer Research Centre, McGill University, Montreal, Canada

Background: We have provided initial evidence on the clinical usefulness of the cancer cachexia stages (CCS) proposed by Fearon et al. However it is still unclear if particular molecular phenotypes are also associated with these stages, or with relevant clinical outcomes.

Methods: A candidate list of cytokines (Actevin A, Eotaxin, FGF, G-CSF, GDF15, GM-CSF, IFN-g, IL-10, IL-12, IL-13, IL-17, IL-1b, IL-1ra, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IP-10, MCP-1, MCAF, MIP-1a, MIP-1b, PDGF-bb, RANTES, TNF-a, VEGF) was measured in 210 blood samples from patients with advanced lung and gastrointestinal cancers, via Luminex and ELISA methods. Non-parametric t-test, Kaplan-Meier and Kruskal-Wallis analyses were used to test the association between cytokine levels with CCS, Patient-Generated Subjective Global Assessment scores (PG-SGA) and survival.

Results: Using non-cachectic patients as controls, Activin A, GDF15, IL-6, IL-8 and VEGFa were significantly up-regulated in cachectic (p<0.001) patients. Activin A (p<0.001), GDF15 (p<0.001) and IL-8 (p<0.001) plasma levels correlated with PG-SGA. Quartiles of Activin A, GDF15, IL-6 and IL-8 plasma levels correlated (p<0.001) with survival curves.

Conclusions: Activin A, GDF15, IL-6 and IL-8 appear to be useful aids for the diagnosis of cachexia stages in advanced cancer. Because of their correlation with nutritional and survival outcomes, these cytokines may also represent useful targets for the treatment of cancer cachexia.

References: (a) Viganò et al., Crit Rev Oncog 2012; (b) Fearon et al. Lancet Oncol 2011; (c) Viganò et al. Clin Nutr 2011.

Ryanodine receptor 1 remodeling in cancer associated muscle dysfunction
David L. Waning2, Khalid S. Mohammadi2, Daniel C. Andersson3, Sutha K. John1,2, Patricia Juarez-Camacho1,2, Steven Reiken4, Andrew R. Marks4,6 and Theresa A. Guise1,2
Cancer cachexia is a condition of unintentional body weight loss through a reduction in skeletal muscle and adipose tissue mass. Cachexia is associated with increased morbidity and inability to treat patients with appropriate chemotherapies. Currently there are no fully effective treatments for cachexia and ascertaining molecular targets to maintain muscle mass in cancer patients is of great importance. Our laboratory has recently discovered a new isoform of the canonical transcriptional coregulator PGC-1α referred to as PGC-1α4. PGC-1α4 induces muscle hypertrophy through increased muscle IGF-1 expression and a reduction in myostatin expression, thus making it an excellent candidate to explore for anti-cachexia therapies. The purpose of this study was to determine if forced expression of muscle PGC-1α4 can attenuate muscle wasting during cancer-induced cachexia. Lewis Lung Carcinoma Cells (LLC), an established cachectic cell line were injected into wild-type and transgenic mice expressing the PGC-1α4 isoform then studied for 28 days throughout the progression of cachexia. The gastrocnemius muscle was examined for gene expression and cellular signaling. PGC-1α4 transgenic mice showed an attenuated loss in body weight, muscle mass and functional strength after 28 days of inoculation. Transgenic mice showed a decrease in muscle myostatin gene expression and increase in IGF-1 corresponding with the reduction in atrogenes MAFbx1 and MuRF1. Improvements in muscle mass observed in PGC-1α4 TG mice were associated with increased voluntary movement throughout the progression of cachexia. In addition, glucose intolerance, a common pathology during cachexia is improved in PGC-1α4 transgenic mice. These data show PGC-1α4 can regulate muscle wasting and improve functionality during cancer-induced cachexia.

