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ABSTRACTS

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001

How does the new diagnostic criteria for protein-energy wasting in chronic kidney disease compare with the ICD-10 AM definitions?

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Background and aims: Malnutrition is associated with higher levels of morbidity and mortality in patients with CKD. The aim of this study was to determine the prevalence of protein-energy wasting (PEW) using new diagnostic criteria compared with ICD-10 AM definitions in patients with CKD.
Methods: Forty-two patients (22 male, 20 female; mean age, 65.7 (SD 17.6)years) from a private haemodialysis unit participated. To determine PEW, four categories are assessed: biochemistry serum albumin (<38 mg/l) or serum cholesterol (<100 mg/100 ml); body mass index (BMI) <23 or total body fat <10%; muscle mass, reduced mid-arm muscle mass area; and reduced dietary intake, protein <0.8 g/kg/day or energy <100 kJ/kg/day. At least three of the four categories must be present to diagnose PEW. The ICD-10 AM definitions of malnutrition include BMI <18.5 or evidence of weight loss, decreased intake and presence of fat loss and muscle wasting. This was assessed using subjective global assessment. Dietary intake was analysed using Foodworks (version 5). Fat mass was determined using bioelectrical impedance spectroscopy.
Results: Ten patients (24%) met the criteria for PEW compared to eight patients (19%) using the ICD-10 AM criteria; however, only three patients met the criteria for both definitions. Thirteen patients had low biochemistry; 14 had low BMI; 19 had low muscle mass; 21 had low intake; two had BMI <18.5; and six had evidence of weight loss, decreased dietary intake, presence of subcutaneous fat loss and muscle wasting. Time taken for the PEW diagnosis was considerably longer than ICD-10 AM.
Conclusions: The new diagnostic criteria for PEW require further validation studies in patients with CKD.

002

Practice-guiding classification of cancer cachexia in palliative cancer care (PCC): clinical pilot evaluation of the SIPP assessment tool

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Background: The understanding of mechanism and impact of cancer cachexia is evolving; a new generic definition of wasting/cachexia includes weight loss (WL) and five additional criteria. Current nutritional assessment tools identify

patients at risk for malnutrition, but guide less practice. A common clinical classification, with simple, robust assessments specific for cancer and the PCC context, is required to improve care decisions, outcomes, and clinical trial design. The aim of this study is to develop a cancer-specific, practice-guiding cachexia classification and assessment system, based on the generic cachexia definition.

Methods: Three systematic literature reviews, two focus group rounds, and a formal Delphi process among clinical academic cancer cachexia experts were conducted to reach an agreement, based on current evidence and consensus on definition, diagnosis, classification, and practical assessment of cancer cachexia. A resulting classification/assessment (SIPP system) is pilot tested in PCC clinics.

Results: Consensus on assessments is reached for domains, but not (yet) for selection of measures. The SIPP contains storage (gap of usual to current weight, WL duration, control for fluid retention or obesity, and specific nutrients' deficit), intake (anorexia, early satiety, percentage of normal intake, 1–2 days dietary record, and secondary nutrition impact symptoms), potential (tumor (catabolic) activity and C-reactive protein), and performance (performance status, cachexia-related suffering, and prognosis). Three phases (pre-cachexia (risk for cachexia), cachexia syndrome, and late unlikely-to-be-reversible cachexia) are proposed. Feasibility, content validity, and independent clinicians agreement on multidimensional interventions are promising (n=10; age, 46–72 years).

Conclusion: The SIPP system seems promising to guide practice in PCC, final agreement on assessments is required, and prospective testing to refine cancer cachexia phases and phenotypes is needed.

003
Cachexia-related suffering: development of an assessment system guiding clinical interventions

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Introduction: Interventions for cancer cachexia (CC) patients typically focus on pharmacological and nutritional approaches, rarely on the under-diagnosed CRS: psychosocial suffering related to eating and weight loss (WL). The aim of the study is to develop an assessment instrument for CRS.

Methods: Based on patients' and proxies' focus groups (Strasser, Palliat Med 21:129–137, 2007) and a systematic literature review, a CRS item pool was developed. A multidisciplinary team (nurses, nutritionists, and palliative care physicians; n=9) reviewed and rated items (agreed, not agreed, and discuss) in two rounds, resulting in 121 items. We test their face validity, completeness, and practicability (negative emotions) in a cross-sectional design, providing each patient two-item sets (n=25). Demographics include living situation, age, nationality, education, religion, BMI, cachexia staging, cognition, Edmonton Symptom Assessment Scale, Hospital Anxiety Depression Scale, subjective (0=none, 10=maximal) importance of eating, eating with a partner, amount of eating, and eating-related suffering (ERS).

Results: Thirteen patients (age, 48–74 years; five female; weight loss, 3–12.5%; HADS-A, 6; HADS-D, 9.5; ERS patient, 7.7; ERS family, 7.5) judged at 15 interviews 36 items (eight items, ≥2 patients) as insufficient comprehensible before explanation. These items contained subordinate clauses or complicated words and were reworded. Twenty-two items (four items, ≥2 patients) released desire to talk or mild emotions. Findings lacked an association to demographic data. No items about ERS/CRS are missing.

Discussion: This is the first study investigating systematically items for a subjective assessment of cachexia-related suffering. The item pool will be further refined by multicenter calibration and analysis; the resulting CRS questionnaire is likely to guide decision and practical interventions.

004
General fatigue in hemodialysis patients

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Background and aims: Hemodialysis patients suffer from generalized fatigue and lack of energy. It has been suspected that the observed excessive fatigue do not depend only on muscle atrophy and weakness but also depend on other factors. The aim of the current study was to investigate whether factors related to quality of life and general health contribute to the level of general fatigue in patients receiving hemodialysis therapy.

Methods: Fifty-two hemodialysis patients (15 female/37 male; age, 55±17; BMI, 25.4±4.4; years in dialysis, 2.4±1.2) consented to participate in this study. Hemodialysis patients were assessed for their functional capacity; body composition (DEXA); levels of central adiposity, muscle quality, and liver fat by CT; insulin resistance (OGTT); perceived quality of life parameters; and fatigue by various questionnaires.
Results: The overall quality of life score was below 70% (67±18), implying a low level of perceived life quality. Fatigue significantly correlated with gait speed (−0.397, P=0.01), exercise performance (−0.338, P=0.01), abdominal girth (−0.357, P=0.02), visceral adiposity (−0.421, P=0.01), liver fat levels (0.528, P=0.01), indices of insulin sensitivity (0.432, P=0.01), sleep diary (−0.435, P=0.01), daily sleepiness (−0.354, P=0.01), and depression (−0.526, P=0.01). Lean body mass and percentage of body fat did not correlate significantly with fatigue score, while the thigh muscle cross-sectional area correlated weakly (0.277, P=0.08).
Conclusions: Hemodialysis patients present with increased levels of generalized fatigue, leading to a considerable reduction of physical activity, impaired insulin action, and glucose disposal, implicating various metabolic alterations with a common denominator: the increased cardiovascular risk and a high mortality rate.

005
Metabolic gene expression in skeletal muscle of chronic heart failure patients: the effect of exercise training

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Background: Chronic heart failure (CHF) patients are characterized by insulin resistance and impaired energy metabolism. Adiponectin is an insulin-sensitizing adipocytokine. In CHF, high adiponectin confers poor prognosis. We investigated the expression of adiponectin, the adiponectin receptor, and genes involved in downstream lipid and glucose metabolism in the skeletal muscle of CHF patients before and after exercise training (ET).
Methods: Nine CHF patients and ten healthy subjects underwent clinical assessment, echocardiography, and CPET. Muscle biopsies (m. vastus lateralis) were taken to assess mRNA expression of adiponectin, AdipoR1, and downstream metabolic genes. The effect of combined endurance-resistance ET (ambulatory training, three sessions/week, and 60 min duration) in CHF was assessed after 4 months.
Results: Adiponectin expression in CHF patients was higher compared to healthy subjects (P<0.01), whereas AdipoR1 was downregulated (P=0.04). In addition, the expression of genes involved in lipid (PPAR-alpha, ACADM) and glucose metabolism (AMPK-alpha1, HK2) was reduced in CHF.
ET increased maximal workload and muscle strength and lowered mRNA expression of adiponectin (P=0.02), whereas AdipoR1 (P=0.01) expressed was increased to levels comparable to healthy subjects. Furthermore, both AMPK-alpha1 (P=0.02) and PPAR-alpha mRNA expression (P=0.05) were upregulated.
Conclusion: CHF patients are characterized by increased adiponectin expression and decreased expression of AdipoR1 and downstream metabolic genes. ET normalizes the expression of adiponectin and AdipoR1 and reverses the disorders in lipid and glucose metabolism. These alterations in metabolic gene expression following exercise might help to understand the beneficial effects of ET in CHF.

006
Increased proteasome activity in skeletal muscle of patients with chronic heart failure—a fluent transition to cachectic disease state?

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Background: In chronic heart failure (CHF), weight loss and the development of cardiac cachexia represent frequent late state complications. The associated muscle wasting accelerates the downward spiral of symptomatic status and particularly poor prognosis. We investigated proteasome activity in skeletal muscle as an indicator of protein catabolism in cachectic and non-cachectic CHF patients and healthy controls.
Methods: We studied 26 stable CHF patients (age, 65±2.2 years (mean±SEM); BMI, 26.3±1.4; NYHA I/II/III, n=

2/21/11) and 30 healthy controls of similar age and BMI. Muscle biopsies were obtained from the vastus lateralis of the quadriceps using the Bergstrom needle technique. The obtained samples were homogenized in lysis buffer. Of protein, 20 μg was used for the fluorogenic assay (substrate:benzyloxycarbonyl-Leu-Leu-Glu-7-amido-4-methylcoumarin-Z-LLE-AMC, Biomol), in which substrate turnover was determined. The activity was calculated by using free amidomethylcoumarin (AMC) as working standard.

Results: Proteasome activity was significantly increased in CHF patients compared to controls (0.35±0.16 vs 0.04±0.01 units/mg; p=0.038). Among CHF patients, seven subjects have been diagnosed as cachectic following the current consensus-based definition. The highest proteasome activity was observed in the cachectic subgroup (0.61±0.46 units/mg) as compared to non-cachectic CHF (0.24±0.13 units/mg) and to controls (Con: 0.04±0.01 units/mg; ANOVA, p=0.04).

Conclusion: Our study shows that proteasome activity is elevated in patients with CHF as compared to healthy subjects of similar age. This finding is in accord with the observed overall increased catabolic drive in CHF. Proteasome activity is highest in the cachectic subgroup. Increased proteasome activity may therefore be an underlying mechanism of muscle wasting in CHF.

007

Skeletal muscle measured at the time of ICU admission may be determinant of clinical outcomes

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Introduction: Patients admitted to the intensive care unit (ICU) often lose weight during hospital stay; however, specific changes in body composition features of ICU patients have not been quantified. We investigated the relationship between muscularity and length of hospital stay in ICU patients.

Methods: The charts of 54 ICU patients participating in a nutrition study were searched retrospectively for CT images that had been taken at the third lumbar vertebrae (L3) for diagnostic purposes. Of the 54 patients, 24 (14 males, ten females) had CT images taken at L3 close to the time of entry into ICU (within 14 days). CT images were analyzed for muscle cross-sectional area using Tomovision's Sliceomatic software. Previously published cutoff points (males, <55.4 cm^2/m^2; females, <38.9 cm^2/m^2) were used to identify sarcopenic patients.

Preliminary results: Sixteen of 24 patients (67%; nine males, seven females) were classified as sarcopenic near the time of admittance to ICU. Sarcopenic patients were in hospital twice as long as non-sarcopenic patients (44±48 vs 17±9 days; p=0.043) and also tended to be in ICU longer (19±20 vs 9±5 days; p=0.085).

Conclusions: Patients who are sarcopenic at the time of ICU admission are in hospital for a longer duration than non-sarcopenic ICU patients. Further research into nutritional and rehabilitation interventions in this high-risk group is warranted. Ultimately, CT scans may be used to identify patients who require nutritional intervention and may be used to evaluate such interventions.

008

Novel changes in anabolic and catabolic pathways considered to regulate mass in muscle of critically ill patients

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Background and aims: A significant clinical feature of critically ill patients (CIP) is the loss of skeletal muscle mass. We aimed to compare the expression levels of genes and proteins, purported from mainly animal work to control muscle protein synthesis and breakdown, in muscle biopsy samples from CIP and control volunteers.

Methods: Ten CIP (mean APACHE II score, 17.6±4.8 (SD)) and six control volunteers participated in a study approved by the Local Ethics Committee. Gene (real-time PCR) and protein (Western blotting) expression was determined in biopsies obtained from vastus lateralis muscle. Significance (one-way analysis of variance) was set at P<0.05.

Results: An increase in IL6 (P<0.01) and TNF-alpha (P<0.01) mRNA was seen in CIP compared to controls. There was a decline in phosphorylation of Akt1 (P<0.01), GSK3-beta (P<0.05), mTOR (P<0.01), p70s6k (P<0.01), and 4EBP1 (P<0.01) protein in CIP, which was paralleled by an increase in Akt1 (P<0.05), GSK3-beta (P<0.001), mTOR (P<0.001), p70s6k (P<0.01), and 4EBP1 (P<0.001) mRNA expression. Concurrently, MAFbx (P<0.001), MuRF1 (P<0.001), 20S proteasome (P<0.001),

cathepsin-L ($P<0.01$), and myostatin ($P<0.05$) mRNA increased in CIP and was matched by an increase in MAFbx ($P<0.05$), MuRF1 ($P<0.05$), 20S proteasome ($P<0.05$), and myostatin ($P<0.001$) protein expression.
Conclusions: This study shows that a decrease in anabolic signaling protein phosphorylation occurs in CIP, which was accompanied by increased mRNA expression of the genes encoding these proteins. Additionally, CIP presented with increased MAFbx, MuRF1, 20S proteasome, and myostatin mRNA expression, which was paralleled by increased expression at the protein level.

009
Muscle wasting in patients with chronic obstructive pulmonary disease (COPD)

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Background and aims: Malnutrition is frequent in COPD patients. However, little is know about the implications of the depletion in different body compartments and dietary intakes on pulmonary function.
Patients and methods: Consecutive outpatients with stable COPD were included. Variables include age, tobacco consumption, body mass index (BMI), muscle mass estimated by mid-arm circumference and bioelectrical impedance (fat-free mass index (FMMI)), fat stores measured by triceps skin-fold thickness (TSF), muscle function by handgrip strength (HG), pulmonary function, dyspnoea degree, number of exacerbations last year, food intake assessed by 24-h recall and food questionnaire and energy and protein requirements.
Results: Ninety-six patients were included (95% men), with a mean age of 62±21 years and smoking habits of 59.9±31.6 packets/year. Of patients, 4.2% had low BMI, although muscle wasting was present in 5% by MAMC and 37% by FFMI. Fat depletion was present in 23% by TSF. Muscle wasting was present in 79.3% of normal weight patients and in 19% of overweight. Loss of muscle mass correlated with pulmonary function (FFMI with FEV1/FVC, $r=0.238$, $p=0.021$; MAMC with FEV1, $r=0.211$, $p=0.041$). HG and TSF correlated with morbidity (dyspnoea degree, $r=-0.61$, $p<0.001$; number of exacerbations, $r=-0.282$, $p=0.012$). Protein intake was reduced in 77.1% of patients with FEV1<50% compared with 58.0% in those with FEV1 >50%, $p=0.0026$.
Conclusion: Muscle wasting and low fat stores were more prevalent and linked with functional capacity and outcome in COPD patients than low body weight. Protein requirements are compromised and need dietetic counseling.

010
Acute phase response (APR) cachexia and systemic zinc redistribution

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Background and aims: Several diverse factors such as Ang-II, PIF, LPS, TNF-α, IL-6, and conditions such as chronic inflammation, sepsis, and cancer induce cachexia. We have hypothesized that systemic redistribution of Zn and its accumulation in muscle tissue plays a key role in muscle wasting. To help assess the validity of this hypothesis, we suggest a link between cachexia inducing conditions/factors and zinc redistribution.
Methods: The hypothesis is based on data on the fluctuation and regulation of Zn homeostasis in animal and man and the extensive literature on acute phase response (APR).
Results: APR is the host's normal acute response to trauma, inflammation, and infection. APR is characterized by protein catabolism and appears to play a central role in cachexia, but APR's mechanism of action in cachexia is unresolved[1]. APR's salient characteristics include the steep decline of serum zinc levels and the redistribution of internal zinc stores[2,3]. Several factors mediate APR, and at least TNF-α, IL-6, and LPS significantly reduce serum zinc levels and drive the redistribution of internal zinc stores.
Conclusion: Chronic APR is a well-known host defense mechanism commonly observed in cachexic patients. APR is characterized by significant Zn redistribution and hypozincemia. We have hypothesized earlier that in cachexia, systemic Zn redistribution and hypozincemia cause anorexia, growth failure in children, inflammation, insulin resistance, and hypogonadism. We suggest that chronic APR may explain the mechanism behind these symptoms. APR also suggests a mechanism for how Zn is mobilized for PKR-mediated muscle wasting (see Figure).
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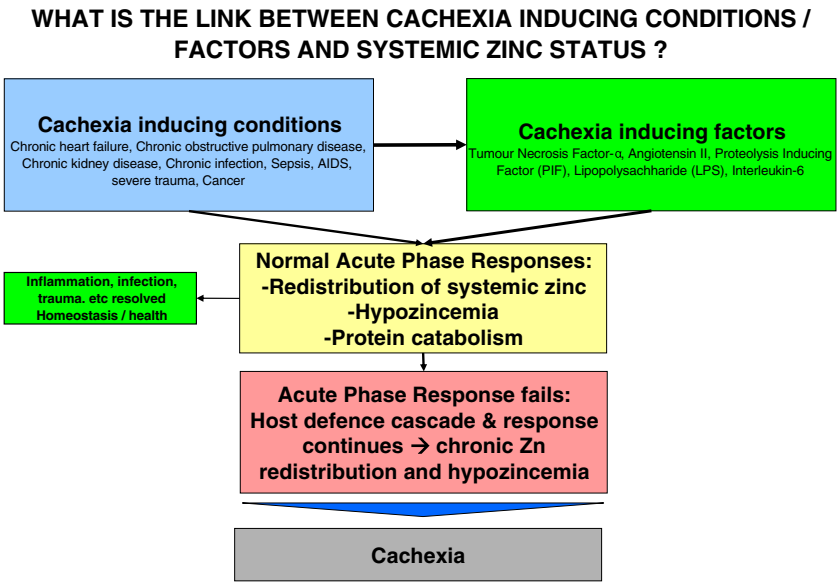

011

Flexible modeling improves assessment of prognostic value of C-reactive protein in advanced non-small cell lung cancer

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C-reactive protein (CRP) is gaining credibility as a prognostic factor in different cancers. A conventional Cox's model is usually used to assess prognostic factors. However, this model imposes a priori assumptions that (1) the hazard ratio associated with each prognostic factor remains constant across the entire follow-up interval (PH assumption), and (2) the relationship between the continuous predictor and the logarithm of the mortality hazard is linear (linearity assumption), which are rarely tested. We tested these assumptions for CRP, using a flexible statistical model, while adjusting for other known prognostic factors, in a cohort of 269 patients newly diagnosed with non-small cell lung cancer (NSCLC). In the conventional Cox's model, high CRP increased the risk of death (HR=1.11 per each doubling of CRP value; 95% CI, 1.03–1.20, p=0.008). However, both the PH assumption (p=0.033) and the linearity assumption (p=0.015) were rejected for CRP measured at the initiation of chemotherapy, which kept its prognostic value for only about 18 months. Our analysis demonstrates that flexible modeling provides new insights about the value of CRP as a prognostic factor in NSCLC, and that a conventional Cox's model underestimates early risks associated with high CRP.

012

Studies on intestinal blood flow in patients with chronic heart failure compared to healthy control subjects

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Introduction: Chronic heart failure (CHF) is characterized by reduced circulatory blood flow due to low cardiac output, sympathetic activation, and impaired vasodilator capacity. Impaired tissue perfusion may likely occur as well in the intestinal vascular bed. This hypoperfusion may contribute to impaired intestinal barrier function, increased bacterial translocation, chronic inflammation, malnutrition, and cachexia as observed in CHF. Intestinal arterial blood flow in comparison to limb blood flow has not yet been studied in CHF.
Methods: We prospectively investigated 16 patients with CHF (age, 64.8±2.3 years; LVEF, 29.3±1.5%; peak VO_2, 15.7±1.2 mL/kg/min; NYHA class, 2.3±0.1; BMI, 27.2±

1.2 kg/m^2) and 17 control subjects of similar age and gender (peak VO_2, 27.1±1.6 mL/kg/min; LVEF, 66.4±2.1%; BMI, 26.4±1.2 kg/m^2). Forearm blood flow at rest and post-ischemic peak blood flow were assessed by venous occlusion plethysmography. Intestinal peak systolic blood flow was calculated from peak systolic velocity and vessel diameter of the mesenteric arteries, as well as of the coeliac trunk using high-resolution ultrasound and Doppler sonography. We assessed small intestinal permeability by lactulose–mannitol test, bowel wall thickness by transcutaneous sonography, and mucosal bacterial biofilm by FISH in biopsies taken during sigmoidoscopy.

Results: In CHF, we found a lower intestinal peak systolic blood flow in the inferior mesenteric artery in CHF patients compared to controls (6.03±0.72 vs. 8.40±0.74 mL/s, $p<0.04$). Systolic blood flow in the coeliac trunk in CHF patients was 39.38±6.45 vs. 56.20±6.20 mL/s in controls ($p=0.09$). Intestinal systolic blood flow of the superior mesenteric artery was similar in CHF and control subjects (42.46±4.28 vs. 47.47±4.06 mL/s, $p=0.4$). CHF patients had lower forearm resting blood flow (2.83±0.41 vs. 4.74±0.86 mL/100 mL/min, $p<0.05$) compared to controls and lower peak post-ischemic blood flow (13.84±1.53 vs. 19.57±2.01 mL/100 mL/min, $p=0.03$), indicating impaired vasodilator capacity. Furthermore, CHF patients showed a lower blood mid-regional proadrenomedullin concentration as a biomarker of impaired endothelial function (0.82±0.09 vs 0.51±0.02 nmol/L, $p=0.003$). Impaired intestinal blood flow in the inferior mesenteric artery was in accord with previously observed greater bowel wall thickness (all $p<0.02$ for all bowel segments), increased paracellular intestinal permeability, and higher concentrations of adherent bacteria within mucus on biopsies taken during sigmoidoscopy in patients compared to controls (permeability index 0.022±0.002 vs 0.016±0.002, $p<0.05$, and 230×106/mL (1×106/mL and 80,320×106/mL) vs. 0.1×106/mL (0.1×106/mL and 290×106/mL), $p=0.02$), respectively.