Although enzymes involved in ubiquitin conjugation have been implicated 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 protects them from a catabolic stimulus. To test the relevance of this in vivo, we inactivated USP19 in mice and characterized the wasting response induced by denervation, fasting, glucocorticoids and cancer and also measured expression of USP19 in human muscle samples. In response to fasting, denervation, dexamethasone, 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 fasting, fractional synthesis rates were 260 % and 135 % higher in the KO in the sarcoplasmic and myofibrillar fractions respectively. In fasting, denervation and cancer, expression of MuRF1 and some autophagy genes in the KO were <50 % that in WT. USP19 may exert these effects on both protein synthesis and degradation by regulating signaling upstream of both processes. Indeed in the KO, the induction of muscle myostatin upon fasting and cancer was abolished whilst there were no effects on IGF-1 expression. In muscle samples of patients with cancer (20 with non-small cell lung cancer and 100 with mostly abdominal cancers), expression of USP19 correlated with expression of the MURF1 and MAFbx liganes.

**Conclusions:** USP19 is required for the catabolic response in diverse wasting conditions. It suppresses protein synthesis and enhances protein degradation (ubiquitin proteasome system and autophagy) and this appears in part due to induction of myostatin expression. These results and its expression in human cancer patients identify USP19 as a potential drug target in treatment of cachexia.
and growth of adult muscles. Modulation of satellite cell self-renewal (proliferation) and lineage commitment (differentiation) affects adult muscle growth and repair in animal models. Here, similar to what has been reported in Colon-26 cachexia, we observed a large increase in Pax7-positive cells in Lewis Lung Carcinoma cachexia model. Further analysis showed that Ki67-positive cell numbers were doubled in the muscle of LLC tumor-bearing mice, suggesting that the increase in Pax7-positive cells might be associated with proliferation. Co-staining for Pax7 and MyoD showed that the majority of Pax7+ cells (~92 %) found in normal muscle co-expressed MyoD, while a drastic decrease in the proportion of Pax7+/MyoD- cells was observed in LLC tumor-bearing mice. Gene-ontological analysis of microarray data from murine and human cancer cachexia revealed that muscle contraction and differentiation genes were the most down-regulated. The expression of myogenic regulatory factors (MRFs) was also down-regulated in cancer cachexia models. Overall, our findings revealed that the elevated proliferation level of muscle progenitor cells and the suppression of muscle terminal differentiation were highly associated with muscle wasting in cachexia models, suggesting that blockade of terminal differentiation of myogenic progenitors may in part contribute to muscle wasting in cancer cachexia. If so, strategies to promote differentiation might be a means of preserving muscle mass.

A new cancer cachexia animal model substantiates the causative relationship between cachexia and systemic inflammation

Lingbing Zhang, Dongdong Feng, Lynda X. Yu, Jeffrey A. Norton and Kangla Tsung
Department of Surgery, Stanford University School of Medicine, 1201 Welch Rd. Stanford CA 94305, USA

Although previous studies have linked cancer cachexia to some inflammatory cytokines, the relationship of cachexia with inflammation still remains elusive which hampers the development of effective therapeutic approaches. Part of the reason is lack of appropriate animal cancer cachexia models since many of the cachexia associated debilitation and systemic inflammation related pathogenesis observed in cancer patients were not reflected in those early animal models. Thus a clinically relevant cancer cachexia animal model is urgently needed. Recently we have developed a new mouse cancer cachexia model (CHX207). After a subcutaneous inoculation of CHX207 cells (one million), 40 % of mice experience a loss of more than 7 % total body weight in two successive days (defined as cachexia onset by us) by 2 weeks when tumors are 7–10 mm in diameter, and 80 % by 3 weeks. After the onset of cachexia, the overall health of mice deteriorated quickly as indicated by 3–6 % step-wise daily drop of body weight and appearance of ruffled fur, arched back and lethargy. Most of the cachectic mice become moribund within 3–10 days following cachexia onset. Necropsy of moribund mice revealed a variety of pathological changes among multiple organs including liver, spleen and lung etc. Consistent with clinical observations in cachectic cancer patients, systemic inflammation characterized by severe lymphopenia, leukocytosis and massive infiltration of neutrophils in multiple vital organs is found to accompany cachexia. While systemic inflammation has long been speculated to be one of the major underlying mechanisms of cachexia, there is no previous experimental investigation to support this speculation. We substantiated this speculation for the first time by the demonstration that cachexia can be reversed by suppressing systemic inflammation with palliative dose of certain chemotherapy drugs without inhibition of tumor growth. Thus we have not only established a new cancer cachexia model which faithfully recapitulates the debilitating medical condition in cancer cachexia patients from visible symptoms to underlying systemic inflammation related pathogenesis, but also pointed out targeting systemic inflammation with palliative dose of chemo drug is a promising approach to the treatment of cancer cachexia.