Conclusion: Impaired organ and tissue perfusion in CHF occur in the limb (forearm) and in the intestinal vascular bed. Mesenteric malperfusion may contribute to intestinal hypoxia and may hence underlie increased bacterial translocation with chronic inflammation, malnutrition, and cachexia in CHF.

013
Increased adipose tissue catabolism, insulin resistance and chronic heart failure

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Background: Impaired insulin sensitivity (IS) is a common finding in chronic heart failure (CHF) patients contributing to symptomatic status and mortality. Impaired glucose metabolic efficiency is associated with increased fatty acid utilisation. We tested the hypothesis that adipose tissue catabolism is increased in CHF patients which might contribute to impaired IS.

Methods: After an overnight fast, adipose tissue metabolism was studied by microdialysis on CHF patients and in age and body weight-matched healthy controls (both $n=8$) at rest and during an oral glucose tolerance test (oGTT). Dialysates were analysed for glucose, lactate, glycerol and ethanol in order to assess changes in tissue metabolism and perfusion (ethanol dilution technique).

Results: Serum glucose and insulin profiles and also baseline and postprandial tissue perfusion and glucose supply did not differ significantly between CHF patients and controls (all $p>0.2$). In contrast, baseline adipose tissue dialysate (lactate) was 0.51±0.10 and 0.26±0.06 mmol/l ($p<0.01$) and (glycerol) 116±18 and 50±8 μmol/l ($p<0.001$) in CHF patients and controls, respectively, indicating increased adipose tissue glycolysis and lipolysis. During oGTT, adipose tissue dialysate (lactate) increased by about 50% and 160%, and dialysate (glycerol) decreased by about 60% and 50% in CHF patients and controls, respectively, but values were always significantly higher in CHF patients vs. controls (all $p<0.05$).

Conclusion: Our data indicate increased catabolic stimulation in adipose tissue of CHF patients and increased anaerobic drive at rest and after an oral glucose load. Increased lipid mobilisation may contribute to impaired glucose utilisation and insulin resistance observed in CHF patients.

014
Adipose tissue—a special target organ in patients with chronic heart failure

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Adipose tissue in case of CHF and especially cardiac cachexia obtains new qualities and changes regulation mechanisms.

Aim: The aim of this study is to evaluate weight, BMI, levels of leptin in blood, lean mass, and percentage of adipose tissue in patients with CHF I-IV FC NYHA.
Methods: In 60 patients with CHF of ischemic genesis, 10 patients have I FC, 15 have II FC, 15 have III FC, and 20 have IV FC. Height, weight, BMI, lean mass, percentage of adipose tissue (Durnin methodic) and leptin, and NT-pro-BNP in plasma were evaluated. At baseline, level of NT-pro-BNP was evaluated.
Results: Patients with CHF III–IV FC were admitted to hospital due to decompensation of CHF and have a level of NT-pro-BNP >3,000 ng/l; patients with CHF I–II FC were admitted to hospital due to arterial hypertension. At baseline, BMI includes I FC, 31.8±2.1 kg/m^2; II FC, 31.6±2.7 kg/m^2; III FC, 28.4±1.9 kg/m^2; and IV FC, 25.8±2.3 kg/m^2. Lean mass includes I FC, 56.8±4.5 kg; II FC, 55.7±3.8 kg; III FC, 58.4±4.3 kg; and IV FC, 56.7±4.2 kg. Percentage of adipose tissue includes I FC, 32.3±2.3%; II FC, 34.1±2.5%; III FC, 26.9±2.1%; and IV FC, 18.9±1.9%. Leptin level includes I FC, 12.3±3 ng/ml; II FC, 24.1±2.1 ng/ml; III FC, 36.9±2.5 ng/ml; and IV FC, 58.9±1.1 ng/ml.
Conclusions: In patients with III-IV FC CHF, we found mainly fat mass lost in association of leptin increase.

015
Morphology changes of adipose tissue in patients with chronic heart failure

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Chronic heart failure (CHF) is one of the most frequent causes of cardio-vascular death. Patients with end stage of CHF usually develop cardiac cachexia, which associated with very poor prognosis.

Aim: The aim of this study is to evaluate morphology and histological changes in adipose tissue in CHF I–IV FC (autopsy material).
Methods: Forty-five autopsies with CHF of ischemic genesis were analyzed: 10 cases, I FC; 10, II FC; 10, III FC; and 15, IV FC. Course of death in patients with CHF of III–IV FC was progression of heart failure, in patients with CHF of I–II FC, acute coronary syndrome. Thickness of adipose tissue (2 cm below umbilical), weight of sealing gland, and histological analysis (morphometria, collagen percent, and level of lymphocyte infiltration) of adipose tissue were performed.
Results: Thickness of adipose tissue (2 cm below umbilical) includes I FC, 2.9±0.7 nm; II FC, 3.6±0.6 nm; III FC, 3.05±0.5 nm; and IV FC, 1.35±0.4 nm. Weight of sealing gland includes I FC, 378±89 g; II FC, 511±104 g; III FC, 319±76 g; and IV FC, 84.5±44 g. In histological analysis, in autopsy cases of CHF III–IV FC lymphoid infiltration, collagen accumulation in perivascular spaces was found.
Conclusions: In patients with III–IV FC of CHF, not only amount of adipose tissue changes but also morphology, with development of fibrosis, inflammation, and possibly apoptosis of adiposities.

016
Putative role of zinc-alpha 2 glycoprotein (ZAG) in lipolysis in cardiac cachectic patients

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ZAG is a serum factor involved in adipose tissue metabolism, and higher levels have been reported in cancer cachectic patients. Altered mitochondrial function and beta-3 adrenergic receptor pathway activation have been proposed as potential mechanisms of ZAG effects. Our study aimed at evaluating the role of ZAG in cardiac cachexia (CXC) and at determining its relationships with adipose metabolism in a cohort of chronic heart failure patients. Sixty-four advanced heart failure (AHF, NYHA functional class III–IV) patients have been recruited in a tertiary university hospital over a period of 4 years, 18 of whom showed CXC with an unwilling and oedema-free reduction in body weight of more than 7.5% in the last 6 months. Abnormal thyroid function, previous or current cancer disease, severe chronic obstructive pulmonary and severe renal or hepatic failure have been excluded. No significant differences in age, gender proportion, ejection fraction and blood pressure between the two groups have been observed. Higher plasma free fatty acid (FFA) concentrations were measured in CXC patients as compared to AHF; ZAG serum concentration (ELISA, Biovendor, Germany) was similar in CXC and AHF, both groups showing higher plasma levels versus an age-matched healthy group of subjects. ZAG and FFA levels showed a positive correlation in CXC patients (R^2, 0.29; p=0.02), whereas such a relationship was missing in AHF group. Since there are no differences in ZAG values between CXC and non-CXC AHF groups, these preliminary data seem to

suggest an increased sensitivity to ZAG in stimulating lipolysis in CXC.

017

Zinc-alpha2-glycoprotein in adipose atrophy in human cancer cachexia

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Background: Profound loss of adipose tissue (AT) is a hallmark of cancer cachexia, but the underlying mechanisms remain elusive. Zinc-alpha2-glycoprotein (ZAG), a recently identified adipokine, has been suggested as a candidate in the regulation of adiposity. We investigated the potential role of ZAG in adipose atrophy in human cancer cachexia.

Methods: Twenty-five gastrointestinal (n=12) and pancreatic (n=13) cancer patients with cachexia (mean weight loss being 14.7%) or without were recruited. ZAG mRNA levels (real-time PCR) and protein expression (Western blotting) were assessed in subcutaneous AT. Tissue morphology was examined using light microscopy. Serum ZAG levels were quantified by ELISA. The effect of recombinant ZAG on lipolysis was evaluated in human primary adipocytes.

Results: AT remodelling in cancer cachexia patients (weight loss ≥5%) was characterised by shrunken adipocytes with increased fibrosis evident by strong collagen-fibril staining in the tissue matrix. ZAG mRNA levels in AT were increased in cancer cachexia (2.7-fold, P=0.028). Concurrently, ZAG protein expression was enhanced in cachectic cancer patients. ZAG mRNA showed a negative correlation with BMI (r=−0.43, P=0.016), but a positive correlation with weight loss (r=0.48, P=0.033). Furthermore, ZAG mRNA was positively correlated with serum glycerol levels (r=0.58, P=0.0026). Serum ZAG levels did not differ between weight-losing and weight-stable patients. In vitro, ZAG production by differentiated adipocytes was abundant. Treatment with recombinant ZAG stimulated glycerol release by human adipocytes.

Conclusions: Our results suggest that adipokine ZAG is involved in adipose atrophy in human cancer cachexia. This could be, at least partly, attributable to its lipid-mobilising effect in human adipocytes.

018

Plasma retinol binding protein 4 (RBP4) but not its adipose expression is associated with systemic inflammation and monocyte TNF-alpha expression in non-diabetic dialysis patients

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Background and aims: Adipose-secreted retinol binding protein 4 (RBP4) circulates in free and transthyretin (TTR)-bound forms and may be associated with obesity-related inflammation. Involvement of RBP4 and its adipose expression in systemic inflammation independently of obesity and diabetes is unknown. Inflammation occurs, impairs nutritional state, and reduces survival in chronic kidney disease (CKD; particularly in hemodialysis (MHD), and plasma RBP4 may increase with renal dysfunction.

Methods: We determined whether plasma RBP4 changes are associated with CKD-related systemic inflammation and whether adipose RBP4 expression contributes to plasma RBP4 elevation and inflammation independently of obesity and diabetes. Plasma and adipose RBP4, systemic inflammation (plasma CRP), and passive RBP4 accumulation (creatinine and TTR) were measured in non-obese, non-diabetic CKD undergoing conservative (CT, n=10) or MHD treatment (n=25), controls (C, n=11), and renal transplant recipients (n=5).

Results: Plasma RBP4, TTR, and CRP increased with renal dysfunction and were highest in MHD (P<0.05). Adipose RBP4 mRNA was however comparably low in CT and MHD (P<0.05). All parameters were normalized in transplant recipients (P<0.05 vs MHD). In all subjects (n=51), creatinine and TTR (P<0.05) but not RBP4 mRNA were related to high plasma RBP4. Plasma RBP4 was associated positively (P<0.05) with CRP independently of creatinine-TTR in all subjects (n=51) and with blood cell TNF-alpha mRNA and TNF/IL10 ratio in MHD.

Conclusions: Clustering of RBP4 and inflammation occurs in MHD independently of obesity, diabetes, and renal dysfunction; could involve monocyte TNF-alpha expression; and is normalized by transplantation. Involvement of adipose tissue RBP4 in CKD-associated inflammation is not supported by these results.

019

Loss of adipose tissue and plasma phospholipids: relationship to survival in advanced cancer patients

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Background: Loss of adipose tissue and low plasma phospholipids (PL) are features of cancer cachexia. It is not known whether these processes coincide across the cancer trajectory nor has their relationship with survival been defined. Changes in adipose tissue mass and plasma PL were characterized during the 500 days prior to death and prognostic significance assessed.
Methods: Adipose tissue (AT) rate of change (percent/100 days) was determined using two lumbar computed tomography scans taken in the last 500 days of life in a retrospective cohort of patients who died of colorectal and non-small cell lung cancers (n=108). Plasma PL fatty acids were measured prospectively in a similar cohort of patients with metastatic cancer (n=72). Cutpoint analysis was used to classify patients into high vs low risk of death. Multivariate Cox regression was conducted to determine survival.
Results: Accelerated loss of AT (subcutaneous, visceral, and intramuscular) begins at 7 months from death reaching average losses of 29%/100 days of total, which equates to a 1,100-kcal (4,600 kJ) per day energy deficit. Plasma PL fatty acids were 35% lower in patients closest to death vs those surviving >8 months (p=0.003). Median survival was 140 days shorter in subjects with n-3 fatty acids in plasma PL below the cutpoint (RR=2.1, p=0.004). Loss of PL fatty acids and adipose tissue occurs in tandem, and both are predictive of survival.
Conclusion: Depletion of plasma PL indicates a deficit of essential fatty acids in the periphery, which may contribute to loss of adipose tissue mass.

020

Prognostic effect of body weight and involuntary weight loss in cancer patients with solid tumours: an updated, population-based scoring system

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Background: Existing guidelines to define clinically important weight loss in cancer patients are heterogeneous, do not consider current trends in body weight, tend to lack a point of reference (habitual body weight or time frame), and as a result are often confusing for clinicians. We aim to characterize the relationship of body mass index (BMI) and weight loss to overall survival and develop a population-based scoring system.
Patients and methods: Newly diagnosed cancer patients completed a standardized nutrition assessment. Prospectively collected data included self-reported height, weight, 6-month weight loss history, and functional status. The relationships of BMI and percent weight loss to overall survival were defined and entered into a multivariate analysis controlling for age, gender, diagnosis, cancer stage, and functional status. Scores for BMI and percent weight loss were assigned proportional to their prognostic weights.
Results: Patients (n=2214) were overweight (mean BMI, 25.5) and weight losing (7.0% in past 6 months). Risk of death increased with decreasing BMI and increasing weight loss. Variables independently prognostic at the multivariate level included age (p<0.001), gender (p=0.01), diagnosis (p<0.001), cancer stage (p<0.001), functional status (p<0.001), BMI (p=0.004), and percent weight loss (p<0.001). Scores for BMI (≥22.0=0, <22.0=1) and percent weight loss (<0.0–7.9%=0, 8.0–13.9%=2, 14.0–19.9%=3, and ≥20.0%=4) were summed (range, 0–5), and four risk groups with significantly different (p<0.001, log-rank test) survivals were defined.
Conclusion: An updated, population-based scoring system for BMI and percent weight loss effectively separates patients with different survivals, and may be useful for making decisions regarding treatment and initiation of clinical trials.

021

Two stage specific prognostic indices to estimate survival of patients with non-small cell lung cancer (NSCLC)

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Objective: The objective of this study is to improve prognostification of patients with advanced non-small cell

lung cancer (NSCLC) by creating two prognostic indices (PIs), one for stage 3 and one for stage 4.
Methods: We studied 266 patients with NSCLC. We did two separate analyses for stage 3 and 4 with follow-up to 24 and 12 months, respectively. Cox proportional hazards regression models were used to create the PIs and Kaplan–Meier curves to estimate each PI.
Results: The patients' mean age was 65±11.04 years, and the numbers of patients in stage 3 and stage 4 were 26% (69/266) and 74% (197/266), respectively. The multivariate analysis for stage 3 revealed that a lactate dehydrogenase (≥300) and C-reactive protein concentrations (>10 mgl^{-1}) were associated with an increased risk of death, while for stage 4, C-reactive protein (>10 mgl^{-1}) absolute neutrophil count (≥11), ECOG (2 and 3), lactate dehydrogenase (≥300), and albumin (≤35) were associated with an increased risk of death. The PIs, for patients with stage 3 and 4, were constructed using the significant predictors divided into three categories {0, 1, 2}. The median (95% CI) [p value] survival in months for stage 3 with a PI=0, 1, and 2 were >24 (13.8, −), 8 (8.5, 18.23), and 6.8 (2.1, −) [$p<0.001$] and for stage 4 were >24 (9.7, −), 9.2 (7.1, 10.7), and 3.7 (2.0, 4.9) [$p<0.0001$], respectively.
Conclusion: Two different prognostic indices, one for each stage (3 and 4), may be a better way to estimate survival of patients with advanced NSCLC.

022
Obesity paradox in type 2 diabetes mellitus (DM) of the PROactive study population: weight reduction but not weight gain is associated with increased mortality in patients with type 2 DM and high cardiovascular risk

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Background: Weight reduction is a recommended treatment goal in type 2 diabetes mellitus (DM) to improve metabolic balance and reduce cardiovascular risk. In contrast, the so-called obesity paradox suggests reduced mortality in overweight patients with pre-existing chronic cardiac disease. The impact of weight and weight change in a type 2 DM population with pre-existing cardiovascular disease on total mortality is analysed in the PROactive study population.
Methods: PROactive was a double-blind, placebo-controlled cardiovascular outcome study including 5,238 patients with type 2 DM and evidence of pre-existing cardiovascular disease. Patients were randomized to Pioglitazone (n=2605) or placebo (n=2633) in addition to their concomitant glucose-lowering and cardiovascular medication. Mean follow-up was 34.5 months. Baseline weight and percent weight change were analysed in univariable and multivariable models to predict all-cause mortality.
Results: Documented weight reduction during the study period was associated with increased mortality in the total study population (HR per 1% weight loss, 1.14 (95% CI, 1.11–1.16)), as well as in both treatment groups separately in univariable Cox analysis (Pioglitazone, HR 1.13 (1.10–1.16); placebo, HR 1.14 (1.11–1.17); all $P<0.0001$). In contrast, weight gain was not associated with increased mortality. In multivariable Cox proportional analysis including further cardiovascular risk factors (HR 1.13 (1.11–1.15), $P<0.0001$), weight reduction but not weight gain remained a significant predictor of increased mortality risk. In Cox proportional analysis of body mass index (BMI) at baseline, the lowest risk for all cause mortality was observed for patients with BMI 30–35 kg/m^2. In comparison, patients with BMI <22 (HR 1.93 (0.90–4.14), $P=0.09$) and BMI 22–25 (HR 1.53 (1.04–2.25), $P=0.03$) had an increased risk of all-cause mortality.
Conclusion: Weight reduction but not weight gain is associated with increased all-cause mortality in patients with type 2 DM and pre-existing cardiovascular disease. This suggests the presence of the obesity paradox in patients with type 2 DM who have a high risk of cardiovascular events.

023
Serum creatinine as a surrogate of muscle mass and 5-year survival in over 130,000 long-term hemodialysis patients: 2001 to 2006 national US cohort

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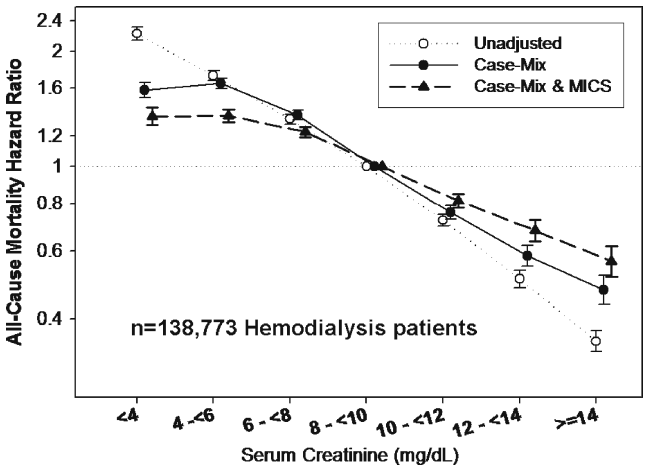
Background: In long-term hemodialysis (HD) patients with minimal to no residual renal function, serum creatinine measured prior to each HD treatment is a reflection of muscle mass. We hypothesized that higher pre-HD serum creatinine intake is an incremental predictor of greater survival.

Methods: Using Cox survival models, we examined survival predictability of monthly measured pre-HD serum creatinine—averaged into calendar quarterly values—in 138,773 patients undergoing thrice-weekly MHD in all DaVita clinics during the period July 2001 to June 2006, after adjustment for case-mix and measures of malnutrition-inflammation cachexia syndrome (MICS).
Results: HD patients were 61.9±15.5 years old and included 45% women, 32% African Americans, 15% Hispanics, and 50% diabetics. Higher serum creatinine values were incrementally and linearly associated with greater survival in all models, within any selected period of time (1- to 5-year cohorts) and after virtually any level of multivariate adjustment (see Figure):



Conclusions: Assuming higher serum creatinine is a surrogate of larger muscle mass in HD patients; larger muscle mass is incrementally and linearly associated with greater survival in this patient population. Nutritional trials to examine this effect of changes in muscle mass on survival are warranted.

024
Prognostic factors in advanced cancer patients: refinement of survival prediction using nutritional variables

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Purpose: The purpose of this study is to determine nutritional variables prognostic of survival in advanced cancer patients and to assess their predictive ability in the development and validation of a prognostic scoring index (PSI).
Patients and methods: In this prospective nested cohort study, advanced cancer patients were accrued from different units of a Regional Palliative Care Program. Patients completed a standard nutritional assessment upon admission. Variables collected included age, sex, cancer site, functional status, and nutrition features (height, weight, weight history, dietary intake, and 13 nutrition impact symptoms). These variables were evaluated using univariate and multivariate analyses, and those variables that were independently prognostic at the multivariate level were assigned PSI scores proportional to prognostic weight.
Results: A training set of patients in a palliative home care service (n=1164) was used to identify prognostic variables. A U-shaped relationship was seen between survival and percent weight change, with increased risk of death with increasing weight loss, or with short-term weight gain, compared with stable weight. Obesity was protective, and risk of death increased for BMI.
Conclusion: More accurate prognostication is feasible and can be achieved by combining clinical and demographic features with relevant nutritional features related to food intake and body weight.

025
Cachexia in patients with chronic pancreatitis is different from cachexia in pancreatic cancer

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Background and aim: Pancreatic cancer and chronic pancreatitis are the most common reasons for operative treatments on the pancreas. Cachexia, defined as loss of more than 10% of stable weight, in pancreatic cancer has a significant influence on resection rate and survival. The aim of the study was to compare the prevalence of cachexia in chronic pancreatitis and cancer patients with a special interest to show differences.
Methods: In 605 patients who underwent operation in the Department of Surgery, University of Heidelberg—437 with

pancreatic cancer and 168 with chronic pancreatitis—an evaluation of the preoperative clinical course and perioperative data was available.

Results: Cachexia was present in 42% of CP and 37% of cancer patients. Although the amount of weight loss did not differ significantly, there was a more pronounced systemic effect of cachexia in PDAC patients, with significantly reduced protein, albumin, and hemoglobin levels and elevated CRP levels. Cachexia developed over a significantly longer period in CP patients. Cachexia in cancer was significantly associated with metastases leading to a significantly reduced resection rate. With or without tumor resection, cachexia had an ongoing effect on survival.

Conclusions: Although there are significant effects on nutritional status, neither morbidity nor 30-day mortality is increased in patients with cachexia. There is a similar prevalence of cachexia in patients with CP and PDAC and no difference in the amount of weight loss between the groups, but many more significant systemic effects are detected in cancer patients with cachexia.

026

Does the new operational definition of cachexia (ODC) help with profiling nutrition and performance characteristics in advanced cancer patients?