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

Graham Robertson1, Ryland Taylor2, Dominic Burg3, Lucy Jankova3, Arran Painter1, Maria Tsoli1, Shiba Dolai2, Stephen Clarke1 and Mark Molloy2
1 Cancer Pharmacology Unit, ANZAC Research Institute, Sydney, Australia; 2 Australian Proteome Analysis Facility; Macquarie University; Sydney, Australia

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

Methods: Utilising a multi-platform approach including microarray and MS-based iTRAQ analysis, as well as novel ATP-binding protein enrichment technology coupled with label free MS-quantitation, we have profiled gene and protein expression patterns of livers from C26 tumor-bearing mice displaying cachexia. RESULTS. The transcriptomic and proteomic datasets revealed high correlation between the three approaches, with very few instances of incongruity. Pathway analysis utilizing several software packages indicated that central metabolic processes including lipid handling, glycolysis/glucogenesis, amino-acid metabolism, TCA cycle and mitochondrial electron transport chain are reduced in cachectic mice. Linking these metabolic pathways to upstream regulatory events, transcriptional activation is reduced within the RXR canonical pathway (e.g. CAR, LXR, FXR, TR, PPARα/β/γ), associated with cytokine signalling through activated JAK/STAT pathway, SOCS3 and IL-1/LPS-BP signalling. Repressed expression of genes and proteins in key energy generation pathways is counter-intuitive to the expected role of the liver in settings of food restriction/weight-loss – i.e. to adaptively utilize amino acids, carbohydrates & fatty-acids and activate ketone body production & glucogenesis. As a counterpoint to this dramatic disruption in metabolic pathways, we see enhanced acute phase protein production and a concomitant increase in protein translation, potentially mediated through phosphorylated 4E-BP downstream of mTOR.

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

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

Graham Robertson1, Dominic Burg1, Melissa Moore1, Arran Painter1, Nigel Turner2, Sarah Lockie3, Brian Oldfield3, Greg Cooney2, Stephen Clarke1 and Maria Tsoli1
1 Cancer Pharmacology Unit, ANZAC Research Institute, Sydney, Australia; 2 Garvan Institute of Medical Research, Sydney, Australia; 3 Department of Physiology, Monash University, Melbourne, Australia

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 &
fatty acid β-oxidation and their corresponding target genes revealed dramatic molecular changes indicative of active BAT. Increased Ucp1, Pbe and Cpt1α 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 PGCoα 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.

Altered regulation of circadian rhythm and lipid metabolism associated with inflammatory signalling in white adipose tissue (WAT) in cancer cachexia
Graham Robertson1, Anne S Vanniasinghe1, Arran Painter1, Stephen Clarke2 and Marta Tsoli1
1Cancer Pharmacology Unit, ANZAC Research Institute, Concord RG Hospital, NSW, 2139, Australia 2Northern Clinical School, University of Sydney, RNS Hospital, St Leonards, NSW, Australia

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

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

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

Parameters of cardiovascular function and exercise capacity in patients with advanced pancreatic cancer
Stephan von Haehling, Christoph Heinz, Yuriko Mori, Thomas Kung, Larissa Cramer, Tatjana Stoijkovic, Günter Fauler, Jens Stieler, Bert Hildebrandt, Wolfram Doehner, Helmut Oettle, Jochen Springer, Stefan D Anker, Mathias Rauchhaus
Applied Cachexia Research, Department of Cardiology, Charité Medical School, Campus Virchow-Klinikum, Augustenburger Platz 1, 13353 Berlin, Germany

Background: Pancreatic cancer (PCA) is associated with low quality of life, a prevalence of cachexia, and an unacceptably high number of treatment failures. We hypothesized that patients presenting with advanced PCA display cardiovascular perturbations such as endothelial dysfunction and exercise limitation.