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Background and aims: The aim of the study is to compare the use of the ODC (Evans, 2008) with the recording of weight loss (WL) for profiling nutrition and performance characteristics of advanced cancer patients (ACP).

Methods: Two-hundred fourteen newly diagnosed ACP with non-small cell lung and gastrointestinal primaries were categorized as *cachectic* according to the ODC (cachexia=WL ≥5% over ≤12 months (or BMI <20) plus ≥3/5 criteria including decreased muscle strength (hand grip), fatigue (≥3/10 on ESAS), anorexia (≥3/10 on ESAS), sarcopenia by DXA, and abnormal biochemistry (CRP >5.0 mg/l or IL-6 >4.0 pg/ml or Hgb <12 g/dl or albumin <3.2 g/l)) or as *weight losing* if they had >5% WL over ≤6 months.

Results:

	Cachectic vs. non cachectic patients		WL vs. non-WL patients	
	β^a	95% CI	β^a	95% CI
McGill QOL score 0–10 (0 worst)	−1.54	−2.18 to −0.89	−0.95	−1.64 to −0.26
ESAS QOL score 0–10 (10 worst)	1.53	0.80 to 2.26	1.07	0.32 to 1.81
ECOG PS 0–4 (4 worst)	0.75	0.46 to 1.03	0.60	0.31 to 0.89
Quadriceps ext 60°/s peak torque (Nm)	−21.88	−42.08 to −1.68	−20.32	−41.72 to 1.07
Total fat mass by DXA (kg)	−8.05	−12.60 to −3.51	−8.74	−13.04 to −4.44
Percent body fat by DXA	−6.95	−11.57 to −2.32	−7.64	−12.03 to −3.25
	HR[a]	95% CI	HR[a]	95% CI
Survival (weeks)	1.94	1.36 to 2.75	1.85	1.28 to 2.66

[a]Controlling for gender, age, diagnosis, treatment (radio/chemo), survival, and medications

Conclusions: The ODC does not appear to have diagnostic and prognostic advantages over the simple recording of WL.

027

The cachexia score: a new tool for staging of cachectic patients

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According to a recent consensus, cachexia is defined as "a complex metabolic syndrome associated with underlying illness and characterized by loss of muscle with or without loss of fat mass. The prominent clinical feature of cachexia is weight loss in adults (corrected for fluid retention) or growth failure in children (excluding endocrine disorders). Anorexia, inflammation, insulin resistance, and increased muscle protein breakdown are frequently associated with cachexia. Cachexia is distinct from starvation, age-related loss of muscle mass, primary depression, malabsorption, and hyperthyroidism and is associated with increased morbidity" [1]. Although this definition is accompanied by diagnostic criteria, it does not consider the problem of staging. Indeed, stratification of patients is important when

considering therapy. In fact, in the very first stages of wasting, body weight loss is not necessarily involved—a state known as pre-cachexia. The aim of the present score is to overcome the problem of patient staging. The score considers five main different factors: body weight and lean body mass loss, anorexia, inflammatory, immunological and metabolic disturbances, physical performance, and quality of life. The score's scale goes from 0 to 100: mild cachexia (less than 25), moderate (more than 25 and less than 50), severe (more than 50 and less than 75), and terminal phase (more than 75 and up to 100). The score also takes into consideration pre-cachexia. In conclusion, the present score will facilitate cachexia staging and therefore will allow for a more convenient therapy.

[1] Evans WJ, Morley JE, Argilés J, Bales C, Baracos V, Guttridge D, Jatoi A, Kalantar-Zadeh K, Lochs H, Mantovani G, Marks D, Mitch WE, Muscaritoli M, Najand A, Ponikowski P, Rossi Fanelli F, Schambelan M, Schols A, Schuster M, Thomas D, Wolfe R, Anker SD (2008) Cachexia: a new definition. Clin Nutr 27(6):793–799

028
Cachexia: a public health perspective

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Background: Cachexia and body wasting is encountered in advanced stage of many malignant and non-malignant chronic disease. Once present, it is associated with grim prognosis. The therapeutic approach to cachexia to date must be considered as undefined, with few exceptions. Public health primarily seeks for population-based strategies to tackle diseases with major impact on health care system.
Epidemiology and management challenges: Several definitions with different criteria and cut-offs were used, and only recently, a consensus for clinically relevant cachexia was reached. Epidemiological data is therefore speculative, based on chronic disease prevalence and assumption that 10–80% of patients, depending on the condition and standards of care, eventually develop cachexia. Geographical variations in prevalence of chronic disease are substantial and conform with socioeconomic status and available medical care. Our management approach should therefore consider all circumstances including those beyond the chronic disease and adopt the interventions with greatest impact potential for selected community. Fighting infectious disease and poverty is important and would transiently decrease cachexia patient pool. However, since chronic disease continuum is more and more present in developing countries, this will eventually be counter- or ever over-balanced, and cachexia will presumably remain an important public health issue. It is therefore even more important to intensify our therapeutic efforts to stop or even to reverse the deadly cascade of events leading to cachexia and improve the quality of life and clinical outcomes for our patients.
Clinical implications: Cachexia meets several criteria that need to be addressed by public health practitioners. Preventive and educational strategies, particularly in communities with high prevalence of HIV infection, malnutrition, and rheumatic fever-related cachexia, may be beneficial. Public health strategies in developed countries where cachexia is mainly related to cancer, chronic cardiopulmonary, and kidney disease need to target other issues than in non-developed countries.

029
Anti-tumor and antioxidant effects of antioxidant nutrients in breast cancer-implanted rats

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Background and aims: In the present study, we examined the anti-tumor and anti-oxidative stress effects of antioxidant nutrients in breast cancer-implanted rats, in order to investigate the validity of antioxidants-containing enteral formula.
Materials: Female F344 rats were divided into two groups: control diet and antioxidant (AO) diet. After 7 days of feeding each nutrient diet, breast cancer Mat B-III cells were transplanted subcutaneously, and each diet was continued until sacrifice in each group. The body weights, intake volume of each diet, and tumor size were measured daily, and the rats were sacrificed on post-transplantation days 7 and 14. Blood samples were collected to measure serum total protein, albumin, tumor necrosis factor-alpha (TNF-alpha), 2,2′-azinobis-(3-ethylbenzthiazoline sulphonate) (ABTS) radical-scavenging activity, superoxide dismutase (SOD), and catalase (CAT) activity. These parameters were compared between

the two groups by Student's t test and Dunnett test, and the statistical significance was defined as $p<0.05$.
Results: Nutrient intake decreased in the control diet group significantly on days 1, 3, 4, and 11 after transplantation. The size of implanted tumor increased from post-implantation day 4 in both groups, however, significantly lower in AO diet group. There was a significant correlation between SOD and CAT activities in the AO diet group ($r=0.88$, $p=0.02$), but not in the control diet group ($r=0.12$, $p=0.78$).
Conclusion: Our results suggest that the effects of nutrition with antioxidants in breast cancer-bearing rats contribute to a suppression of cancer growth and cachexia by maintaining the balance of reactive oxygen scavenging enzymes.

030
Cancer cachexia impairs heart function and alters cardiac muscle structure in mice

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Background and aims: Cachexia is a common syndrome in advanced cancer patients and significantly impairs quality of life. It remains elusive whether cancer cachexia causes heart failure. In this study, we investigated the effect of cancer cachexia on heart function and cardiac muscle structure in a mouse model for cancer cachexia.
Methods: Male CD2F1 mice were inoculated with either 1×106 colon-26 adenocarcinoma cells (tumor group) or vehicle (PBS; no tumor group). Another group of mice received PBS and was pair-fed to tumor group. An indicator of heart function, fractional shortening was measured in vivo using transthoracic M-mode echocardiography. At necropsy (day 17 after tumor/PBS inoculation), hearts were collected for histology and qRT-PCR analysis.
Results: Mice from tumor group displayed a significantly reduced fractional shortening compared to mice in no tumor and pair-fed groups. In hearts of tumor mice compared to other groups, there were marked increased fibrosis revealed by Masson's trichrome staining. Gene expression of troponin I, a regulator of cardiac muscle contraction, was reduced. Moreover, the fetal gene program was reactivated as demonstrated by decreased gene expression of the structural protein myosin heavy chain (MHC)α (adult isoform) and increased expression of MHCβ (fetal isoform).
Conclusions: Heart function was diminished in colon-26 tumor-induced cachectic mice, and this impaired function was associated with increased fibrosis and altered gene expression of contractile and structural proteins of cardiac muscle.

031
Investigation of endogenous pro-oxidant and antioxidant pathways contributing to cardiac muscle wastage in a mouse model of cancer-induced cachexia

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Background and aims: Studies have shown that reactive oxygen species (ROS) in patients and animal models of cancer cachexia are increased and are linked to the activation of proteolytic pathways. This study aims to investigate pro- and antioxidant pathways as possible contributors to cardiac atrophy in cancer cachexia.
Methods: Eight-week-old nude mice received either an intraperitoneal injection of MAC16 (murine adenocarcinoma cells; $n=16$) producing cancer-induced cachexia, MAC13 ($n=12$) cells used as no cachexia control or an equal amount of cell media ($n=8$) as no tumour control. The levels of DNA damage by oxidation were examined, mRNA levels for xanthine dehydrogena se, NADPH oxidase subunits p47, p67, NOX2, NOX4 and total superoxide dismutase (SOD) enzyme activity were analysed. A Western blot protein analysis was carried out examining both NOX2 and P47 expression.
Results: Food and water intake did not vary between groups. Heart weight was significantly decreased in the MAC16 group by 15%. A marker for DNA oxidative damage 8-OH-2dG was significantly increased in the MAC16 group. Gene transcripts for xanthine dehydrogenase were significantly increased in cachectic heart muscle. Significant decreases were found with total SOD enzyme activity.
Conclusions: The significant increase in 8-OH-2dG and gene expression of an ROS producer in the myocardium, xanthine dehydrogenase may imply an important role for this system in the atrophy of the cachectic heart. Furthermore, decreases in total SOD activity may indicate an inability for handling ROS. Overall, oxidative stress may switch on proteolytic systems in the cachectic heart leading to significant atrophy.

032

Efficiency of oxidative phosphorylation in liver mitochondria is decreased in cancer cachexia: a role for cardiolipin and reactive oxygen species (ROS)?

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Background and aim: Cancer cachexia is a complex syndrome, and the underlying mechanisms are not fully understood. Whether mitochondria function is altered during this disease and may participate to hypermetabolism is not clear. The aim of our study was to analyze liver mitochondrial function, especially the efficiency of ATP synthesis and its regulating mechanisms (proton leak, oxidative stress, and cardiolipin) in a rat model of cancer cachexia.
Methods: Oxygen consumption (oxygraph), ATP synthesis (spectrophotometer), membrane potential (TPMP+ electrode), ROS production (fluorimeter), and cardiolipin content (fluorimeter) were measured in isolated liver mitochondria from cachectic (peritoneal carcinosis (PC)) and pair-fed control rats.
Results: Our results showed that efficiency of ATP synthesis was lower (−24%) in liver mitochondria from PC than control rats. Proton leak was higher in PC than control rats as state 4 oxygen consumption was increased (+22%) and state 4 membrane potential was decreased (−5%). ROS production was increased (12-fold), and cardiolipin content was higher (+55%) in liver mitochondria from PC than control rats.
Conclusions: Our results demonstrate that PC induces important energy wasting in liver mitochondria. In consequence, more nutrients are necessary to synthetize a given quantity of ATP. Because liver is accounting for 25% of total body energy expenditure, this could participate to hypermetabolism and the weight loss associated to cancer cachexia. Our results suggest that cardiolipin and ROS could partly explain the decrease in ATP synthesis efficiency in liver.

033

Does energy wasting in skeletal muscle mitochondria play a role in hypermetabolism observed in cancer cachexia? Study in a model of peritoneal carcinocis-induced cachexia

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Cancer cachexia is a composite syndrome with a combination of anorexia and hypermetabolism, which leads to a drastic loss in body weight. Mechanisms of hypermetabolism remain to be fully elucidated. We hypothesize that energy wasting in mitochondria of skeletal muscle (30% of resting metabolism rate) could contribute to hypermetabolism. Peritoneal carcinosis (PC), a frequent complication of digestive cancer, was generated by an intraperitoneal injection of PROb cells in BDIX rats. A group of rats was pair-fed for PC rats in order to discriminate between the effect of anorexia or cachexia. PC rats were sacrificed at a severe cachexia state (food intake reduced to −80%). Mitochondrial oxygen consumption and ATP synthesis were measured (by oxygraphy and spectrophotometry, respectively), and the efficiency of ATP production (ATP/O ratio) was assessed on mitochondria isolated from skeletal muscle. PC rats displayed a lower body weight gain as well as lower muscle (−17%) and fat (−40%) masses than pair-fed rats, suggesting a hypermetabolism state. Besides, serum TNF-alpha was significantly increased in PC rats (27-fold). Muscle mitochondria did not exhibit modification in the ATP/O ratio. However, data also showed a decrease in oxygen consumption linked to ATP synthesis in PC rats mitochondria (480±69 vs 600±65 natomsO$min^{-1}mg^{-1}$ in PC and pair-fed rats, respectively). Contrary to our hypothesis, there was no energy wasting in skeletal muscle mitochondria in PC rats. However, as cancer cachexia is a progressive syndrome, we cannot rule out that ATP/O ratio alterations are present in earlier cachectic state.

034

Role of dermcidin in cancer cachexia

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Background and aims: Many host and tumor-secreted products act as mediators of cancer cachexia. The dermcidin (DCD) gene is overexpressed in various types of cancer, and its products may have biological functions in cancer etiology and host pathophysiology. This study evaluates the role of DCD in causing cancer cachexia in murine xenograft models.
Methods: Groups of C57BL/6 or Balb/c nude mice (16–25 g) were subcutaneously injected with murine melanoma B-16, human melanoma G-361 cell lines, human

breast carcinoma MCF-7, and MDA-MB-361 cell lines with stably overexpression of the gene or their derived clones constitutively expressing shRNA against DCD mRNA (knockdown) as well as the daily i.p. injection of rhDCD. The body weight changes, tumor volume, and performance status were monitored daily from 10 to 120 days.
Results: The body weights remained steady or did not gained weight in the B16-DCD-, MCF-7-DCD-, and MDA-361-implanted mice despite of larger tumors as compared to controls. On the contrary, mice bearing G-361 melanoma cells underwent a progressive loss of body weight, which could be partially suppressed by expressing constitutively shRNA against DCD or twice-weekly injection of rabbit polyclonal antibody against DCD. The twice-a-week exogenous administration of rhDCD for 10 days did not cause any symptom associated with cachexia.
Conclusion: It is proposed that cancer cachexia is not directly associated with the expression or activities of DCD 11 kDa protein but by others host and tumor cells products in which the expression is regulated by DCD.
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035
Involvement of autophagy in the pathogenesis of cancer-induced muscle wasting

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Background and aims: Muscle atrophy associated with chronic diseases mainly results from protein hypercatabolism. In particular, the degradation of myofibrillar proteins occurring in cancer cachexia eventually depends on the ubiquitin-proteasome system. Recently, the possible contribution of the autophagic/lysosomal pathway has been proposed. The present study has been aimed to evaluate whether autophagy is involved in cancer-induced skeletal muscle atrophy.
Methods: Balb-c mice were divided into controls (C) and tumor bearers (TB). The latter were inoculated s.c. with 5× 105 C-26 tumor cells. After 13 days, mice were sacrificed, and the gastrocnemius (GSN) muscle was excised and stored for further analysis. C2C12 myocytes were exposed to TNF-alpha during 48 h. Levels of LC3B-II and p62, two accepted markers of autophagy, were analyzed by Western blotting (WB) and immunofluorescence (IF).
Results: GSN weight loss in TB was associated with increased levels of both LC3B-II and p62. To investigate if proinflammatory cytokines may contribute to such increase, LC3B-II and p62 levels were evaluated also in TNF-alpha-treated C2C12 myotubes. Both WB and IF analysis showed high levels of LC3B-II, associated with an increased number of autophagic vacuoles. Similarly to the GSN of TB mice, also TNF-alpha-treated myotubes displayed an accumulation of p62.
Conclusions: The results shown in the present study suggest that autophagy may be hyperactivated in the skeletal muscle of TB mice, as well as in C2C12 myotubes exposed to the proinflammatory cytokine TNF-alpha. Further analysis will be useful to understand the role played by autophagy in muscle wasting.

036
Gut microbiota modifications are associated with cancer-induced cachexia

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Background and aims: We know that the gut microbiota is implicated in energy metabolism, and its role has been mostly studied upon obesity. Here, we set the hypothesis that the gut microbiota could also be implicated in metabolic alterations associated with cancer, cachexia.
Methods: This hypothesis was assessed in BALB/c mice intravenously injected with mouse proB BAF3 cells transfected with BCR-ABL gene in order to allow the development of chronic myelogenous leukemia (CML). Muscles (tibialis and gastrocnemius), liver, intestine, and adipose tissues were withdrawn 2 weeks after injection for further biochemical and histological analysis. Gut microbiota composition was assessed by RT-qPCR.
Results: BCR-ABL expressing CML constitutes a new model of cancer cachexia, as proven by a decrease in adipose and muscle tissue weights. In both male and female, *Lactobacillus* spp. levels in cecal content drastically decrease, independently of food intake ($p<0.001$). Moreover, this decrease is highly correlated to muscle markers of atrophy, such as Atrogin-1 mRNA ($r=-0.8885$, $p<0.0001$). Finally, increasing the level of *Lactobacillus* spp. by dietary prebiotics allows to lessen Atrogin-1 mRNA induction in the muscle.

Conclusions: In this new model of cancer cachexia, we highlight that selective gut microbiota modifications occur and are associated with muscle atrophy. Therefore, we suggest that the gut microbiota may constitute a new target in the treatment of this crucial metabolic disorder associated with cancer development.

037
Exercise counteracts cachexia and extends life span in tumor-bearing mice

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Cachexia, experienced by most cancer patients, is a negative prognostic factor, interfering with therapy and worsening quality of life. Recent studies showed that physical activity after cancer diagnosis lowers the risk of both cancer-specific and overall mortality, although no specific assessment of cachexia was performed in these studies. With the aim to assess whether exercise may be beneficial against cancer cachexia, we analyzed the effects of spontaneous physical activity (wheel running) in colon carcinoma (C26)-bearing mice. Two exercise protocols were used: 5-day running or 19-day running (starting after tumor implant). We found that while cachectic muscle showed fiber damage and necrosis, wheel running did not exacerbate muscle damage. The beneficial effects of exercise in tumor-bearing mice were induced only by the 19-day running protocol and included the rescue of body weight loss, muscle mass loss, fiber atrophy, and enhanced protein catabolism. Besides, exercise was an appetite stimulant. We noted that cachexia resulted in loss of muscle resistance to fatigue and not of specific force, and we found that exercise rescued muscle resistance of tumor-bearing mice to healthy control levels. The effects of exercise resulted in an average 50% life span extension in tumor-bearing mice. In conclusion, according to all the diagnostic criteria derived from the current consensus definition of cachexia, wheel running started at the onset on pathology efficiently counteracts cancer cachexia in mice and extends their life span.

038
Anti-inflammatory effect of exercise training in cachexia: role of interleukin (IL) 10

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Cachexia is a multifaceted syndrome whose aetiology is extremely complex and is directly related to the reduction of survival and to poor prognosis. Changes in lipid metabolism in cancer cachexia include marked reduction of total fat mass, increased lipolysis, total oxidation of fatty acids, hyperlipidaemia, hypertriglyceridaemia and hypercholesterolaemia. These changes are believed to be induced by inflammatory mediators, such as tumour necrosis factor-alpha (TNF-alpha). As an endocrine and inflammatory organ, white adipose tissue (WAT) is an important source of pro-inflammatory cytokines, which may trigger several changes in WAT and systemic homeostasis. In addition to pharmacological approaches, lifestyle modifications have been advocated for the treatment of chronic inflammation. In order to counteract inflammation in chronic disease, our group has currently adopted chronic endurance exercise in experimental models of cancer cachexia (Walker 256 carcinosarcoma) and chronic heart failure. Since it is clear that white adipose tissue is strongly implicated in the secretion of both pro- and anti-inflammatory factors in disease, we chose to address its contribution to cachexia-related inflammation and the effect of endurance training on the capacity of cytokine expression and secretion by this tissue. Our results show a reduction of tumour mass and infiltrating macrophage cell number in WAT. An enhancement of IL-10 adipose tissue and skeletal muscle content, with concomitant increased IL-10/TNF-alpha ratio, was also demonstrated after endurance training. Taken together, our results suggest that the increase in IL-10 concentration might be induced by PPAR-$f\times$ activation induced by exercise training leading to a shift towards decreased inflammation.

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039
Exercise increases liver fatty acid oxidation and prevents steatosis in tumour-bearing rats

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The effect of endurance training on hepatic carnitine palmitoyltransferase (CPT) system activity was studied in tumour-bearing (Walker 256) rats. Animals were assigned

to a sedentary control (SC), sedentary tumour-bearing (ST), or exercise control (EC) or exercise tumour-bearing (ET) group. Trained rats ran on a treadmill (60% VO_2 max) 60 min/day, 5 days/week, for 8 weeks. Gene expression of CPT I and II, L-FABP and COX-2, and the maximal activity of CPT I and CPT II were assessed in the liver. PGE2 content was measured in the serum, liver and tumour. CPT I and CPT II activity was decreased ($p<0.01$) in ST (2.66±0.0 and 0.16±0.10 $nmol\,min^{-1}\,mg^{-1}$ protein, respectively) when compared with SC (3.66±0.38 and 1.68±0.16 $nmol\,min^{-1}\,mg^{-1}$ protein, respectively). In contrast, liver COX-2 mRNA of cachectic animals was increased, as well as PGE2, in serum (2.526±0.132 ng/mL, $p<0.05$), as compared to SC (1.901±0.259 ng/mL). Liver PGE2 was increased (2.030± 0.047 $ng\,\mu g\,protein^{-1}$, $p<0.05$) when compared to SC (0.997±0.036 $ng\,\mu g\,protein^{-1}$). Exercise restored CPT I and CPT II activity in tumour-bearing rats (10.61±1.62 and 9.25±1.12 $nmol\,min^{-1}\,mg^{-1}$ protein, respectively, $p<0.0001$), when compared to ST. Training decreased hepatic PGE2 levels in ET (1.150±0.053 $ng\,\mu g\,protein^{-1}$, $p<0.05$) and decreased tumour PGE_2 content (ET, 2.750±0.096 $ng\,\mu g\,protein^{-1}$ vs ST 4.580±0.088 $ng\,\mu g\,protein^{-1}$, $p<0.01$). COX-2 mRNA expression was decreased in the liver of ET when compared with the sedentary counterparts. In conclusion, endurance training was capable to promote the reestablishment of liver mitochondrial carnitine palmitoyltransferase (CPT) system activity due to a decrease in PGE2 levels in cachectic tumour-bearing animals, preventing steatosis.

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040
Anti-inflammatory effect of exercise training in the adipose tissue of cachectic rats

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The white adipose tissue (WAT) has been recently recognised as a source of many endocrine and pro-inflammatory factors, being a potential contributor to cachexia-related inflammation. Moderate intensity exercise training is known to counteract chronic inflammation and affects the expression of inflammatory factors in WAT. We compared three different adipose pads (retroperitoneal (RPAT), mesenteric (MAT) and epidiymal (EAT)) in regard to the effects of training (TBEx, 6 weeks treadmill, 60–65% VO_2 max) upon inflammation in cachectic Yoshida AH-130 tumour-bearing rats (TB), with special focus on the NF-kappaB pathway. The results show that training prevented the infiltration of mononuclear cells and fibrosis in WAT (qualitative light microscopy) but was not able to counteract the increase in NF-kappaB to DNA (ELISA) in MAT due to cachexia, although a tendency ($p<0.055$) to decreased NF-kappaB mRNA expression (real-time PCR) was observed. In EAT, solely, training induced an augment in phosphor-NF-kappaB expression (Western blot), together with enhanced depot weight, as compared with TB. For TARP, no significant differences were found between groups. The marked decrease of weight expected due to cachexia was prevented ($p<0.0001$) in the three muscles examined (soleus, gastrocnemius and tibilalis) by training. We conclude that the short period training protocol was able to affect the NF-kappaB pathway in WAT in a heterogeneous manner, as well as preventing inflammatory cell infiltration. The preservation of muscle mass herein reported strongly suggests the potential of exercise as an adjuvant therapy in the control of wasting in cachexia.

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041
Heterogeneous response of rat and human adipose tissue in cancer cachexia

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White adipose tissue (WAT), whose morphology and physiology vary according to depot anatomical localisation, is markedly affected by cachexia. We demonstrated that in the Walker 256 model of cachexia, the retroperitoneal (RPAT), mesenteric (MAT), and epididymal (EAT) pads respond differently: while EAT shows the greatest decrease in weight, the other depots present pronounced changes regarding adipocyte shape and size. Fatty acid composition is modified in MAT and EAT, but not in RPAT. Ultrastructural abnormalities were observed in RPAT and EAT, solely. In a subsequent investigation, we found that the pads were markedly infiltrated with macrophages resembling “fo am cells” in cachectic rats. The extent of infiltration was greater in MAT, which showed higher TNF-alpha production, although the depots responded similarly in regard to decreased leptin secretion. The IL10/TNF-alpha ratio, a marker of inflammation, was also found to vary heterogeneously. These results encouraged us to examine whether human WAT would also show heterogeneous response to cachexia. The omental (OAT) and subcutaneous (SC)

pads of 18 cachectic cancer (gastric and colon, CCAC) patients from the university hospital were studied and compared with the tissue of non-cachectic cancer patients (n= 8, CC) and with eight "control" subjects (non-cachectic, hernia, or cirrhosis patients, CT). Results show macrophage infiltration, along with a marked increase in WAT collagen type I in both pads, alterations which were only evident in CCAC. In SC of CACC, increased collagen type III was found. CCAC showed decreased OAT adipocyte perimeter and area in relation to CC and CT (p<0.01).

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042
Changes in interleukin (IL) 10 and tumour necrosis factor (TNF) alpha of rat white adipose tissue in cachexia

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Background and aims: The white adipose tissue (WAT) is severely affected by cachexia, a paraneoplastic inflammatory syndrome, in which cytokines, such as tumour necrosis factor-alpha (TNF-alpha), are known to play an important role. In response to the cachexia-related pro-inflammatory cytokines, there is concomitant enhanced production of anti-inflammatory factors, such as interleukin 10 (IL-10). Recently, the TNF-alpha/IL-10 ratio has been recently postulated as a marker for the assessment of the degree of inflammation, which correlates with disease-associated morbidity and mortality.
Methods: We measured (ELISA) the local concentration of these cytokines in three WAT depots: retroperitoneal (RPAT), epididymal (EAT), and mesenteric (MES) of Walker 256 tumour-bearing rats in a time interval of 7 (T7) and 14 (T14)days after tumour-cell inoculation (5×10^{-5} cells/mL).
Results: Higher concentration of TNF-alpha was found on T14 (30%) of MES of cachectic rats, compared with T7. EAT and RPAT IL-10 levels were reduced on T14 (86% and 60%, respectively) compared to T7. Regarding the TNF-alpha/IL-10 ratio, there was an increase in EAT (15.4-fold) and RPAT (2.4-fold) after 14 days (T14), compared with T7.
Conclusion: The results suggest a heterogeneous local response of both TNF-alpha and IL-10 expression, with an accentuated reduction in IL-10 mRNA levels and little change in TNF-alpha mRNA expression. In addition, changes in TNF-alpha/IL-10 ratio were more evident in EAT and RPAT of the cachectic rats. We conclude that the degree of inflammation of WAT is dependent of anatomical localization of the pad.

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043
Cachexia decreases adipokine expression in Walker 256 tumour-bearing rats

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Cancer cachexia markedly affects the white adipose tissue (WAT), an important endocrine organ, releasing a plethora of factors, the adipokines. Among these, leptin and adiponectin are fundamental for metabolic and body composition regulation. Interleukin-15 (IL-15) is an anti-inflammatory cytokine showing direct effects on mature adipocytes, decreasing leptin and increasing adiponectin secretion. IL-15 expression in WAT has never been addressed in cancer cachexia, while the effect of this paraneoplastic syndrome upon adipokine secretion remains controversial. We examined the expression of these factors by WAT, with special focus on the heterogeneous response of fat pads. Animals were assigned to a tumour-bearing group (Walker 256, TB, n=9) or control group (C, n=6). Plasma leptin and adiponectin (RIA), as well as gene expression (real-time PCR) of leptin, adiponectin, interleukin 15 (IL-15), nuclear-factor kappaB (NFkappaB), and inhibitory protein kappaB-alpha (IkappaB-alpha), were determined in the retroperitonial (RPAT) and epididymal (EAT) adipose tissue. Adiponectin and leptin mRNA levels were reduced in both depots studied (around 99% in RPAT and 78% and 96%, respectively, in EAT), as well as IL-15 expression (96% in RPAT, p<0.05 and 75% in EAT, p< 0.005). Plasma adiponectin (p<0.001) and leptin (p<0.001) also decreased in TB. The present results show that in advanced experimental cachexia WAT, capacity to produce the studied factors is compromised, and this effect is more pronounced in the RPAT. As IL-15 has been associated with NFêB activation in neutrophils, we investigated whether its decrease would concomitantly diminish the expression of this transcription factor in WAT, having failed to detect changes.

044
Altered circadian regulation of metabolic pathways in liver and fat of C26 cachectic mice

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Background and aims: The pathogenesis of cancer cachexia is complex involving perturbations in the homeostatic control of energy balance in fat, skeletal muscle and liver. We explored the links between altered regulation of metabolic pathways in liver and fat and tumour-derived cytokines such as IL-6. As diurnal rhythm influences metabolic control, the circadian expression patterns of metabolic genes were characterised.
Methods: Colon-26 cells were implanted s.c. into mice and tissues harvested on day 14 for molecular analyses at six circadian time points for comparison with ad lib-fed and pair-fed controls. Expression of genes involved in metabolic pathways, nuclear receptors and other metabolic regulators was analysed by qPCR. Liver proteins were profiled by multiplexed mass spectroscopy using iTRAQ labels.
Results: Extensive changes in lipid utilisation were evident in liver and fat with alterations in PPAR-alpha and delta, PGC1-alpha and genes (lpl, CD-36, perilipin, L-FABP, PBE, CPT1-alpha and HADHA/B) involved in fatty acid mobilization and beta-oxidation. Genes involved in lipid accumulation (PPAR-gamma, C/EBP-alpha, FAS, SCD-1 and CD36) were similarly affected. These changes occurred at specific times in the diurnal cycle, with distinctive amplitude and phase shifts. Proteomic profiling by iTRAQ quantified >1,500 hepatic proteins, confirmed these defects in lipid handling and also showed a general impact on mitochondria function. Plasma IL-6 levels progressively increased to ~120 pg/ml, and in all organs analysed, IL-6 signaling was activated. Pair-feeding experiments showed that these changes could not be attributed to reduced feed intake.
Conclusions: Cachexia in the C26 mouse model is associated with aberrant circadian regulation of metabolic pathways, especially lipid handling. In the liver ATP, generation may be severely compromised, while many acute phase proteins are elevated.

045
Cancer cachexia induced molecular and morphological changes in bone

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Background and aims: Bone lytic effects due to cancer cachexia are largely unknown. Cancer cachexia syndrome (CCS) is a hypermetabolic disorder, affecting multiple tissues in cancer sufferers. CCS is prevalent in up to 50–80% of advanced cancer patients and directly contributes to 20–30% of cancer patient mortality. The upregulation of catabolic processes and concurrent reduction in anabolic pathways both contribute to weakened muscle and bone. However, the molecular progression of cachexia that drives these changes in bone metabolism and endocrinology is poorly understood. Studies focusing on molecular regulators, which control bone resorption during CCS, are presented. Furthermore, it was anticipated that such changes were coupled to morphological changes in bone during overt cachexia.
Methods: The colon-26 adenocarcinoma mouse model of cachexia has been established to investigate such tumour-mediated changes in bone. Particular focus has been given to the impact of tumour-derived factors in a tumour setting by measuring changed molecular expression patterns of IL-6; signalling molecules, e.g. ERK 1/2 MAPK; as well as osteogenic bone regulators, e.g. bone morphogenic protein-1 (BMP-1). Changes in osteoclast numbers were assessed using TRAP staining.
Results and conclusions: The findings suggest that during cancer cachexia, tumour-derived factors impact on regulators of bone formation by changing molecular expression patterns leading to increased osteoclasts and dysregulated bone remodelling.

046
In early colorectal cancer only minor muscle wasting occurs but is preceded by depression of muscle protein synthesis (MPS) with no evidence of elevated muscle protein breakdown (MPB)

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Background and aims: Baracos et al. have developed a useful method with clinical CT images to investigate muscle wasting. We applied this technique retrospectively to colorectal cancer patients, in a subset of whom we had sought disordered muscle metabolism.
Methods: We analysed CT images (psoas major, L-3; gluteus maximus, coccyx) for 65 patients (73±11 years) with colorectal cancers and 56 patients (71±11 years) who proved normal on investigation. In a subset (n=18, 74±10 years) of the cancer patients, we also characterized muscle metabolism by state-of-the-art stable tracer and molecular methods.

Results: In the healthy patients, psoas thickness was 30 (28, 31)mm (median (95% CI)) and 31 (26, 30)mm in patients with cancers. Normal gluteus thickness was 30 (31, 36) mm and 29 (32, 38)mm in patients; normal psoas cross-sectional area (CSA) was 2,873 (2,708, 3,299)mm^2 and in patients was 3,049 (2,561, 4,155)mm^2. In patients in whom protein metabolism was studied, psoas thickness was 30 (28, 35)mm, gluteus thickness was 36 (32, 38)mm and psoas CSA was 2,973 (2,442, 3,074)mm^2. Thus, in patients with early colorectal cancer (diagnosed <6 weeks of referral), significant wasting could not be detected by comparison with age-matched controls. However, in those whose muscle metabolism was investigated, despite only minor losses of leg muscle (2–15 g/day by successive DXA scans over 8 weeks), muscle protein metabolism was conspicuously deranged, showing "anabolic resistance" of MPS (fed state, 0.030±0.003%/h vs. 0.071±0.003%/h), with no evidence of elevated MPB or of mRNA or protein "markers" of MPB.
Conclusion: Establishment of muscle metabolic dysfunction precedes major sarcopenia in colorectal cancer.

047
Sarcoplasmic reticulum-mitochondrial abnormalities related with muscle atrophy in cancer cachexia

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Background and aims: Cachexia is a wasting condition that manifests itself in several life-threatening diseases, and the most prominent phenotypic is the profound loss of muscle mass. The goal of this work was to evaluate the putative role of mitochondrial in the skeletal muscle and sarcoplasmic reticulum in cachexia animal models.
Methods: Transmission electron microscopy (TEM) was carried out in muscles from control and tumour-bearing rats (Yoshida AH-130 ascites hepatoma). The pattern of gene expression was performed by RT-PCR and protein expression by Western blot.
Results: The morphological analysis was performed through TEM and revealed mitochondrial abnormalities such as swelling and cristae disruption. Moreover, a dilated sarcoplasmic reticulum, together with glucogen accumulation, was observed, thus supporting clear dystrophic muscle morphology. Expression of genes involved in mitochondrial dysfunction and muscle atrophy was also assessed. The results showed differences in the pattern of gene expression related with apoptosis, mitochondrial biogenesis, muscular proteolysis and calcium metabolism.
Conclusion: Altogether, the results presented indicate that during cancer cachexia, there are changes in genes involved in stress/apoptosis that seem to be related with mitochondrial and sarcoplasmic abnormalities that lead to muscle atrophy.

048
A central serotonin 2C receptor pathway regulates gastrointestinal motor activity via ghrelin-neuropeptide Y signaling, but does not require downstream activation of melanocortin 3/4 receptors in rats

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Background and aims: Serotonin (5-HT) plays a key role in the regulation of appetite and emotion in the brain. 5-HT2c receptor agonists exert most of their anorectic effects through downstream melanocortin 4 receptors (MC4R) in the hypothalamus. We hypothesized that a central 5-HT2cR pathway regulates physiological gastrointestinal (GI) motor activity.
Methods: The 5-HT2cR agonist, m-chlorophenylpiperazine hydrochloride (m-CPP) or fenfluramine, was administered intracerebroventricularly (i.c.v.) to rats. GI motility was measured in rats by a strain gauge force transducer method. The effects of SB242084 (5-HT2cR antagonist), HS014 (melanocortin 4 receptor (MC4R) antagonist), SHU9119 (MC3/4R antagonist), neuropeptide Y (NPY), ghrelin, phentolamine hydrochloride (alpha-adrenergic receptor blocker), and hesperidine (the active flavonoid ingredient in Rikkunshito) on the GI motility induced by fenfluramine were examined. Plasma acyl ghrelin levels were determined by enzyme immunoassay.
Results: Intracerebroventricular administration of m-CPP and fenfluramine to fasted rats altered the fasted motor activities in the antrum and duodenum to fed-like motor activities, and the phase III-like contractions were decreased. The 5-HT2cR antagonist, but not the 5-HT1bR and 5-HT2bR antagonists, restored the fasted motor patterns in the antrum and duodenum of fenfluramine-treated rats. SB242084 also inhibited the decrease in plasma acyl ghrelin induced by fenfluramine. The MC4R and MC3/4R

antagonists failed to modify the decreased motor activities induced by fenfluramine. In contrast, ghrelin and NPY restored the fasted motor patterns in the antrum and duodenum of fenfluramine-treated rats. Phentolamine and hesperidine, which stimulate the secretion of endogenous acyl ghrelin, restored the fasted motor activities in fenfluramine-treated rats.
Conclusions: These results demonstrate direct neuroendocrine control over GI function by central 5-HT2c receptors, which inhibit ghrelin-NPY signalling.

049
Akt activation is not reduced in the diaphragm of emphysematous hamsters

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Background: Diaphragm weakness in COPD patients is associated with loss of contractile protein. Protein loss results from an imbalance between protein breakdown and protein synthesis. We recently showed that increased protein breakdown is involved, since proteasome inhibition restores contractile protein content and improves diaphragm force generation in an animal model for COPD. However, modulation of pathways concerning protein synthesis was not studied. The aim of this study is to investigate the activation of Akt, an important promotor of protein synthesis, in the diaphragm of emphysematous hamsters (EH). In addition, we studied the effect of proteasome inhibition on Akt activation.
Methods: Emphysema was induced by intratracheal instillation of elastase. Four groups were studied; normal hamster control (NH-Co), NH treated with Bortezomib i.v. (NH-Bt), EH-Co, and EH-Bt. Diaphragm was excised, and Western blotting and optical densitometry were used to determine cytoplasmic protein contents of total Akt and phosphorylated Akt (p-Akt).
Results: Content of p-Akt was 190% higher in diaphragm from EH-Co animals than in NH-Co ($p<0.05$). Also, content of total Akt was higher in EH-Co diaphragm, 170% of NH-Co ($p<0.05$). Total Akt and p-Akt levels in EH-Bt and NH-Bt were not significantly different from respective controls. The p-Akt to total Akt ratio did not significantly differ between groups.
Conclusion: Emphysema does not reduce Akt activation in the hamster diaphragm. Instead, absolute levels of activated Akt (p-Akt) are elevated in EH diaphragm. These findings support the concept that contractile protein loss results from increased proteolysis rather than from inhibited protein synthesis. Furthermore, unaffected Akt activation by bortezomib treatment suggests that the positive effects of bortezomib on contractile protein content result from reduced protein breakdown and not from enhanced protein synthesis.

050
Inactivation of the MyD88 gene protects from cancer anorexia cachexia

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The anorexia–cachexia syndrome, characterized by a rise in energy expenditure that paradoxically is associated with loss of appetite and decreased food intake, contributes significantly to the morbidity and mortality in cancer. While the pathophysiology of cancer anorexia–cachexia is poorly understood, evidence indicates that pro-inflammatory cytokines are key mediators of this response. Although inflammation is recognized as an important component of cancer anorexia–cachexia, the molecular inflammatory pathways involved are largely unknown. We addressed this issue in mice carrying a deletion of the gene encoding MyD88, the key intracellular adaptor molecule in Toll-like and interleukin-1 family receptor signaling. Wild-type and MyD88-deficient mice were transplanted subcutaneously with a syngenic methylcholanthrene-induced MCG 101 tumor, and daily food intake and body weights were recorded. While wild-type mice typically display reduced food intake at about 5 days after tumor transplantation and had lost 2% in body weight at the end of the experiment, MyD88-deficient mice did not develop anorexia–cachexia despite similar tumor growth as the wild-type mice. These data show that MyD88-dependent mechanisms are involved in the metabolic derangement during cancer anorexia–cachexia and suggest that innate immune signaling is important for the development of this syndrome.

051
Age-related lean tissue loss is attenuated by treatment with a form of soluble activin receptor type IIB

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Background: Normal aging results in loss of muscle mass, deterioration of muscle quality, and decline in muscle strength. Frailty resulting from age-related lean tissue loss is correlated to an increase in falls and fractures and an increase in loss of independent living. Multiple TGF-beta superfamily ligands that negatively regulate muscle mass mediate their effects through the activin receptor type IIB (ActRIIB). Previous studies in normal animals demonstrated that administration of a non-signaling decoy ActRIIB promotes gain of muscle mass. To examine whether treatment with a soluble ActRIIB could offset the aging-induced changes in lean tissue mass and strength, we examined the effects of RAP-435, a fusion protein comprised of an optimized extracellular domain of ActRIIB linked to a murine Fc, in aged mice.
Materials and methods: Seventy-six-week-old male C57BL/6 mice were treated with either vehicle (VEH) or 10 mg/kg RAP-435 (RAP) for 8 weeks. NMR scans to measure body composition and forelimb grip strength were performed longitudinally across the study.
Results: Over the course of the study, the RAP group gained muscle mass (+19.6%), while the VEH cohort lost lean tissue mass (−2.74%) compared to baseline. Starting at study week 2 and continuing though the last trial at week 7, the RAP group had significantly greater forelimb grip strength compared to the VEH mice ($p<0.01$), supporting the idea that the increased muscle mass resulted in increased muscle function.
Conclusions: These data support the hypothesis that a soluble ActRIIB would be efficacious in attenuating sarcopenia associated with normal aging.

052
Attenuation of tumour-induced skeletal muscle atrophy by a zinc-chelator: D-myo-inositol 1,2,6-triphosphate

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Background and aims: Muscle loss remains one of the major factors influencing morbidity and mortality in cancer patients. The aim of this study is to investigate the effectiveness of D-myo-inositol 1,2,6-triphosphate (alpha trinositol (AT)) in counteracting muscle atrophy in cancer cachexia and the mechanisms involved.
Methods: In vivo studies were performed in mice bearing the cachexia-inducing MAC16 tumour, while mechanistic studies were conducted in murine myotubes.
Results: When administered at the optimal dose of 40 $mgkg^{-1}$ to cachectic mice, AT attenuated the loss of body weight through an increase in non-fat carcass mass. This was due to an increase in protein synthesis in skeletal muscle associated with attenuation of the elevated auto-phosphorylation of dsRNA-dependent protein kinase (PKR), eukaryotic initiation factor-2-alpha (eIF-2-alpha) and eukaryotic elongation factor 2 (eEF2). There was also a decrease in protein degradation due to decreased activity of the ubiquitin-proteasome pathway and casapses-3 and -8. The effect of AT was attenuated by co-administration of Zn^{2+}, and AT inhibited the increased zinc concentration in skeletal muscle with increasing weight loss. AT also attenuated the decrease in protein synthesis and increase in protein degradation in myotubes in response to proteolysis-inducing factor, angiotensin II, tumour necrosis factor-alpha and lipopolysaccharide, and this effect was also reversed by Zn^{2+}.
Conclusions: These results suggest a common signalling mechanism amongst a group of catabolic activators involving Zn^{2+}, AT and other chelators of Zn^{2+} after a potential therapy for muscle wasting in a range of catabolic conditions.

053
Targeting the androgen receptor to treat muscle-wasting disorders

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Background and aims: Modulation of the androgen receptor has the potential to be an effective treatment for hypogonadism, andropause, and associated conditions such as muscle wasting, osteoporosis, and sexual dysfunction. Side effects associated with classical anabolic steroid treatments have driven the quest for drugs that demonstrate improved therapeutic profiles. Novel, non-steroidal compounds that show tissue selective activity and improved pharmacokinetic properties have been developed at GSK.
Methods: Structure-based drug design was used to identify a small molecule androgen receptor modulator. This compound was profiled in vitro assays to determine its potency, selectivity, and molecular fingerprint. Full pharmacokinetic analysis was implemented to determine the molecule's suitability to profile in in vivo studies. Extensive in vivo studies were conducted comparing the small molecule to dihydrotestosterone (DHT).
Results: GSK420A has an ED50 in an ORX rat model of ca. 0.03 mg/kg. GSK420A and DHT almost completely

restored the levator ani muscle to eugonadal levels in 28 days in the ORX model. DHT completely reversed ORX-induced prostate atrophy, while stimulation by GSK420A is less than 10% of that in the DHT-treated animals. GSK420A reduces prostate weight in the intact rat. HPG axis, LH and FSH, suppression is dose dependent. In the 28-day ORX rat study, GSK420A showed a clear trend of increases in other anabolic parameters, such as lean and total body mass, lumbar spine BMD/BMC, and hematocrit.
Conclusions: GSK420A represents a drug molecule with features that make it an attractive candidate for clinical investigations targeting muscle-wasting disorders.