Methods: We studied 74 patients with PCA and 57 control subjects. All patients underwent a full non-invasive cardiological examination including resting electrocardiogram, echocardiography, treadmill exercise testing, body composition, venous strain-gauge plethysmography, assessment of a set of biomarkers and assessment of several pro-inflammatory cytokines.

Results: Analysis of body composition revealed a significantly lower total lean tissue mass in patients with PCA than in controls (20.9±4.6 kg vs. 23.1±5.9 kg, p<0.017). Patients with PCA compared to control subjects showed a significantly lower peak oxygen uptake (21.0±5.9 vs. 27.8±7.7 ml/min/kg, p<0.0001) and forced expiratory volume in 1 s (FEV1, 91±17 % vs. 99±16 %, p=0.013). We found significant differences in both arm and leg blood flow at rest with higher values in patients with PCA than in controls (arm: 5.89±3.82 vs. 4.67±2.15 ml/100 ml/min, p=0.047; leg: 4.76±3.7±vs. 2.87±1.68 ml/100 ml/min, p=0.0036). We detected elevated levels of MR-proADM and CT-proET-1 in patients with PCA as compared to controls (both p<0.0001). Cytokine levels of TNFα, TNFR-1, TNFR-2 and IL-6 were significantly elevated in patients with PCA as compared to controls (all p<0.05).

Discussion: We have shown that patients with PCA display many aspects of cardiovascular illness. Pro-inflammatory cytokines may not only be associated with the development of endothelial dysfunction but also with loss of lean tissue. Decreases in forced expiratory volume in 1 s in our patients with PCA may likewise point to decreased muscle strength. These characteristics may worsen the patients’ exercise capacity and therefore may have a role in the development of fatigue.

Leptin serum levels in colorectal cancer patients
Katia Barao1, Tiago D. Silva1, G.A. OzorioA1, M.A. Vicente1, Lila M. Oyama2, Nora M. Forones1
1Grupo de Oncologia, Departamento de Medicina. Universidade Federal de São Paulo, São Paulo, Brasil; 2Departamento de Fisiologia, Universidade Federal de São Paulo, São Paulo, Brasil

Backgrounds: Leptin, an orexigenic hormone involved in body mass regulation, might play a role in cancer cachexia development. Hormones produced by adipocytes are associated with cancer progression. Elucidating the mechanisms by which obesity may increase cancer risk may lead to the identification of treatment and also prevention targets. We aimed to compare the leptin serum levels in colorectal cancer patients and healthy individuals and also to correlate leptin concentration with body mass index.

Material and methods: Eighty-four patients with colorectal cancer and 80 healthy controls were enrolled and subdivided according to their BMI in underweight, normal weight or overweight. Serum leptin levels were measured as ng/ml by enzyme linked immuno-sorbent assay (ELISA) method in all subjects.

Results: There were no differences in gender and age. Serum leptin concentration of underweight cancer group was significantly lower...
than underweight controls (4.79±3.9 vs. 22.3±21.8) ($p=0.005$). On the other hand, on overweight cancer group were higher compared to overweight control (24.8±29 vs. 10.5±15) ($p=0.022$). Between normal weight cancer group and health individuals there were no differences ($p=0.550$). Comparing cancer group to the control group, without consider the BMI, we did not find any difference ($p=0.229$).