054

Extracellular signal-regulated kinase (ERK) inhibition prevents muscle atrophy in experimental cancer cachexia

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Background and aims: Cancer cachexia is a syndrome characterized by loss of skeletal muscle protein, depletion of lipid stores, and hormonal perturbations. The role of MAP/ERK kinase in the regulation of muscle mass is still debated. Data in the literature show that ERK inhibition prevents IGF1-induced muscle hypertrophy, while others demonstrate that inhibition of the ERK pathway results in a hypertrophic phenotype similar to that elicited by IGF1 treatment. The aim of the present study has been to evaluate the effects of ERK inhibition in two different experimental models of cancer cachexia.
Methods: Tumor-bearing (TB) rats received 108 Yoshida AH-130 cells i.p. and were sacrificed 7 days after transplantation. TB mice received 5×105 colon-26 adenocarcinoma cells s.c. and were sacrificed 13 days later.
Results: A marked loss of body and muscle weight was detected at the end of the experimental period, and ERK phosphorylation increased in the gastrocnemius of TB rats and mice. Administration of the ERK inhibitor PD98059 (3 mg/kg) to TB rats resulted in improved body and muscle weight loss. Similarly, PD98059 treatment (1 mg/kg) in TB mice partially prevented the loss of body weight, muscle mass, and strength. Moreover, PD98059 administration to TB mice resulted in decreased atrogin-1 muscle expression.
Conclusions: In the present study, ERK inhibition reduced muscle wasting in experimental cancer cachexia, suggesting that this kinase may be considered a new therapeutic target. Further studies are needed to unravel the role of ERK in the modulation of both anabolic and catabolic pathways (i.e., IGF-1/Akt and myostatin/follistatin) in the skeletal muscle.

055

Gastric electrical stimulation increases ghrelin production: a new approach to anorexia–cachexia

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Background and aim: Gastric electrical stimulation (GES) has emerged recently as a therapeutic option for refractory gastroparesis. Beside amelioration of nausea and vomiting, GES results in improved appetite; however, the mechanisms of this improvement remain unknown. The present study was designed to determine these mechanisms.
Methods: During laparotomy, two electrodes were implanted in the rat stomach, and typical for gastroparesis, GES with Enterra parameters was applied for 1 h. Expression of digestive and hypothalamic hunger and satiety peptides was assayed. Presence of c-Fos protein in the stomach and in the brain with relation to ghrelin-producing cells or to catecholaminergic neurons, respectively, was studied by immunohistochemistry.
Results: GES increased c-Fos in the gastric myenteric plexus and increased expression of ghrelin mRNA and protein in the stomach as well as plasma ghrelin level. In the arcuate nucleus of the hypothalamus, GES increased c-Fos and agouti-related protein mRNA expression. GES reduced the number of c-Fos-positive cells throughout the nucleus of the solitary tract including catecholaminergic neurons.
Conclusions: This study shows that GES may improve appetite mainly via stimulation of ghrelin production and its downstream pathways in the hypothalamus, as well as by reducing activity of catecholaminergic brainstem neurons. These results suggest that GES with parameters used for treatment of gastroparesis could potentially improve appetite in patients with other pathological conditions characterized by chronic anorexia and cachexia.

056

Effect of SUN11031 (a synthetic human ghrelin) on pulmonary and systemic abnormalities in the cigarette smoke-induced chronic obstructive pulmonary disease (COPD) model

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Cigarette smoking is well known as a major risk factor for chronic obstructive pulmonary disease (COPD) and causes

not only the pulmonary abnormalities but also the extrapulmonary pathophysiological changes such as malnutrition, loss of body weight (BW), systemic inflammation, and skeletal muscle wasting. Previously, it has been reported that ghrelin improves body composition and exercise tolerance in patients with COPD cachexia. We investigated whether ghrelin improves the respiratory function and catabolic changes in the animal model of COPD induced by chronic cigarette smoke (CS) exposure. Rats were exposed to CS or air twice a day and received SUN11031 (human ghrelin, manufactured by Asubio Pharma Co., Ltd., 1 mg/kg, subcutaneous, b.i.d.) for 12 weeks. BW and food intake were measured throughout the study. After 12 weeks exposure, respiratory function, pulmonary emphysema, and muscle strength were evaluated. Compared with the air-exposed rats, BW gain, food consumption, epididymal fat weight, and peak air flow were significantly lower, while lung capacity was significantly higher in the CS-exposed rats. The grip strength of the forelimb tended to be lowered by CS exposure. In contrast, SUN11031 significantly increased both BW gain and food efficiency, but not fat weight. SUN11031 also significantly improved peak air flow and lung capacity. Moreover, a trend for the improvement of grip strength was observed in the SUN11031-treated rats. These results suggest that SUN11031 may improve not only the energy balance but also the respiratory function and the systemic abnormality in COPD model induced by chronic CS exposure.

057

Acyl ghrelin improves selective serotonin reuptake inhibitor-induced gastrointestinal motility in rats

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Background and aims: Nausea, vomiting, and anorexia are the common side effects caused by selective serotonin reuptake inhibitors (SSRIs) and lead to early discontinuation of treatments in patients with depressive disorder. We examined the effects of SSRIs on gastrointestinal (GI) motility and whether acyl ghrelin improves SSRI-induced GI symptoms in rats.

Methods: SSRIs (fluvoxamine, paroxetine, fluoxetine, and fenfluramine) were intraperitoneally administered to 24 h food-deprived rats, respectively. GI motility was measured in conscious, freely moving rats by a strain gauge force transducer method. Plasma acyl ghrelin levels were determined by EIA. Phenol red retention in the stomach at 15 min was calculated to assess the gastric emptying. Effects of acyl ghrelin (and Rikkunshito, a traditional Japanese medicine which releases endogenous acyl ghrelin) on these functions were examined.

Results: The fasted motor patterns in both the antrum and duodenum were disrupted by the treatments of SSRIs and replaced by the fed-like pattern. The motor activity was increased in the antrum, whereas decreased in the duodenum, and the duodenal phase III-like contractions of fasted motility, was decreased. All of the SSRIs significantly decreased plasma acyl ghrelin concentration. When acyl ghrelin or Rikkunshito was administered to fluvoxamine or fenfluramine-treated rats, the motor activity in the antrum was not changed, while the motor activity and the phase III-like contractions in the duodenum were significantly increased. In addition, acyl ghrelin or Rikkunshito improved the decrease in food intake and gastric emptying rate. The pretreatment with GHS-R antagonist (D-Lys3) GHRP-6 blocked the effects of Rikkunshito on theses parameters.

Conclusions: These results indicate that SSRIs decrease plasma acyl ghrelin and induce GI motility similar to anorexigenic peptides. Acyl ghrelin may be effective in relieving SSRI-induced GI symptoms.

058

Efficacy of orally available selective melanocortin-4 receptor antagonists in animal models of cancer cachexia

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Background and aims: Cachexia is among the most debilitating and life-threatening aspects of cancer and includes anorexia, fat, and muscle tissue wasting. The anorexigenic peptide alpha-MSH is believed to be crucially involved in the regulation of food intake. It has been proposed that blockade of its central physiological target, the melanocortin-4 receptor (MC4-R), might provide a promising treatment strategy. This concept is supported by the fact that agouti-related protein (AgRP), the endogenous antagonist at the MC4-R, was found to affect two hallmark features of cachexia, namely to increase food intake and to reduce energy expenditure. Based on this rationale, we pursued the discovery and development of small-molecule MC4-R antagonists for the treatment of cancer cachexia.

Methods: A recently discovered, selective and orally bioavailable MC4-R antagonist, which penetrates the blood–brain barrier, was studied. Specifically, stimulation of food intake in healthy mice and efficacy in rodent models of cancer cachexia were investigated.

Results: Our compound, which was very well tolerated in mice and rats, was found to distinctly increase food intake

in healthy animals. Moreover, in tumor-bearing animals, repeated oral administration ameliorated tumor-induced weight loss and diminished loss of lean body mass and fat mass. Concommitantly, expression of atrogenes in gastrocnemius muscle was reduced compared to vehicle-treated tumor-bearing animals.

Conclusions: In contrast to previously reported peptidic or small-molecule MC4-R antagonists, the compound described here was effective after oral administration and was very well tolerated. In light of patient care, orally applied drugs might offer a considerable advantage for the treatment of cancer cachexia.

059

Right on target: melanocortin (MC) 4 receptor mediated increase in food intake and decrease in energy exependiture with orally bioavailable small molecule antagonists

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Background and aims: The melanocortin system is crucially involved in the regulation of food intake and energy expenditure in the healthy, physiological state. In a pathophysiological situation like cachexia, these two parameters are dysregulated, i.e., food intake is decreased and energy expenditure is increased, a vicious combination leading to a catabolic state. Blockade of the melanocortin-4 receptor (MC-4R) might provide a promising treatment strategy for cachexia because agouti-related protein (Ag RP), the endogenous antagonist at the MC-4R, was found to increase food intake and to reduce energy expenditure. Based on this rationale, we designed small-molecule MC-4R antagonists for the treatment of cancer cachexia.

Methods: We studied the efficacy of our recently discovered, in vitro selective and potent MC-4R antagonists in an in vivo situation. Specifically, stimulation of food intake and regulation of energy expenditure measured as oxygen consumption were investigated. Furthermore, using MC-4R-deficient mice, we confirmed that the effects of our compounds are indeed MC-4R mediated.

Results: Single and multiple oral administration of our compounds significantly increased food intake and decreased energy expenditure in wild-type mice. In MC-4R-deficient mice, however, these effects were absent.

Conclusions: We demonstrated that our small molecule MC-4R antagonists were orally effective on both food intake and energy expenditure, and that these effects were MC-4R mediated. These findings open up the possibility of treating two hallmarks of cachexia with one drug.

060

A pharmacologically active monoclonal antibody against the human melanocortin-4 receptor as a possible treatment of cachexia

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Background: Blockade of the melanocortin-4 receptor (MC4R) by low molecular weight antagonists has been shown to be effective in preventing anorexia and cachexia in rodents. Comparable results have been obtained in our previous studies with active immunization against the MC4R. In the present experiments, we validated the pharmacological profile of a monoclonal antibody (mAb) against the MC4R.

Methods and results: We produced an mAb (1E8a) directed against the N-terminal domain of the human (h) MC4R. This mAb showed specific binding in HEK-293 cells expressing the hMC4R and blocked the activity of this receptor in vitro. When mAb 1E8a (1 μg) was injected intracerebroventricularly (i.c.v.) into the third ventricle of rats, their 24-h food intake increased (26.6±0.7 vs 21.4±1.0 g, $p<0.05$). After 7 days of continuous i.c.v. administration of mAb 1E8a via osmotic minipumps in rats cumulative food intake (160±5 vs 134±9 g), body weight change (+8.4±5.8 vs −8.6±9.5 g), and fat pad weights (3.9±0.2 vs 3.0±0.4 g) were significantly ($p<0.01$) increased. Intravenous (i.v.) injection of mAb 1E8a was ineffective, but a single chain variable fragment (scFv) derived from this mAb increased significantly ($p<0.01$) food intake (24.5±0.2 vs 19.7±1.0 g) and body weight (+0.3±0.7 vs −6.0±1.2 g) within 24 h after i.v. administration (300 μg/kg).

Conclusion: The positive results obtained with scFv 1E8a after i.v. administration suggest that such derivatives of mAbs against the MC4R may have therapeutic potential in the treatment of anorexia or cachexia.

061

Myostatin inhibition attenuates atrophy and loss of muscle function in mice with cancer cachexia

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Background and aims: Cancer cachexia describes the progressive skeletal muscle wasting and weakness in many cancer patients. Cancer cachexia impairs mobility, causes severe fatigue, and accounts for >20% of cancer-related deaths. We tested the hypothesis that antibody-directed myostatin inhibition would attenuate the atrophy and loss of function in skeletal muscles of tumor-bearing mice.
Methods: Twelve-week-old C57BL/6 mice received a subcutaneous injection of saline (control) or 7.5×10^5 Lewis lung carcinoma (LLC) tumor cells. One week later, mice began once-weekly injections of saline (control, $n=12$; LLC, $n=9$) or a mouse chimera of anti-human myostatin antibody (PF-354, 10 mg/kg/week, Pfizer Global Research and Development, Groton, USA; LLC+PF-354, $n=11$), which continued for 5 weeks.
Results: Compared with controls, LLC mice had a lower muscle mass (8–10%, $P<0.05$) which was prevented with PF-354 ($P>0.05$). Peak tetanic in situ force production of the tibialis anterior (TA) muscles of LLC mice was reduced by 8% ($P<0.05$), but this reduction was attenuated with PF-354 ($P>0.05$). In contrast, normalized peak tetanic force of diaphragm muscle strips in vitro was not different between groups ($P<0.28$). TA muscles and diaphragm muscle strips from LLC mice exhibited reduced fatigability compared with controls ($P<0.001$), which was prevented with PF-354.
Conclusions: Antibody-directed myostatin inhibition attenuated the skeletal muscle atrophy and loss of muscle force-producing capacity in a murine model of cancer cachexia. The findings highlight the therapeutic potential of myostatin inhibition for cancer cachexia.
Supported by research grant funding from the National Health and Medical Research Council (Australia) and Pfizer Inc. (USA).

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Activin type II receptor fusion protein (ACVR2B-FC) preserves skeletal muscle and adipose tissue in two models of cancer cachexia

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Background and aims: Myostatin is a potent tonic inhibitor of skeletal muscle growth that binds the activin type II receptor ACVR2B. An ACVR2B-Fc fusion protein has been shown to bind myostatin and induce muscle hypertrophy in mice. We sought to determine how inhibition of myostatin by ACVR2B-Fc might affect muscle wasting in two models of cancer cachexia.
Methods: Athymic nude mice were injected with CHO cells expressing ACVR2B-Fc or no recombinant protein as a control. Mice were also injected with either Lewis lung carcinoma (LLC) or colon-26 (C26) adenocarcinoma cells to induce cachexia.
Results: ACVR2B-Fc administration improved skeletal muscle and fat mass in LLC cachexia. Triceps mass was 25% greater ($p<0.05$), and pectoralis mass was 28% greater ($p<0.05$) in ACVR2B-Fc-treated mice versus controls. Adipose tissue was also protected, with epididymal fat pad mass 2,127% larger ($p<0.01$) in the ACVR2B-Fc-treated group. LLC and CHO tumor weights were not different between groups. ACVR2B-Fc administration also inhibited C26 cachexia. ACVR2B-Fc-treated mice had 12% greater tumor free body weight ($p<0.01$), and gastrocnemius and quadriceps masses were 26% greater ($p<0.001$) than in control mice. ACVR2B-Fc mice in this model also demonstrated 154% increased adipose tissue versus controls ($p<0.05$). CHO and C26 tumor weights were not different between groups.
Conclusions: The myostatin-family inhibitor, ACVR2B-Fc, potently inhibited muscle wasting and protected adipose stores in two models of cancer cachexia, without affecting tumor growth. Thus, targeting myostatin-family proteins may be a potential clinical therapy for patients with muscle wasting in cancer cachexia.

063

Novel effects of myostatin inhibition on skeletal muscles of aging mice: implications for sarcopenia

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Background and aims: Sarcopenia is the progressive loss of muscle mass with advancing age associated with a significant age-related decline in muscle strength and power. We tested the hypothesis that antibody-directed myostatin inhibition would improve the mass and function of skeletal muscles from aged mice.
Methods: Eighteen-month-old C57BL/6 mice were treated for 14 weeks with saline (control, $n=9$) or a mouse chimera of anti-human myostatin antibody (PF-354, 10 mg/kg/week, Pfizer Global Research and Development, Groton, CT, USA; $n=12$).
Results: PF-354 completely prevented the age-related reduction in body mass ($P<0.05$). Treatment with PF-354 increased muscle mass by 8–18% and increased muscle fiber size by 12% ($P<0.05$). Consequently, peak tetanic in

situ force production of tibialis anterior (TA) muscle was improved by 35% after treatment ($P<0.03$), without affecting the muscle's susceptibility to contraction-mediated injury ($P=0.48$). PF-354 treatment resulted in a shift to a greater proportion of type IIa fibers by 114% ($P<0.01$) and enhanced muscle fiber oxidative capacity by 39% ($P<0.01$). Treatment with PF-354 also reduced apoptosis by 56% ($P<0.03$) and caspase-3 mRNA expression by 65% ($P<0.04$), and tended to reduce protein abundance of the cleaved form of caspase-3 ($P<0.06$).

Conclusions: Antibody-directed myostatin inhibition improved the mass and function of skeletal muscles of aging mice. Apoptosis was reduced with myostatin blockade, identifying a novel role of myostatin for apoptotic signaling. These findings support the therapeutic potential of myostatin inhibition for sarcopenia.

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064

In vivo antagonism of activin receptor type IIB (ActIIB) pathway reverses muscle wasting and prolongs survival in experimental models of cancer cachexia

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Cancer cachexia affects up to 80% of patients with advanced cancers and is the cause of more than 20% of cancer-related mortality. A key feature of cachexia is the progressive depletion of skeletal muscle, which occurs in concert with the loss of adipose tissue. Muscle wasting has long been postulated to be a key risk factor for cancer-related death, but to date, there has been no direct experimental research evidence to substantiate this hypothesis. Here, we demonstrate that in two independent preclinical models of cancer cachexia, the colon-26 tumor-bearing mice and inhibin-alpha-deficient mice, pharmacological blockade of ActRIIB-mediated signaling pathway not only prevents muscle wasting but also reverses muscle loss, thereby significantly prolonging survival. We show that mechanistically, ActRIIB pathway antagonism is able to prevent the induction of critical components of the Ub-proteasome system in skeletal muscle and also stimulates muscle stem cell growth. These findings (1) establish a crucial link between the ActRIIB signaling activities and the pathogenesis of muscle wasting and cancer cachexia and (2) show that pharmacological antagonism of the ActRIIB pathway may hold therapeutic potential for the treatment of cancer cachexia.

065

A myostatin decoy receptor prevents loss of muscle mass and function in cancer cachexia

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Background: Cachexia is a complex syndrome which is characterized by decreased protein synthesis and increased protein degradation. Cytokines and other secreted factors are believed to mediate muscle wasting, although the exact mechanisms are not completely understood. Myostatin, a negative regulator of muscle mass and member of the TGF-beta/BMP superfamily, has been implicated in cachexia. To determine if myostatin activity is important for cachexia, we tested a soluble myostatin decoy receptor (ActRIIB-Fc) in a mouse ascites tumor model.

Methods: Ten-week-old SCID female mice were injected with S180 cells to initiate ascites tumor formation or PBS. The mice were then subdivided into two cohorts and given weekly injections of vehicle or 10 mg/kg ActRIIB-Fc for 2 weeks.

Results: S180-induced cachexic mice lost 15% gastrocnemius and quadriceps muscle mass and showed a 27% decline in grip strength compared to controls after 2 weeks. However, cachexic mice treated with ActRIIB-Fc did not lose muscle mass or show decline in grip strength. Analysis of muscle extracts from cachexic mice revealed a ~25% increase in proteasome activity and a decrease in FOXO1 phosphorylation which was not reversed by ActRIIB-Fc treatment. However, the increased mRNA expression of muscle-specific E3 ubiquitin ligases, Murf-1, and atrogin/MAFbx in the untreated cachexic mice was significantly reduced following ActRIIB-Fc treatment. Further, a maker of protein translation, 4EBP1, was increased in ActRIIB-Fc-treated mice compared to vehicle-treated cachexic mice.

Conclusions: The data suggests that ActRIIB-Fc maintains muscle mass and function by balancing the catabolic activity during cachexia by increasing protein translation.

066

Theophylline is able to partially revert cachexia in tumour-bearing rats

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Background and aims: Cancer cachexia occurs in the majority of cancer patients before death and seems to be responsible for the death of 22% of cancer patients. The abnormalities associated with cancer cachexia include anorexia, weight loss, muscle loss, atrophy, anemia, and alterations in carbohydrate, lipid, and protein metabolism. Different nutraceuticals, i.e., w3 fatty acids of marine origin, have been proposed to counteract muscle wasting during cancer. The aim of the present investigation was to examine the anti-wasting effects of theophylline (present in tea leaves) on a rat model of cancer cachexia.
Methods: Individual muscle weights, muscle gene expression, body composition, and cardiac function were measured in rats bearing the Yoshida AH-130 ascites hepatoma, following theophylline treatment.
Results: Theophylline treatment resulted in a clear anti-proteolytic effect on muscle tissue (soleus and heart), which was associated with a decrease in circulating TNF-alpha levels. Treatment with the nutraceutical also resulted in an improvement in body composition and cardiac function.
Conclusion: Theophylline—alone or in combination with drugs—may be a candidate molecule for the treatment of cancer cachexia.

067
Therapeutic potential of proteasome inhibition in Duchenne muscular dystrophy

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Bakground and aims: Duchenne muscular dystrophy (DMD) is a severe progressive disease resulting from mutations of the dystrophin gene and leading to progressive muscle degeneration and weakness. Several evidences suggest that enhanced activation of proteasomal degradative pathways underlies critical steps in the pathogenesis of DMD dystrophic process. We previously demonstrated that treatment with the proteasome inhibitor MG-132 rescued the protein levels and cell membrane localization of dystrophin and the dystrophin glycoprotein complex (DGC) in *mdx* mice, an experimental model of DMD. This work was aimed to define the therapeutic potential of Velcade, a drug selectively blocking the ubiquitin-proteasome pathway, in dystrophinopathies. Velcade is particularly intriguing since, being FDA-approved for the treatment of multiple myeloma, its side effects in humans are already known.
Methods: Dystrophic *mdx* mice were systemically administered Velcade (0.8 mg/kg) over a 2-week period.
Results: Velcade proved efficient in inhibiting proteasome activation, as well as serum CK level increase. Moreover, it reverted the membrane expression of dystrophin and DGC members, prevented both NF-kB nuclear translocation and the inflammatory reaction, enhanced muscle regeneration, and improved the dystrophic phenotype by increasing muscle CSA and reducing the number of both centralized nuclei and necrotic fibers.
Conclusions: In the present work, we reported that Velcade-induced proteasome inhibition partially prevents muscle degeneration in dystrophic mice and promotes the expression and membrane localization of dystrophin and dystrophin-associated proteins. Future studies will investigate the efficacy of Velcade in combination with other agents, such as corticosteroids, as well as their effects on muscle strength and functional motility.

068
The fast skeletal troponin activator, CK-2017357, increases skeletal muscle force and reduces muscle fatigue in vitro and in situ

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CK-2017357 is a member of a class of fast skeletal troponin activators that were identified by high throughput screening of skeletal sarcomere preparations. We sought to understand how this compound altered isometric force development in skinned and live muscle fibers. Treatment of skinned muscle fibers with 0.1–10 μM CK-2017357 caused a left shift of the force–pCa relationship without altering the maximum force, consistent with a calcium-sensitizing effect on force production. In rat flexor digitorum brevis (FDB) muscle in vitro, CK-2017357 (10 μM) caused increases in sub-tetanic force (150% at 10 Hz) without altering maximum force. Similar experiments were performed using rat extensor digitorum longus (EDL) muscle in situ, where nervous and vascular connections were left intact and the muscle was stimulated via the peroneal nerve. Infusions of CK-2017357 up to 10 mg/kg increased sub-tetanic force (200% at 30 Hz). One cause of muscle fatigue is reduced Ca^{2+} release from the sarcoplasmic reticulum. We tested whether CK-2017357 would alter the rate of fatigue in FDB fibers. Muscle was pretreated with 5 μM CK-

2017357 and stimulated at a frequency giving 50% maximal force every 6 s for 15 min (30°C). Treatment significantly reduced the rate of fatigue (terminal force, 33.7±5% vs 53.9±5%). In summary, we have identified a skeletal troponin activator that sensitizes the sarcomere to calcium, increases sub-maximal force development, and reduces fatigue. We believe that this may translate to improved physical power and efficiency in diseases where muscle function is compromised due to injury, disease, or age.

069

Formoterol: a potential therapeutic use in cancer wasting

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Background and aims: Perhaps the most common manifestation of advanced malignant disease is the development of cancer cachexia. Indeed, cachexia occurs in the majority of cancer patients before death and seems to be responsible for the death of 22% of cancer patients. The abnormalities associated with cancer cachexia include anorexia, weight loss, muscle loss, atrophy, anemia and alterations in carbohydrate, lipid and protein metabolism. β_2-adrenergic agonists are potent muscle growth promoters in many animal species, resulting in skeletal muscle hypertrophy, while they cause a reduction of the body fat content. Formoterol is a highly potent, β_2-adrenoceptor-selective agonist combining the clinical advantages of rapid onset of action with duration of action. The aim of the present investigation was to examine the anti-wasting effects of formoterol.
Methods: Individual muscle weights, total physical activity and grip force were measured in rats bearing the Yoshida AH-130 ascites hepatoma, following formoterol treatment.
Results: Administration of the β_2-agonist formoterol to tumour-bearing rats resulted in an important reversal of the muscle-wasting process, as reflected by individual muscle weights. The anti-wasting effects of the drug were also observed in terms of total physical activity and grip force.
Conclusion: Formoterol treatment resulted in an improvement in physical performance in cachectic tumour-bearing rats.

070

The effects of the catabolic/anabolic transforming agent MT-102 on body weight, body composition, food intake, physical activity and survival compared to imidapril and bisoprolol in a rat model of cancer cachexia

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Background: The ACE inhibitor imidapril has been investigated as an anti-cachexia drug in a phase III trial. Depending on the cancer type, a small weight gain was observed. Beta-blockers such as bisoprolol have been shown to reduce the onset of cardiac cachexia and are generally known to increase body weight (i.e. mainly fat mass) in patients with cardiovascular illness. In this study, both compounds were compared to MT-102, a new catabolic/anabolic transforming agent (CATA). MT-102 has a pharmacologic profile that includes muscle anabolic effects, appetite stimulation, reduction in energy expenditure, lipolysis inhibition and cardio-protection.
Methods: Wistar rats (weight approximately 200 g) were inoculated intra-peritoneally with AH-130 hepatoma cells or saline (sham). Animals were treated with 0.4, 1 or 10 mg/kg/day imidapril; 0.5, 2, 5 or 50 mg/kg/day bisoprolol; 0.3 or 3 mg/kg/day MT-102; or placebo. Food intake and locomotor activity per day were assessed before tumor inoculation and on day 11 of the 16-day protocol. Weight and body composition (NMR scan) were assessed on day 0 and after sacrifice (maximum day 16, without tumor).
Results: Animals of all groups showed similar baseline values for weight, body composition and heart function. All imidapril and bisoprolol doses, as well as low-dose MT-102, significantly reduced loss of lean tissue mass. Only high-dose MT-102 induced lean tissue gain compared to baseline. The same pattern was found for body weight overall. Imidapril did not improve quality of life (food intake and spontaneous activity). The higher doses of bisoprolol and both doses of MT-102 did improve quality of life. The two doses of MT-102 had the best results for physical activity. Mortality was higher in imidapril-treated groups compared to placebo. Five and 50 mg/kg/day bisoprolol significantly improved survival, as did high-dose MT-102. Low-dose MT-102 displayed a strong trend (p=0.064) (see Table).

	N	ΔBW (g)	Δlean (g)	Δfat (g)	Food (g/24 h)	Activity (counts/24 h)	Hazard ratio vs placebo (95% CI)
Placebo	78	−53.7±1.8	−39.8±1.6	−12.4±0.4	4.3±0.5	29,509±1,775	–
0.4 mg Imidapril	10	−30.0±14.1***	−21.6±10.5**	−8.9±2.7*	7.9±3.0	29,379±7,205	1.23 (0.49–3.09)
1 mg Imidapril	14	−24.8±6.9***	−13.4±4.8***	−5.8±1.8***	5.8±3.4	21,170±7,148	4.03*** (1.55–10.52)
10 mg Imidapril	15	−21.9±6.8***	−14.9±4.8***	−5.31.3***	6.3±4.0	22,732±9,073	6.35*** (2.37–17.01)
0.5 mg Bisoprolol	13	−37.8±7.5**	−25.3±5.7**	−9.3±1.2**	5.8±2.0	33,701±6,559	1.35 (0.58–3.14)
2 mg Bisoprolol	14	−25.6±13.2***	−20.4±9.4***	−6.4±3.0***	16.7±6.9**	38,777±6,981	0.54 (0.26–1.13)
5 mg Bisoprolol	23	−21.8±10.6***	−16.7±7.7***	−5.9±1.9***	10.9±2.0***	43,755±3,741***	0.32*** (0.18–0.59)
50 mg Bisoprolol	20	−30.6±9.8***	−24.4±6.9***	−8.4±1.6***	10.1±2.0**	43,124±5,722**	0.44* (0.23–0.83)
0.3 mg MT-102	14	−20.3±14.6***	−9.7±11.3***	−9.5±2.4*	6.4±2.9**	46,872±6,684***	0.52**** (0.25–1.04)
3 mg MT-102	15	+17.8±17.9***	+15.1±13.8***	−4.3±2.8***	12.9±3.1***	51,268±6,521***	0.29*** (0.15–0.57)

BW body weight
*$p>0.05$; **$p<0.01$; ***$p<0.001$; ****$p=0.064$ vs placebo

Conclusion: The catabolic/anabolic transforming agent MT-102 demonstrates a strong anti-cachexia treatment profile. Only MT-102 maintained true weight gain in this model of severe cancer cachexia. MT-102 improved measures of quality of life and survival. In our model system, imidapril was not a successful anti-cachexia therapy. Bisoprolol reduced weight loss and, at higher doses, resulted in improved physical activity, food intake and survival.

071
The xanthine oxidase inhibitors oxypurinol and allopurinol reduce wasting and improve cardiac function in experimental cancer cachexia

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Background: Cachexia is a common co-morbidity in cancer patients. Uric acid is a danger signal and associated with inflammation. In chronic heart failure, high serum uric acid levels are associated with metabolic illness and poor survival. We hypothesised that xanthine oxidase (XO) inhibition can reduce tissue wasting and improve survival in cancer cachexia.
Methods: Rats (weight approximately 200 g) were inoculated intra-peritoneally with AH-130 hepatoma cells and treated with 4 mg/kg/day ($n=11$) and 40 mg/kg/day ($n=12$) oxypurinol (Oxy), 40 mg/kg/day ($n=11$) allopurinol (Allo) or placebo ($n=49$). Heart function (echocardiography), food intake and locomotor activity were assessed before inoculation and on day 11 of the 16-day protocol. Weight and body composition (NMR scan) were assessed on day 0 and day 16 after sacrifice (without tumour). XO activity for generation of reactive oxygen species (ROS) was assessed by EPR after purification of XO. Activity was assessed without Oxy or Allo. Proteasome activity was measured by turnover of flurogenic substrates in gastrocnemius homogenates.
Results: Wasting of both fat and lean tissue was significantly reduced (see Table) by 4 mg/kg/day oxypurinol and to a somewhat lesser degree by allopurinol. The food intake and spontaneous activity were significantly higher in the low dose oxypurinol and allopurinol groups. Also, cardiac function was improved. Sham animals ($n=16$) showed an increase in body weight (59.8±2.1 g), lean mass (41.6±2.0 g) and fat mass (9.1±0.9 g). The values for reactive oxygen species (ROS) were 3.23±0.99 nmolROS/μg protein/min. The proteasome activity (174.4±15.9 nmol/mgprotein/min), IL-6 (50.5±9.6 pg/ml), TNF-α (14.5±2.6 pg/ml) and NF-κB (100±24.9%) were lower in sham animals. Uric acid level were significantly lower (1.58±0.32 mg/dL) compared to tumor-bearing animals.

	Placebo (n=49)	4 mg/kg/day Oxy	40 mg/kg/day Oxy	4 mg/kg/day Allo	40 mg/kg/day Allo
Delta BW (g)	−50.2±2.2	−15.4±14.2***	−45.3±11.8	−31.9±14.9*	−26.4±17.1*
Delta fat (g)	−11.2±0.4	−6.8±2.6**	−14.4±1.9*	−9.9±2.0	−8.1±2.9
Delta lean (g)	−37.1±2.0	−10.6±10.4***	−33.0±11.8	−28.4±10.9	−19.9±12.6*
Uric acid (mg/dL)	2.57±0.23	0.40±0.08***	0.26±0.02***	0.62±0.17***	0.21±0.02***
Urea (mg/dL)	182.4±17.4	122.6±32.2	140.5±38.8	117.5±51.1	108.7±28.6*
Albumin (g/L)	7.9±0.2	8.3±0.3	7.8±0.2	8.6±0.2	7.7±0.3
NmolROS/μgprotein/min	169.51±52.5	25.6±6.5**	17.3±8.3**	38.2±5.7*	31.2±12.6*
Proteasome activity (mU)	254.8±73.2	37.8±12.7*	112.2±69.3	84.3±33.4	97.1±42.3
IL-6 (pg/ml)	81.72±12.6	37.63±8.2*	37.41±8.0*	51.44±13.6	48.78±8.3
TNF (pg/ml)	23.98±2.9	13.86±1.2*	20.14±2.6	20.74±1.8	17.25±1.6
NF-κB GC (% of sham)	191.3±31.3	92.90±16.7*	153.9±25.7	125.8±22.7	130.7±27.7

BW body weight
*p<0.05; **p≤0.01; ***p<0.001 vs placebo

Conclusion: Inhibition of XO can reduce tissue wasting and improve survival in an animal model of cancer cachexia. ROS generation and proteasome activity are reduced compared to placebo. Low-dose oxypurinol had superior properties compared to tenfold higher dose of allopurinol. Interestingly, the dose–response relationship for oxypurinol was inversed. Studies using even lower doses of oxypurinol are necessary.

072
Erythropoietin (EPO) and insulin-like growth factor (IGF) 1 have limited effects on cancer cachexia

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Background: Loss of lean mass is an important aspect of cachexia, and hence the anabolic IGF-1 and the tissue-protective EPO were chosen in order to reduce wasting.

Methods: Wistar rats (weight approximately 200 g) were inoculated intra-peritoneally with AH-130 hepatoma cells or saline (sham). Food intake and locomotor activity were assessed before inoculation and on day 11 of the 16-day protocol. Weight and body composition (NMR scan) were assessed on day 0 and day 16 after sacrifice (without tumor). Tumor-bearing animals received 100, 500, 2,500, or 5,000 U EPO/kg/day; 0.3 or 3 mg/kg/g IGF-1; or placebo.

Results: Animals of all groups showed similar baseline values for weight, body composition, and heart function. Both anabolic compounds did not increase total tumor cell number. Low-dose IGF-1 as well as 100, 500, and 2,500 U/kg/day EPO reduced wasting. While EPO reduced both loss of lean and fat mass, IGF-1 did not reduce loss of fat mass. Low-dose EPO improved quality of life (activity and food intake). Only 2,500 U/kg/day EPO significantly improved survival compared to placebo (HR, 0.44; 95% CI, 0.22–0.89; p=0.022).

	Placebo	100 U EPO	500 U EPO	2,500 U EPO	5,000 U EPO	0.3 mg IGF-1	3 mg IGF-1
N	78	20	16	14	16	14	15
Cell No. ($\times 10^9$)	2.98±0.27	3.03±0.29	2.91±0.26	2.76±0.32	2.25±0.25	2.84±0.42	2.5±0.28
Delta BW (g)	−53.7±1.8	−30.6±9.6***	−39.6±8.9*	−29.1±10.1***	−47.4±5.9	−39.6±11.1*	−42.7±8.8*
Delta lean mass (g)	−39.8±1.6	−23.7±7.4**	−30.3±6.81*	−21.6±7.7***	−35.4±4.6	−28.8±8.4*	−30.6±7.4
Delta fat mass (g)	−12.4±0.4	−8.0±1.7***	−10.5±1.5	−8.4±1.5***	−11.2±0.9	−12.4±1.9	−11.0±1.3
Food (g/24 h)	4.3±0.48	7.85±1.66**	6.56±1.73	6.57±1.89	5.38±1.19	2.5±1.66	11.8±9.82
Activity (counts/24 h)	29,509±1,775	38,550±4,412*	36,915±3,854	38,893±6,012	40,342±4,630*	35,685±4,637	29,962±5,139

BW body weight
*p<0.05; **p<0.01; ***p<0.001 vs placebo

Conclusion: In this model of cancer cachexia, the anabolic compounds EPO and IGF-1 have limited beneficial effects on survival and body wasting.

073
Overcoming obstacles to conducting trials in the context of palliative care: an exploratory phase II cluster randomized trial to investigate the effectiveness of the Macmillan approach to weight loss and eating difficulties (MAWE)

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Background: The Macmillan Weight and Eating Studies (2000–2008) have developed the first complex psychosocial intervention for weight- and eating-related distress in people with cancer cachexia and their carers: The Macmillan Approach to Weight and Eating (MAWE).
Aim: This paper is about a phase II trial of MAWE. It presents (1) challenges of conducting the exploratory trial in the context of palliative care, and how these were successfully addressed, and (2) evidence that MAWE can help patients and their carers live with advanced cancer.
Methods: The phase II trial, conducted in the UK in 2006–2007, was of cluster randomised design. It used mixed methods to compare an intervention group (n=25), who were supported by MAWE trained Clinical Nurse Specialists (CNSs), with a group who received standard care (n=25), the control group. The trial was a feasibility study. Its purpose was to test (1) recruitment and data collection process, (2) the deliverability and acceptability of MAWE and (3) the effect of MAWE on weight- and eating-related distress.
Results: The study achieved its recruitment target, data collection tools were acceptable to participants and CNSs were able to deliver MAWE in the context of their everyday practice. Contamination of the MAWE group prior to baseline data collection was problematic. However, both quantitative and qualitative data analysis support the proposition that MAWE can help patients and their carers live with cancer cachexia.
Conclusion: The evidence collated supports the argument that an RCT of MAWE is warranted, but it should be of a revised design.

074
What is an appropriate diet for people living with cancer and involuntary weight loss: a sytematic literature review

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Background: Up to 80% of people with cancer experience involuntary weight loss, a symptom of cancer cachexia syndrome (CCS). Feeding these people has little effect on their weight or survival.
Purpose: The purpose of this study is to report a systematic review of the evidence base that can inform dietary advice for patients with CCS.
Methods: Searches were conducted of MEDLINE, EMBASE, PsycINFO, and CINAHL databases for publications about diet and cancer patients off treatment with symptoms of CCS. Limits were English language, 1998 to September 2008, and adults. Seven hundred eighteen abstracts were assessed against inclusion/exclusion criteria, and 90 were selected for full-text independent examination by two researchers. Information, from 60 papers, was extracted, quality assessed, thematically analyzed, and presented as a narrative description.
Results: Two dominant perspectives emerged. The majority of authors advocated a nutrition dense diet, achieved through (1) education on the fortification of foods and (2) advice to feed frequently. The alternative approach was to advise the patient to "eat what they want." There is little robust evidence to justify either approach as able to deliver on the objectives they aim to achieve. Research is needed to establish which subgroups of weight-losing cancer patients can benefit from a nutrition dense diet.
Conclusion: A new model for nutritional care with non-traditional objectives, such as mitigation of eating-related distress, may help identify patients with CCS for whom feeding is not futile. It could also facilitate optimal nutritional intake within the confines of the patient's (1) disease process and treatment, (2) coping approach, and (3) beliefs about food.

075
L-Carnitine induces recovery of liver lipid metabolism in cancer cachexia

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Cachexia is a chronic inflammatory syndrome, characterised by weight loss and abnormalities in intermediary metabolism. Disruption of liver lipid metabolism, comprising higher uptake of long-chain fatty acids (LCFA), along with diminished oxidation and incorporation into very low-density lipoproteins (VLDL), aggravate cachexia. L-Carnitine is required for the transport of LCFA into the mitochondria, and cachectic patients present decreased plasma carnitine concentration. L-Carnitine supplementation is adopted in the treatment of many diseases, and we sought to examine whether cachectic rats would benefit from this strategy. Rats were inoculated with the Walker 256 carcinosarcoma and divided into two groups: supplemented with L-carnitine (1 g/Kg, for 28 days; TBC) and control (receiving saline; TB). Twenty-four non-tumour-bearing rats were divided into a L-carnitine-supplemented group, NC, and the respective control, N. Liver and plasma triacylglycerol (TAG), liver mRNA expression (semi-quantitative RT-PCR) of carnitine palmitoyltransferase I and II (CPT I and II), microsomal triglyceride transfer protein (MTP), fatty acid-binding protein (L-FABP), fatty acid translocase (FAT/CD36) and peroxisome proliferator-activated receptor-alpha (PPARα), and the maximal activity (radioassay) of CPT I and II, were evaluated. Gene expression of MTP and CPT I activity was reduced in TB (6%, $p<0.05$, and 42%, $p<0.01$, respectively). TB also showed increased ($p<0.01$) liver and plasma TAG content in relation to N. Chronic treatment with L-carnitine restored mitochondrial LCFA oxidation ($p<0.01$) and VLDL assembly capacity, suppressing cachexia-related hypertriglyceridemia ($p<0.001$) and steatosis. Attenuation of cachexia was accompanied by reduced tumour weight (62%) after supplementation. The results indicate that L-carnitine improves liver lipid metabolism in cachexia.
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076
Creatine supplementation improves muscular functionality in cancer cachexia

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Creatine plays an essential role in muscular functioning. It has been show that creatine supplementation in a healthy condition leads to increased muscular energy content and lean body mass. We have investigated whether creatine supplementation in a cancer-induced cachectic condition affects muscular performance and body composition. Male CD2F1 mice, 5–6 weeks of age, were divided into body weight-matched groups: (1) control, (2) tumour-bearing and (3) tumour-bearing receiving creatine (10 gkg^{-1} food). Tumours were induced by s.c. inoculation with murine colon adenocarcinoma (C26) cells. Food intake, body mass and tumour size were monitored. At day 20 after tumour/vehicle inoculation, animals were sacrificed, and muscle function was tested ex vivo. Creatine supplementation did not result in differences in organ and skeletal muscle weights, nor in changes in maximal isometric force. In contrast, creatine supplementation resulted in an increase in endurance capacity of the muscle. There was a decrease in loss of maximal force during an intermittent exercise series of high intensity. No differences in glycogen and high-energy phosphates were found. The muscular hydration level was decreased in cachectic mice and partially attenuated by creatine supplementation. Therefore, the observed increase in relative performance capacity might be linked to an improved muscular hydration level. In conclusion, no effects on absolute force output were observed after creatine supplementation, but we clearly demonstrated that the loss of relative muscular functionality during an intermittent exercise series in a murine cachexia model could be partially counteracted by creatine supplementation.

077
Appetite enhancement and weight gain by peripheral tyrosine kinase receptor (TrkB) agonists in non-human primates

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Loss of function mutations in the brain-derived neurotrophic factor (BDNF) or tyrosine kinase receptor TrkB results in hyperphagia and obesity in human and rodents. Conversely, peripheral or central stimulation of TrkB by its natural ligands BDNF or NT4 reduced body weight and food intake in mice (Tsao et al., Endocrinology 149:1038, 2008), supporting the idea that TrkB is a key anorexigenic signal. Here, we will describe the surprising discovery that in non-human primates, TrkB agonists were anorexigenic when applied centrally, but unexpectedly orexigenic, leading to gain in appetite, body weight, fat deposits, and serum leptin levels, when given peripherally (Lin et al., PLoS ONE 3(4):e1900, 2008). The orexigenic and anabolic effects of peripherally administered TrkB agonists appear to be dose dependent, not associated with fluid retention nor with evidence of receptor downregulation. Furthermore, changes in several serum biomarkers in response to TrkB agonist treatment suggest the possible mechanisms of appetite and weight regulation via peripheral TrkB activation. Our findings indicate that TrkB signaling exerts dual control on energy homeostasis in the primates that could be targeted for the treatment of either cachexia or obesity.

078

Acetate of megestrol: new evidences supporting its use in cancer cachexia. An experimental study

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Background and aims: Cancer cachexia occurs in the majority of cancer patients before death and seems to be responsible for the death of 22% of cancer patients. The use of megestrol acetate (MEGACE) for the treatment of cachexia is widely spread despite its adverse effects and the fact that, at least in humans, it seems to only increase fat and water body content and not lean body mass. The aim of the present investigation was to re-examine the anti-wasting effects of MEGACE using an experimental cancer cachexia model.
Methods: Individual muscle weights, muscle gene expression, total physical activity and grip force were measured in rats bearing the Yoshida AH-130 ascites hepatoma, following MEGACE treatment.
Results: Administration of the MEGACE to tumour-bearing rats resulted in an important reversal of the muscle-wasting process, as reflected by individual muscle weights. The anti-wasting effects of the drug were also observed in terms of total physical activity and grip force. MEGACE also decreased the rate of protein degradation in incubated skeletal muscle. Real-time PCR analysis revealed that MEGACE treatment resulted in a decrease in ubiquitin, E2 and atrogin mRNA content in gastrocnemius muscles, therefore suggesting that the main anti-proteolytic action of the drug may be based on an inhibition of the ATP-ubiquitin-dependent proteolytic system.
Conclusion: MEGACE treatment was effective in treating muscle wasting in tumour-bearing rats as reflected by the obtained results that clearly show an improvement in muscle mass and physical performance in cachectic tumour-bearing rats.