**Conclusion:** The higher levels of leptin in cancer obese patients suggest a higher amount of fat mass probably because of fat free mass depletion. Our results showed that leptin may play a role in development and progression of colorectal cancer only in obese subjects. Key words: leptin, colorectal cancer, obesity

---

**Ghrelin, PYY, NPY and AgRP serum levels in colorectal cancer patients**

Katia Barao¹, Tiago D. Silva¹, G.A. Ozorio¹, M.A. Vicente¹, Lila M. Oyama², Nora M. Forones¹

¹Grupo de Oncologia, Departamento de Medicina. Universidade Federal de São Paulo, São Paulo, Brasil; ²Departamento de Fisiologia. Universidade Federal de São Paulo, São Paulo, Brasil

**Backgrounds:** Obesity is a significant cause of mortality and diseases like cancer. On the other hand, cachexia, or pathologic weight loss, is a significant problem. Despite their differences, both processes involve neuropeptides and hormones that regulate food intake and energy expend. Alterations in this mechanism can lead to obesity or anorexia. Thus far, these peptides have mainly been studied in animal models but not in cancer people. The present study aimed to assess the behaviour of anorexigenic and orexigenic neuropeptides and peripheral signals (Ghrelin, PYY, NPY and AgRP) in colorectal cancer patients compared to a healthy group.

**Material and methods:** 164 subjects were enrolled into the study: 84 with colorectal cancer and 80 healthy controls. The peptides and the neuropeptides were measured by enzyme linked immuno-sorbent assay (ELISA) method in all subjects.

**Results:** PYY, NPY and AgRP were lower in the Cancer group. In underweight Cancer subjects the results were the same but in AgRP levels ($p=0.181$). Between normal weight and overweight we found differences in all groups with lower levels in Cancer group. Ghrelin levels were higher in colorectal cancer patients despite of BMI.

**Conclusions:** PYY and NPY seem to have the same regulation and not to be influenced by the BMI. On the other side, AgRP levels were higher in cancer patients only among underweight. The most important finding was that ghrelin were always higher in cancer patients despite of body mass index.

---

**The behaviour of the peptides leptin and ghrelin, PYY, NPY, CART, AgRP and MSH in gastric cancer patients**

Katia Barao¹, Tiago D. Silva¹, G.A. Ozorio¹, M.A. Vicente¹, Lila M. Oyama², Nora M. Forones¹

¹Grupo de Oncologia, Departamento de Medicina. Universidade Federal de São Paulo, São Paulo, Brasil; ²Departamento de Fisiologia. Universidade Federal de São Paulo, São Paulo, Brasil

**Backgrounds:** Peptides that regulate food intake have been studied and associated to some types of cancers. Generally, gastric cancer patients tend to be cachectic with low body mass index (BMI), since most of them have gastrointestinal symptoms such as a nausea and anorexia. The aim of this study was to investigate, in gastric cancer patients, the behaviour of some peptides that regulate food intake.

**Methods:** Serum ghrelin, leptin, PYY, NPY, AgRP, CART and MSH levels were measured by ELISA (enzyme linked immuno-sorbent assay). BMI and weight loss, were determined in all subjects studied. The patients had been classified in 3 groups: cachectic group (gastric cancer patients in treatment), non-cachectic group (gastric cancer patients that have already finished the treatment and are gaining weight) and health controls.

**Results:** among the 127 subjects studied, 19 were in the cachectic group (47 % underweight and 21 % overweight); 28 in the non-cachectic and 80 as health control. PYY, NPY and CART levels were lower in cachectic group followed by non-cachectic. Ghrelin levels, in contrast were lower in the health group ($p<0.001$). Leptin, AgRP and MSH did not show any difference between all groups.

**Conclusion:** Leptin, MSH and AgRP concentrations did not show any difference in gastric cancer patients (cachectic and non-cachetic). Despite of gastric surgery ghrelin levels were still higher in cachectic group followed by non-cachetic. The concentrations of PYY and NPY were lower while ghrelin were higher in cachectic group suggesting a possible mechanism of reversing the cachexia process.