079

Different nutraceuticals are able to inhibit muscle proteolysis in vitro

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Background and aims: Cancer cachexia occurs in the majority of cancer patients before death and seems to be responsible for the death of 22% of cancer patients. The abnormalities associated with cancer cachexia include anorexia, weight loss, muscle loss, atrophy, anemia, and alterations in carbohydrate, lipid, and protein metabolism. Different nutraceuticals, i.e., w3 fatty acids of marine origin, have been proposed to counteract muscle wasting during cancer. The aim of the present investigation was to examine the anti-wasting effects of other nutraceuticals such as geninstein (soybean), resveratrol (grapes), theophylline (tea), epigallocatechin gallate (tea), and diallyl sulfide (garlic). Bearing this in mind, the in vitro effects of these nutraceuticals on proteolysis were examined on muscle cultures.
Methods: C2C12 muscle cell cultures were submitted to hyperthermia to increase the proteolytic rate. The different nutraceuticals were added to the cultures to examine their potential in inhibiting the increased proteolytic rate.
Results: All the nutraceutical used showed an anti-proteolytic effect on muscle cell cultures submitted to hyperthermia. The largest effect was observed with resveratrol (15% inhibition).
Conclusion: The nutraceuticals studied are candidate molecules for future in vivo studies using cancer cachexia models.

080

Effect of beta-hydroxyl-beta-methylbutyrate (HMB) on muscle recovery following hind limb suspension in aged rats

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Bed rest or muscle immobilization causes a rapid loss of muscle force and atrophy. This is particularly exacerbated in aged muscles along with a slow rate of muscle recovery. This is an undesirable consequence of hospitalization in the elderly. In this study, we evaluated the effects of HMB (beta-hydroxyl-beta-methylbutyrate), a leucine metabolite, on muscle recovery following hind limb suspension (HLS) in aged (>32 months) Fisher Brown Norway (FBN) rats. Male rats were fed either control 2018S chow (CON) or Ca-HMB (340 mg/kg bw)-supplemented chow (HMB) over a 14-day HLS (unloading) period, followed by recovery (reloading) for 7 or 14 days. Unloading caused significant reduction in muscle force in all groups. Neither groups recovered their baseline force even after 14 days of

reloading, and both groups lost similar amounts of muscle mass. However, histological staining revealed that during recovery, the HMB groups had significantly larger muscle fiber cross-section area (1,034.1±150.3 μm^2) than the CON (951.1±298 μm^2) , indicating muscle hypertrophy. Along this line, the HMB groups also displayed improved satellite cell activation (BrdU positive nuclei) upon reloading (HMB, 8.1±2%; CON, 4.2±2%). TUNEL assays revealed the HMB groups to have significantly fewer apoptotic nuclei than the CON (HMB, 10±5%; CON, 18±7%). Thus, administration of HMB appears to induce muscle fiber hypertrophy as well as protection against muscle atrophy, possibly by protecting myonuclei from apoptosis. This study demonstrates the potential for using HMB to facilitate muscle recovery following extended limb immobilization.

081
Fish oil and leucine decrease plasma calcium levels in tumour-bearing mice concommitant with changes in plasma levels of interleukin (IL) 6, prostaglandin E2 (PGE2) and interleukin (IL) 4

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Cancer-induced hypercalcaemia induces neuromuscular complications like fatigue. Development of hypercalcaemia involves inflammatory cytokines, like IL-6 and cyclooxygenase-2, while IL-4 has been shown to inhibit osteoclast formation in mice, thus reducing plasma calcium (pCa^{2+}). A specific nutritional combination (SNC) of high protein, leucine (Leu), fish oil (FO) and oligosaccharides increased muscle performance and reduced proinflammatory cytokine levels in a tumour-bearing mouse model. We speculated that this might have a direct ameliorating effect on pCa^{2+} and thus positively affect the cancer-induced fatigue. Male CD2F1 mice were subcutaneously inoculated with tumor cells (0.5× 106 cells) or were sham treated and divided in controls, tumour-bearing (TB) controls and TB receiving experimental diets. At termination of the experiment (day 20), bone mineral density (BMD) was measured (DEXA scan), muscle function was tested ex vivo and blood was taken for analysis of total pCa^{2+} (free+albumin-bound), cytokines and PGE2. BMD decreased and pCa^{2+} increased in TB compared to control mice. Supplementation with either Leu or FO had no effect. However, supplementation with the combination of Leu and FO or supplementation with the SNC did reduce pCa^{2+} (32%; $p<0.05$) and increased BMD (42%; $p<0.05$). Moreover, pCa^{2+} correlated positively with serum TNF-alpha, IL-6 and PGE2 and negatively with serum IL-4 ($R>0.05$; $p<0.005$). Muscle function parameters and daily activity correlated negatively with pCa^{2+} ($R<-0.7$, $p<0.05$ and $R=-0.85$, $p<0.001$, respectively). The SNC increased BMD and reduced plasma pCa^{2+}, which was related to increased ex vivo muscle function and daily activity, possibly due to the SNC-induced changes in serum cytokine levels.

082
Eicosapentaenoic acid administration attenuates arthritis-induced decrease in liver and serum insulin-like growth factor (IGF) 1 in rats

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Background and aim: Adjuvant-induced arthritis is an experimental model of rheumatoid arthritis that decreases body weight and anabolic hormones such as IGF-I. Eicosapentaenoic acid (EPA) is an omega-3 fatty acid that has anti-cachectic actions in several chronic illnesses. The aim of this study was to elucidate whether EPA administration is able to prevent the inhibitory effect of arthritis on IGF-I.
Methods: Arthritis was induced by a Freund's adjuvant injection; rats were gavaged with 1 g/kg EPA or coconut oil daily. On day 15 after adjuvant injection, rats were killed, and liver, gastrocnemius and serum were harvested.
Results: In arthritic rats, EPA administration decreased TNF in liver and gastrocnemius, the external signs of arthritis, and attenuates the decrease in gastrocnemius weight. Arthritis decreased serum concentration of IGF-I and its gene expression in the liver in rats treated with coconut oil ($P<0.01$), but not in rats treated with EPA. In contrast, arthritis did not modify IGF-I mRNA in the gastrocnemius muscle, but it increased IGFBP-3 and IGFBP-5 mRNA in the rats treated with either EPA or coconut oil. In control rats, EPA administration did not modify IGF-I or IGFBPs in serum, liver or gastrocnemius muscle.
Conclusion: Our data suggest that the beneficial effect of EPA administration to arthritic rats on gastrocnemius mass can be due to the increase in serum concentration of IGF-I

as well as its synthesis in the liver, rather to modifications in the muscular IGF-IGFBPs system.
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083
Loss of skeletal muscle during chemotherapy treatment is attenuated by n-3 fatty acid supplementation in non-small cell lung cancer patients

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Background: We recently reported low n-3 fatty acids relate to sarcopenia in patients receiving chemotherapy. The effect of chemotherapy on muscle loss is unknown but may be an important determinant in chemotherapy response and patient outcomes. We aimed to determine how chemotherapy affects muscle mass and evaluate the ability of n-3 fatty acids to attenuate muscle loss during chemotherapy.
Methods: Non-small cell lung cancer patients received four capsules of fish oil per day (2.2 g EPA+240 mg DHA; n=14) or no intervention (n=27) for ~3 months. Skeletal muscle cross-sectional area was evaluated using lumbar computed tomography (CT) images. Previously established cut-points (males below 55.4 cm^2/m^2 and females below 38.9 cm^2/m^2) were used to classify patients as sarcopenic (control n=15, supplementation n=8) or non-sarcopenic (control n=12, supplementation n=6). Muscle change was determined using two CT images from a given time interval (200 days) and expressed as a standard unit (percent change/100 days) to facilitate comparison between individuals. Amounts and types of plasma phospholipid fatty acids were determined using gas liquid chromatography.
Results: Patients receiving n-3 supplementation maintained muscle (−0.02%/100 days) compared to control patients who averaged muscle loss at a rate of −5.4%/100 days (p=0.05). Muscle rate of change was similar between sarcopenic and non-sarcopenic patients in response to n-3 supplementation. Plasma EPA and DHA was positively correlated to muscle rate of change (r=0.57, p=0.04) in the supplementation group. Maximal muscle gain was observed in patients with highest plasma EPA and DHA.
Conclusions: Muscle mass decreases during chemotherapy treatment, but attenuation can potentially be achieved through fish oil supplementation.

084
Effects of omega-3 fatty acids on blood cytokines in the patients with sepsis and cachexia

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Aims: We aim to study changes of blood cytokines in patients with sepsis and cachexia before and after intravenous injection of omega-3 fatty acids.
Methods: Twenty patients with severe sepsis and cachexia (eight men and 12 women), aged from 33 up to 86 years old, located on treatment in ICU, were randomized to two even groups. The patients from the first group on the background of parenteral nutrition were prescribed with lipid emulsion, enriched by omega-3 fatty acids ("Lipoplus" (B. Braun, Germany)). The patients of the second group were prescribed parenteral nutrition without application of lipid. At the beginning of research and on the sixth day, the content in serum blood following cytokines emulsions was identified in all patients. At the beginning of research and on the sixth day, the content in serum blood following cytokines was identified in all patients: IL-2, IL-4, IL-6, IL-8, IL-10, IFN-gamma, and TNF-alpha (Bio-Plex, Bio-Rad, USA).
Results: The content of cytokines in the blood serum of the first group of patients, receiving the parenteral nutrition with inclusion of omega-3 fatty acids, was significantly lower than in the second (control) group of patients. So, IL-6 level in serum blood in the first group was reduced to 65% and, in the second group, increased to 25%. The level of IL-8 at the first group was reduced to 87% and, in the second group, to 28%; the level of tumor necrosis factor (TNF-alpha) was reduced to 15% and 1% suitably.
Conclusions: Omega-3 fatty acids substantially hold down the level of cytokines and the inflammatory reactions of the patients with sepsis and cachexia.

085
Increased amino acid provision does not lead to insulin resistance in lung cancer cachexia

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Background and aims: Studies in healthy persons have suggested that hyperaminoacidemia compromises peripheral

glucose uptake leading to insulin resistance through altered insulin signalling and glucose transport. Insulin resistance is commonly reported in cancer cachexia, and increased protein intakes are recommended to favour protein anabolism and prevent muscle loss. We tested whether a sustained, physiological elevation of circulating amino acids would interfere with glucose metabolism.

Methods: Insulin resistance was assessed in six men with non-small cell lung cancer (NSCLC) and seven age-matched controls with healthy BMI using the hyperinsulinemic (1.25 mU/kgLBMmin), euglycemic (5.5 mM), isoaminoacidemic clamp (IsoAA) for 2.5 h, followed by 2.5 h of hyperaminoacidemia (HyperAA) at postprandial levels. Glucose kinetics was quantified by tracer (3H3-glucose) methodology.

Results: NSCLC patients had 7.3±1.2% weight loss, lower muscle mass, higher CRP and GH levels and lower serum prealbumin and urea than controls. Fasting plasma glucose, insulin and branched-chain amino acids (BCAA) and glucose rate of appearance (Ra, production) and disappearance (Rd, uptake) did not differ. During IsoAA, insulin reached 436±33 in NSCLC vs. 551±36 pM in controls (p= 0.04). Glucose production was equally suppressed in both, but glucose uptake was less stimulated in NSCLC (6.2±0.7 vs. 8.6±0.8 mg/LBMmin, p=0.038, adjusted for insulin) indicating peripheral insulin resistance. During HyperAA (732±70 μM of total BCAA), serum insulin and glucose production did not change significantly. However, glucose uptake increased similarly in both groups, Rd remaining lower in NSCLC (7.3±0.8 vs. 10.0±0.7 mg/LBMmin, p= 0.027). Glucose uptake negatively correlated with fasting TNF-alpha (Spearman's r=−0.71, p=0.033).

Conclusions: Inflammation was associated with insulin resistance in lung cancer patients. However, in both these and in insulin-sensitive controls, providing a high availability of amino acids, equivalent to a physiological postprandial state (~25 g protein), did not interfere with insulin action on glucose metabolism, rather it may enhance glucose uptake. Presence of insulin resistance should not be viewed as a deterrent to recommending higher protein intakes in patients with cancer cachexia.

086

Effects of the insulin sensitizer rosiglitazone on cachexia outcomes in mice with colon-26 tumors

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Background and aims: Insulin resistance is a metabolic derangement associated with cancer cachexia. We previously demonstrated that rosiglitazone (RGZ) improved insulin sensitivity and body weight and suppressed quadriceps mRNA of E3 ligases Atrogin-1 and MuRF-1 before the onset of overt cachexia in tumor-bearing mice. Additionally, adipose IL-6 mRNA was decreased, and adiponectin mRNA was increased. The positive effects of RGZ early in cachexia pathogenesis led us to test the hypothesis that RGZ would also improve cachexia outcomes in late stage cancer.

Methods: Male CD2F1 mice were inoculated with 1×106 colon-26 adenocarcinoma cells and treated with 10 mg/kg/day RGZ or vehicle (PBS). RGZ-treated mice were fed ad libitum or pair-fed to PBS-treated mice.

Results: RGZ delayed the onset of weight loss by 2 days, decreased total weight loss, and increased white and brown adipose mass compared to PBS-treated mice. Pair-feeding negated the effects of RGZ on body weight and white adipose mass, and decreased quadriceps muscle mass. However, the RGZ-induced increase in brown adipose mass was maintained in the pair-fed group. Insulin-stimulated glucose disappearance, muscle fiber diameter, muscle strength, plasma IL-6, and quadriceps E3 ligase mRNA were not different between groups at the end of the study.

Conclusions: Although RGZ delayed weight loss and attenuated adipose atrophy, it did not improve muscle mass, strength, or proteolytic gene expression in severely cachectic mice. Some effects of RGZ appear to be dependent on the increased food intake associated with RGZ, emphasizing the importance of targeting both dysregulated metabolism and anorexia for optimal cachexia treatment.

087

Peroxisome proliferator-activated receptor-α (PPAR-α) agonist fenofibrate ameliorates arthritis-induced upregulation of proteolysis and regenerative response in the gastrocnemius muscle of rats

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Background and aims: Adjuvant induced arthritis is an animal model of chronic inflammation that leads to marked skeletal muscle atrophy, activation of the ubiquitin proteasome pathway, and regenerative response. PPAR-α is an important target in the inflammatory processes. Our aim was to investigate the effect of fenofibrate in experimental arthritis.

Methods: Arthritis was induced in male Wistar rats by an intradermal injection of Freund's adjuvant. Control and arthritis rats were divided into two groups and were daily gavaged with 300 mg/kg bw of fenofibrate or vehicle

during 12 days. Gene expression of TNF-α in the liver, and atrogin-1, MuRF-1, myogenin, MyoD, and PCNA in gastrocnemius muscle were measured by real-time PCR. Protein levels of myogenin, MyoD, and PCNA were measured by Western blot.
Results: Arthritic rats treated with fenofibrate had lower arthritis score, hind paw swelling, and splenomegaly ($P<0.01$). Arthritis-induced increase in liver TNF-α mRNA was blocked by fenofibrate ($P<0.01$). Fenofibrate administration to arthritic rats increased gastrocnemius weight and prevented arthritis-induced increase in atrogin-1 and MuRF-1 mRNA in this muscle ($P<0.01$). There was an increase in myogenin, MyoD, and PCNA gene expression and protein in the gastrocnemius of the arthritic rats ($P<0.01$), whereas fenofibrate normalized this effect ($P<0.01$).
Conclusion: In addition to its anti-inflammatory effect in arthritic rats, activation of the PPAR-α by fenofibrate prevents the upregulation of the ubiquitine proteasome pathway and normalizes the regeneration and differentiation processes in the skeletal muscle.
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088
Acetylation of heat shock protein (HSP) 70 and regulation of Foxo transactivation

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The Foxo transcription factors are upregulated in response to multiple conditions of skeletal muscle wasting and are both sufficient and required for the normal atrophy program. Although Foxo is largely regulated in skeletal muscle through its phosphorylation and inactivation by Akt, we recently demonstrated that Foxo-dependent transcription can be inhibited by heat shock protein 70 (Hsp70). However, the precise mechanism responsible for this inhibition remains unknown. Because acetylation of Hsp70 has been shown to regulate its function in other cell types, the current study sought to determine whether Hsp70 is acetylated in skeletal muscle, and whether this contributes to its repression of Foxo transactivation. Immunoprecipitation of total acetylated proteins from whole muscle revealed Hsp70 to be acetylated during weight bearing conditions, which was significantly reduced following 3 days of immobilization. Subsequent co-immunoprecipitation experiments revealed binding of Hsp70 to the histone acetyltransferase p300. To determine if acetylation of Hsp70 is mediated via p300, and whether this affects Hsp70-mediated repression of Foxo activity, we subsequently transfected whole muscle with a WT p300 expression plasmid or a p300 mutant lacking its acetyltransferase activity (p300DY) plus Hsp70 and a Foxo-responsive reporter prior to weight bearing or immobilization conditions. Expression of WT p300, but not p300 DY, prevented the decrease in Hsp70 acetylation in response to immobilization. Importantly, expression of the p300DY reversed the inhibitory effect of Hsp70 on Foxo transactivation. These findings suggest that modulating the acetylation status of Hsp70 could be an important mechanism of Foxo regulation in skeletal muscle.

089
Toll-like receptor 4 mediates muscle wasting

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Background and aims: Cachectic muscle wasting is a complication of inflammatory diseases. Lipopolysaccharide (LPS) is known to stimulate muscle catabolism by upregulating specific ubiquitin ligases via inducing inflammatory cytokines. Here, we investigated the direct stimulation of muscle protein degradation by LPS via the activation of muscle Toll-like receptor 4 (TLR4).
Methods and results: Incubation of C2C12 myotubes with the TLR4 ligand LPS (100 ng/ml) for 48 h resulted in myosin heavy chain loss and thinner myotubes. Expression of atrogin-1/MAFbx was upregulated by LPS within 1 h and peaked at 2 h. On the other hand, expression of MuRF1 was initially downregulated at 1 and 2 h, and then upregulated at 3 h. To understand the signaling mechanism of the LPS actions, we observed that LPS activated p38 MAPK and AKT within 30 min. The latter resulted in hyperphosphorylation and inactivation of Foxo1/3, which could explain the downregulation of MuRF1. Pretreatment of C2C12 myotubes with p38 inhibitor SB202190 blocked LPS upregulation of atrogin-1/MAFbx, but not MuRF1. Conversely, overexpression of active MKK6 (MKK6bE) upregulated atrogin-1/MAFbx expression. TLR4 knockdown with siRNA abolished LPS activation of p38 and AKT. In mice, pre-administration of SB202190 or TLR4 gene knockout blocked LPS upregulation of atrogin-1/MAFbx expression and stimulation of tyrosine release by muscle.
Conclusions: LPS direct activation of muscle TLR4 accelerates muscle protein degradation by upregulating muscle E3 ligases. TLR4/p38 signaling upregulates atrogin-1/MAFbx expression and contributes significantly to LPS-induced muscle loss. These results depict a new paradigm for the mechanism of cachexia.
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090
Low-dose dexamethasone prevents protein loss and the impairment of carbohydrate oxidation in rat skeletal muscle during endotoxaemia

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Background and aims: We previously suggested a role for cytokine-mediated impairment of Akt/Forkhead box O (FOXO) signalling in the induction of muscle atrophy and impairment of muscle carbohydrate oxidation during lipopolysaccharide (LPS)-induced endotoxaemia. We aimed to determine whether low-dose dexamethasone (Dex; anti-inflammatory agent) infusion during endotoxaemia would dampen LPS-induced impairment of Akt/FOXO signalling, thereby blunting muscle atrophy and the impairment of carbohydrate oxidation.
Methods: Chronically-instrumented rats received a continuous intravenous infusion of either LPS (15 $\mu g kg^{-1} h^{-1}$), Dex (12.5 $\mu g kg^{-1} h^{-1}$), Dex+LPS or saline for 24 h at 0.4 $ml h^{-1}$.
Results: Dex co-administration during endotoxaemia (1) blunted the LPS-induced increase in muscle cytokines, the reduction in Akt and FOXO1 phosphorylation and the decline in the muscle protein:DNA ratio; (2) suppressed LPS-induced cathepsin-L upregulation, but not the increase in MAFbx or MuRF1 mRNA expression; and (3) blunted LPS-induced muscle PDK4 mRNA upregulation and glycogen breakdown and lactate accumulation (see Table).

Measurement	Effect of LPS from control[a]	Effect of Dex in LPS-treated rats[b]
Protein:DNA ratio	↓1.7-fold**	↑63%*
mRNA expression		
Tumour necrosis factor-α (TNF-α)	↑10-fold***	↓51%**
Interleukin-6 (IL-6)	↑14-fold***	↓85%***
Muscle atrophy F-box (MAFbx)	↑4.6-fold***	No change
Muscle RING finger 1 (MuRF1)	↑13-fold***	No change
Cathepsin-L	↑9.7-fold***	↓43%*
Pyruvate dehydrogenase kinase 4 (PDK4)	↑16-fold***	↓47%**
Protein expression		
Phosphorylated-Akt	↓2-fold*	↑128%*
Phosphorylated/total-FOXO1	↓1.9-fold*	↑43% (NS)
Metabolites		
Glycogen	↓1.3-fold*	↑30%*
Lactate	↑1.53-fold**	↓27%**

*$P<0.05$; **$P<0.01$; ***$P<0.001$
[a]Significantly different from control
[b]Significantly different from LPS-treated group

Conclusions: Low-dose Dex infusion blunted muscle protein loss and the impairment of muscle carbohydrate oxidation, possibly through suppression of cytokine-mediated Akt/FOXO inhibition.

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C2 skeletal myoblast survival, death, proliferation and differentiation: regulation by Adra1d

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Background: Apoptosis represents one potential pathway underlying skeletal muscle wasting associated with chronic diseases. Cytokines (e.g. TNF-α) act to trigger catabolism while the insulin-like growth factor (IGF) system influences anabolism and is essential for skeletal muscle regeneration and maintenance. Up-regulation of inhibitors of apoptosis (e.g. IGF) could be beneficial in the treatments of cachexia and sarcopenia. Using C2 skeletal myoblasts, we have reported that while 10 $ng ml^{-1}$ TNF-α-induced apoptosis is rescued by IGF-I, 1.25 $ng ml^{-1}$ TNF-α (which alone is not myotoxic) elicits MAPK-mediated apoptosis when co-incubated with IGF-I (1.5 $ng ml^{-1}$).
Aims, methods, and results: Array technology followed by qRT-PCR identified Adra1d as a potential gene regulating pro- and anti-apoptotic roles of IGF-I. Expression was up- or down-regulated in conditions of survival or apoptosis, respectively. Administration of TNF-α (1.25 or 10 $ng ml^{-1}$) induced a significant ($p<0.05$) decrease (~50%) in Adra1d expression relative to controls. Addition of IGF-I to 10 $ng ml^{-1}$ TNF-α induced significant myoblast survival and a return in Arda1d expression to basal levels. However, IGF-I addition to 1.25 ng ml^{-1} TNF-α induced elevated death and a 55% decline ($p<0.05$) in Adra1d expression. Twenty micromole PD98059 (MEK inhibitor) administration rescued death induced by 1.25 $ng ml^{-1}$ TNF-α and IGF-I and returned Adra1d levels to baseline. Since Adra1d was elevated following survival, we investigated the impact of Adra1d gene silencing. Adra1d knockdown resulted in elevated cell death under all incubation conditions ($p<0.05$).
Conclusion: These novel findings suggest that Adra1d expression is essential for skeletal muscle cell survival and may have implications for cachexia and sarcopenia.

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C2 skeletal myoblast survival, death, proliferation and differentiation: regulation by SIRT1

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Introduction and aim: Sirt1 is a key regulator of cell defences and survival in response to stress. Deletion of Sirt1 abolishes the increase in lifespan induced by calorie restriction or sublethal cytokine stress, indicating that Sirt1 promotes longevity and survival. We have demonstrated that sublethal TNF-α (1.25 ng ml^{-1}) administration inhibits myotube formation, and co-incubation with IGF-I (1.5 ng ml^{-1}) facilitates C2 myoblast death rather than rescuing differentiation. Higher dose TNF-α (10 ng ml^{-1}) resulted in significant apoptosis, which was rescued by IGF-I (1.5 ng ml^{-1}; 50% rescue; $p<0.05$). We aimed to investigate the role of Sirt1 in the conflicting roles of IGF-I.

Methods and results: qRT-PCR revealed that Sirt1 expression was elevated in myoblasts following incubation of 10 ng ml^{-1} TNF-α or 1.25 ng ml^{-1} TNF-α plus IGF-I (5–7.2-fold increases vs. control; $p<0.05$). Of TNF-α, 10 ng ml^{-1} induced ~21±0.7% apoptosis, which was reduced (~50%, $p<0.05$) when administered with IGF-I. Similarly, Sirt1 expression was elevated following 10 ng ml^{-1} TNF-α administration, but was reduced (~30%, $p<0.05$) in the presence of IGF-I. Incubations that induced the greatest myoblast stress/damage resulted in elevated Sirt1 expression; we therefore investigated the effects of Sirt1 gene silencing. Of TNF-α, 10 ng ml^{-1} or co-incubation of 1.25 ng ml^{-1} TNF-α/IGF-I resulted in apoptosis (20.33±2.08% and 19±2.65%), which was increased when myoblasts were pre-administered with Sirt1 siRNA (31±2.65% and 27.33±2.52%; $p<0.05$). Under basal condtions, Sirt1 inhibition had no impact on death.

Conclusion: These findings suggest that Sirt1 expression increases under conditons of stress, functioning to reduce or dampen myoblast death.

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The effects of pro- and anti-inflammatory factors on the interleukin (IL) 6 secretion from the precursors of muscle regeneration

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Background: Critical illness myopathy is a frequent condition in the emergency units. Its pathophysiology and final outcome is complex and depends not only on the extent of muscle damage but also on the efficiency of muscle regeneration, which is triggered by muscle injury. Muscle regeneration starts from the mytotically and metabolically quiescent satellite cells. After injury, they are released from their location and enter the cell cycle as mononuclear myoblasts. Muscle mass formed in the regeneration process critically depends on the myoblast proliferation, and since critical illness is accompanied by extensive cytokine signaling, it is important to know how this signaling affects the mechanisms participating in the myoblast proliferation.

Aim and methods: A great body of evidence supports the concept that with regard to the cytokine signaling, major response of the skeletal muscle is IL-6 secretion. IL-6 promotes myoblast proliferation and is an essential regulator of satellite cell-mediated muscle hypertrophy. Therefore, it is very likely that it plays an important role in muscle regeneration. In this study, we followed the effects of pro-inflammatory (TNF-alpha and LPS) and anti-inflammatory factors (dexamethasone and IL-10) on the IL-6 secretion from the cultured human myoblasts. The effects on proliferating myoblasts were compared with the effects on non-proliferating myotubes.

Results and conclusion: Pro-inflammatory factors TNF-alpha- and LPS stimulated IL-6 release in both myoblasts and myotubes. Anti-inflammatory factor dexamethasone suppressed constitutive secretion of IL-6, while IL-10 decreased secretion of IL-6 but only in myoblasts. Our results suggest complex and differentiation stage-specific control of IL-6 from precursors of human muscle regeneration.

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Systemic inflammation and nuclear factor-kappa B (NF-kB) dependent muscle atrophy in a mouse model of acute pulmonary inflammation

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Background and aims: Skeletal muscle atrophy is a prominent and disabling feature of inflammatory lung disease. The aim of this study was to address whether acute pulmonary inflammation is sufficient to induce systemic inflammation and loss of muscle mass, and whether local NF-kB activity is required for muscle atrophy.

Methods: C57/bl6 mice received a single bolus of *Escherichia coli* LPS by intra-tracheal (IT) instillation to induce acute pulmonary inflammation or 0.9% NaCl as a control.

Results: Neutrophil infiltration in the lungs was maximal at 72 h and almost completely resolved at 120 h post-LPS. Loss of bodyweight (bw) and muscle weight (mw) was maximal 48–72 h post-LPS (10–15% decrease), and recovery was almost complete 120 h post-LPS. Assessment of MuRF1 and atrogin-1 in muscle revealed a transient increase in mRNA abundance (24–72 h post-LPS), preceding maximal loss of mw. Pair-fed experiments revealed that reduced food intake was responsible for only a minor part of the changes observed in bw, mw, and Atrogin-1/MuRF1 expression. Systemic inflammation was evidenced by increased circulating levels of a number of cytokines and chemokines, as well as NF-kB activation in circulating neutrophils and peripheral tissues including skeletal muscle measured in NF-kB-luciferase reporter mice, which was verified by increased KC (IL-8) and IkBa expression. Importantly, muscle specific abrogation of NF-kB signaling strongly attenuated MuRF1 expression and loss of muscle mass following IT-LPS instillation in transgenic mice.
Conclusions: These data demonstrate that acute pulmonary inflammation induces systemic inflammation, and that subsequent muscular NF-kB activation contributes to muscle atrophy, potentially via the induction of MuRF1.

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Morphological and metabolic responses of C2C12 myotubes following food deprivation and refeeding

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Skeletal muscle is a prominent tissue involved in the regulation of metabolic homeostasis by its capacity to oxidize fatty acids and glucose. To test the adaptations of skeletal muscle cells following food deprivation, we developed an in vitro experimental model of C2C12 myotubes submitted to a metabolic challenge consisting in 4 h of food deprivation followed by 20 h of refeeding. During the time course of this metabolic challenge, we determined the morphological and metabolic responses of C2C12 myotubes. Myotube area decreased progressively (~20% at 4 h) during food deprivation and increased progressively to reach control values 18 h after refeeding. Accordingly, the phosphorylation of ribosomal protein S6, which is involved in the regulation of protein synthesis, decreased and increased in a time-dependant manner during food deprivation (~75% at 4 h) and refeeding (~500% at 4 h), respectively. Refeeding was accompanied by a strong increase in glucose consumption (~400% at 15 min), which progressively decreased to control level 20 h after refeeding. During this period, glucose metabolism, which was initially oxidative, progressively switched to a glycolytic metabolism (~35%). Furthermore, food deprivation was accompanied by a transient decrease (~20% at 15 min) in hexokinase and citrate synthase activities, which recovered progressively during food deprivation and refeeding. The expression of sirtuin 1, a NAD^+-dependent histone deacetylase, potentially involved in the regulation of hexokinase expression, decreased during food deprivation (~40% at 4 h). We are currently assessing the contribution of sirtuin 1 in these morphological and metabolic adaptations.

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Redox balance and carbonylated proteins in limb muscles and heart of cachectic rats

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In fast- and slow-twitch limb muscles and heart of cachectic rats, redox balance and muscle structure were explored. Furthermore, the nature of the oxidatively modified proteins was also identified in these muscles. Reactive carbonyls, hydroxynonenal (HNE)-, and malondialdehyde (MDA)-protein adducts and antioxidant enzyme levels were determined in limb muscles and heart of cachectic (7 days

after inoculation of Yoshida A H-130 ascites hepatoma) and control rats. Moreover, carbonylated proteins were identified (proteomics), and fiber-type composition was evaluated (morphometry) in these muscles. In cachectic rats, compared to the controls, (1) HNE- and MDA-protein adducts levels were greater in gastrocnemius, tibialis anterior, soleus, and heart; (2) in the gastrocnemius, type II fiber size was reduced, and the intensity of carbonylated protein immunostaining was significantly greater in these fibers; and (3) proteins involved in glycolysis, ATP production and distribution, carbon dioxide hydration, muscle contraction, and mitochondrial metabolism were significantly more carbonylated in limb muscles and heart. Cancer cachexia alters redox balance in fast- and slow-twitch limb muscles and heart of rats, inducing increased oxidative modifications of key proteins involved in muscle structure and function. Additionally, it induces a reduction in type II fiber size in the gastrocnemius, which is associated with increased protein oxidation.

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Impact of exercise training on myostatin expression in the myocardium and skeletal muscle in a chronic heart failure model and patients with chronic heart failure (CHF)

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Background: In terminal chronic heart failure (CHF), elevated cytokines and cachexia are often observed. Several studies documented that exercise training exerts beneficial effects on skeletal muscle in this setting. Furthermore, it has been shown that the expression of myostatin, a key regulator of skeletal muscle mass, is increased in a variety of cachectic states.
Aims: The aim of the present study was to investigate the expression of myostatin in CHF, the influence of exercise training, and its regulation by tumor necrosis factor-alpha (TNF-a). Furthermore the effects of exercise training on myostatin in patients with CHF were examined.
Methods and results: In an animal model of CHF (LAD ligation model), protein expression of myostatin was elevated 2.4-fold in the skeletal muscle and more than four times in the myocardium as compared to control. Exercise training on a treadmill over 4 weeks lead to a significant reduction of myostatin protein expression in the skeletal muscle and the myocardium of CHF-animals back to baseline levels. In differentiated C2C12 cells, TNF-α induced the expression of myostatin through a p38MAPK-dependent pathway under participation of NF-kB. In patients with CHF, exercise training lead to a significant reduction of myostatin in the skeletal muscle compared to controls.
Conclusion: These alterations of myostatin expression seen under exercise training in the skeletal and heart muscle could be one possible mechanism explaining the beneficial anti-catabolic effects of exercise training in CHF.

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Skeletal muscle transcriptional responses to short-term resistance exercise greatly differ among individuals

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While resistance exercise training increases muscle mass and can be exploited to aid rehabilitation following wasting, our understanding of the molecular events that underpin these processes remains at a rudimentary level. Here, we explored the transcriptional events that occur in healthy skeletal muscle following resistance exercise.
Sixteen, young, healthy males, divided into exercise or control groups, had basal muscle biopsies taken (vastus lateralis, day 0). Subjects in the exercise group then performed 5×30 maximal isokinetic knee extensions (180º/s), which was repeated on days 2 and 4. Control subjects abstained from exercise. Additional biopsies obtained on days 1, 3, and 7 at rest allowed global gene expression changes to be examined using gene microarrays. The data was filtered using Ingenuity's IPA software, constricting analysis to genes considered relevant to muscle processes.
Principal component analysis revealed two disparate transcriptional responses to resistance exercise between individuals on days 1 and 3 (but not day 7). Subjects grouped according to these responses resulted in one cohort (n=5) with 0, 8, and 0 genes significantly (FDR≤ 0.05) altered from basal on days 1, 3, and 7, respectively. In the three remaining subjects, 630, 1,042, and 419 genes were altered over the same period with 20% of identified genes altered at multiple time-points. These observations could not be rationalized by volume of isokinetic work performed or initial muscle mass of volunteers. No significant changes were observed in control subjects.

Resistance exercise elicits a markedly different transcriptional response among individuals, with some showing an exaggerated and prolonged response.

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Curcumin treatment blocks increased proteasome and apoptosome activities in immobilized rat skeletal muscle without inhibiting muscle atrophy

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Background and aim: Sustained muscle wasting ultimately leads to bed rest and immobilization. Designing new strategies that prevent muscle wasting following immobilization is of major importance to maintain autonomy and to reduce public health care costs. Immobilization is characterized by a concomitant activation of the ubiquitin (Ub)-proteasome-dependent proteolytic system and of the mitochondria-associated apoptotic pathway. Increased oxidative stress and inflammatory response also occur in immobilized skeletal muscle. We evaluated the role of curcumin on muscle atrophy in immobilized animals. This compound exhibits anti-oxidant and anti-inflammatory properties and has been reported to block proteasome activation in intact animals.
Methods: Wistar rats were subjected to unilateral hindlimb immobilization for 8 days, and the contralateral muscle of the non-casted leg was used as a control. Half of the animals received a daily administration of curcumin (1 mg/kg, i.p.). Proteolytic and apoptotic pathways were studied in gastrocnemius muscles.
Results: Curcumin did not reduce immobilization-induced muscle atrophy. Similarly, the increase amount of ubiquitinated conjugates and the increased caspase-3 activity that both prevail in immobilized muscles were unchanged in curcumin-treated rats. By contrast, curcumin suppressed the increased chymotrypsin-like activity of the proteasome and apoptosome activities in the immobilized muscles.
Conclusions: The lack of effect of curcumin on muscle wasting in immobilized muscles suggests that curcumin does not inhibit rate-limiting steps for muscle atrophy. Alternatively, the data may suggest that the i.p. mode of administration of curcumin does not provide enough biologically active metabolites on the signaling pathways of muscle proteolysis.

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Loss of USP19 increases transcription of myofibrillar proteins in L6 muscle cells and decreases muscle wasting in response to denervation in mice

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Background and aims: Although many enzymes involved in ubiquitination are activated in atrophying muscle, little is known about the role of deubiquitinating enzymes (DUBs). We previously showed that the USP19 DUB is induced in various conditions of muscle atrophy including cancer. We tested the effects of loss of USP19 in muscles cells and transgenic mice.
Methods: Methods include RNAi of USP19 in muscle cells and gene inactivation in mouse.
Results: Depletion of USP19 by siRNA in L6 myotubes resulted in 1.5–2.5-fold increases in protein levels of myosin heavy chain (MHC), actin, troponin T, and tropomyosin. USP19 depletion also increased MHC and tropomyosin mRNA levels, suggesting that this effect is due to increased transcription. Consistent with this, USP19 depletion increased myogenin protein and mRNA levels by ~2-fold. Lowering myogenin using siRNA prevented the increase in MHC and tropomyosin upon USP19 depletion, indicating that myogenin mediated the increase in myofibrillar proteins. Dexamethasone or TNF-α treatment lowered MHC and increased USP19. USP19 siRNA reversed the suppression of MHC by these agents. Mice lacking USP19 were grossly normal, but lost 25–53% less mass in gastrocnemius/tibialis anterior muscles than WT mice upon denervation.
Conclusions: These studies demonstrate for the first time a role for a DUB in muscle wasting. Interestingly, the induction of USP19 in wasting appears to suppress synthesis of myofibrillar proteins, indicating that the ubiquitin system not only mediates the increased protein breakdown but also is involved in the decreased protein synthesis seen in atrophying skeletal muscle.

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Structural remodeling of human skeletal muscle with chronic inactivity

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Background and aims: Long-term inactivity leads to muscle atrophy and weakness. The aim of the present study was to investigate the mechanisms of human skeletal muscle remodeling in chronic disuse.
Methods: Two paradigms of disuse were used for this study: unilateral lower limb suspension (ULLS) and bed rest (BR). Nine males (aged 22.3±3.8 years) participated to a 23-day ULLS study, while ten males (aged 22.3±2.2 years) took part to a 35-day BR study. Evidence of skeletal muscle remodeling was obtained by measuring vastus lateralis (VL) muscle fibre fascicle length (Lf) using ultrasound. To investigate the mechanism underlying this structural alteration, focal adhesion kinase activity and content (FAK, an integrin-associated phosphoprotein involved in mechanotransduction) were measured on VL muscle biopsy samples obtained in the same individuals. Since FAK is an upstream modulator of protein synthesis, concomitant fractional rate of protein synthesis was measured after infusion of stable isotopes.
Results: VL Lf decreased by 5.5% after just 10 days of unilateral lower limb suspension (ULLS) and by 9% after 35 days of bedrest. This decrease in Lf, indicating a rapid loss of sarcomere in series, was associated with a concomitant decrease in concentration (−20%) and activity (−30%) of FAK. Myofibrillar fractional rate of protein synthesis, measured on the same biopsy samples, dropped by 50% within 10 days of unloading.
Conclusions: Sarcomere remodeling, in response to unloading in humans, is a very fast process associated with marked changes in cytoskeletal proteins involved in mechanotransduction, leading to a dramatic decrease in protein synthesis. The results also suggest that countermeasures aimed at preventing muscle atrophy should start from the very early phases of the disuse period.

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The transcription factor activating transcription factor 4 (ATF4) promotes skeletal myofiber atrophy during fasting

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Background and aims: Prolonged fasting alters skeletal muscle protein metabolism and promotes skeletal myofiber atrophy, but the underlying molecular mechanisms are not fully understood. Here, we examined the potential role of ATF4, a transcription factor that plays an evolutionarily ancient role in protein metabolism and the cellular response to starvation.
Methods: We studied adult male C57BL/6 mice and used electroporation to transfect their tibialis anterior muscles with plasmid DNA.
Results: Reduction of ATF4 expression with an artificial microRNA designed to target ATF4 (miR-ATF4) did not alter myofiber size under nonfasting conditions, but it prevented myofiber atrophy under fasting conditions. Likewise, when we reduced the fasting level of ATF4 mRNA with a phosphorylation-resistant form of eukaryotic intitiation factor 2-alpha, myofiber atrophy was reduced. To determine if increased ATF4 expression was sufficient to reduce myofiber size, we overexpressed ATF4 and found that it reduced myofiber size in the absence of fasting. In contrast, a transcriptionally inactive ATF4 construct did not alter myofiber size, suggesting a requirement for ATF4-mediated transcriptional regulation. To begin to determine the effect of ATF4 on skeletal muscle gene expression, we examined mRNAs encoding atrogin-1, MuRF1, and the protein synthesis inhibitor 4E-BP1, all of which were increased by fasting. Under fasting conditions, miR-ATF4 reduced levels of atrogin-1, MuRF1, and 4E-BP1 mRNAs. However, in the absence of fasting, ATF4 overexpression increased only 4E-BP1 mRNA.
Conclusions: Taken together, these data suggest that ATF4 plays an essential role in modifying skeletal muscle gene expression to promote myofiber atrophy during starvation.

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Intramyocellular lipid content in human cancer cachexia—an ultrastructural analysis

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Background and aims: Fat infiltration in skeletal muscle is thought to be a pathological feature and associated with muscle frailty in several conditions. However, few data exists on its relevance in human cancer cachexia. We hypothesised that intramyocellular lipid content is increased in the muscle of patients with cancer cachexia and could represent a marker of muscle frailty.
Methods: Nineteen patients with upper gastrointestinal cancer (pancreatic, gastric or oesophageal) and six weight-stable non-cancer controls were recruited. Rectus abdominis biopsy was taken at the time of open surgery. Samples were processed, embedded in resin and 60-nm sections were cut and stained with uranyl acetate/lead citrate. Images were taken using a Phillips CM120 transmission electron microscope at ×2,650 magnification. Lipid vacuoles were counted manually in at least two fields per patient, and the average count was recorded. Three groups were analysed (using SPSS ver. 15 software): A, controls; B, weight-stable cancer patients (weight loss <5%) and C, weight-losing cancer patients (weight loss ≥5%).

Results: Mean weight loss was −0.25% in group A, −0.19% in B and 11.6% in C. Control patients were younger than the cancer groups (52 (A) vs 69 (B) vs 63 years (C); $p=0.016$). Mean lipid vacuole count was significantly higher in group C patients compared with group A and B (10.2 vs 1.8 vs 2.2, respectively; $p=0.004$) and correlated positively with weight loss ($R=0.50$, $p=0.014$).
Conclusions: Intramyocellular fat deposition appears to increase in cancer patients with weight loss. This may have implications on muscle function and furthermore could be used as a marker of muscle frailty.

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Reduced muscle quality is observed with progressive weight loss in patients with cancer

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Background and aim: Previous studies have suggested that cancer cachexia is not associated with a reduction in leg muscle mechanical quality when defined as muscle strength per unit cross-sectional area (CSA). We tested this hypothesis in a group of patients with mixed upper gastrointestinal (UGI) cancer.
Methods: Patients with UGI cancer (gastric, oesophageal and pancreatic) were recruited. Maximal voluntary isometric knee extensor strength was measured using dynamometry. Sequential T1-weighted axial images of the thigh were obtained using magnetic resonance (MR) imaging. MR images were analysed offline to obtain quadriceps CSA. A *k*-means clustering technique was used to allow subtraction of intermuscular fatty infiltration. Muscle quality was defined as muscle strength/CSA of the quadriceps (Newtons (N)cm^{-2}). Data was analysed using SPSS statistical software.
Results: Eighteen patients participated, mean (SD) age of the cohort was 62 (10)years and male to female ratio was 7:11 with a mean weight loss of 12.8%. There was a significant negative correlation between percentage weight loss and quadriceps muscle quality ($r=-0.54$, $p=0.020$). Patients with a weight loss of >15% had significantly lower muscle quality compared with patients with weight loss.
Conclusions: Contrary to previous evidence, this study suggests that muscle quality is impaired in cancer patients with significant weight loss. Efforts should thus focus on identifying patients at risk of cancer cachexia early in the disease process in order to allow treatment prior to deterioration in muscle quality.

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Near-infrared measured lean body mass is correlated with serum creatinine in long-term hemodialysis patients

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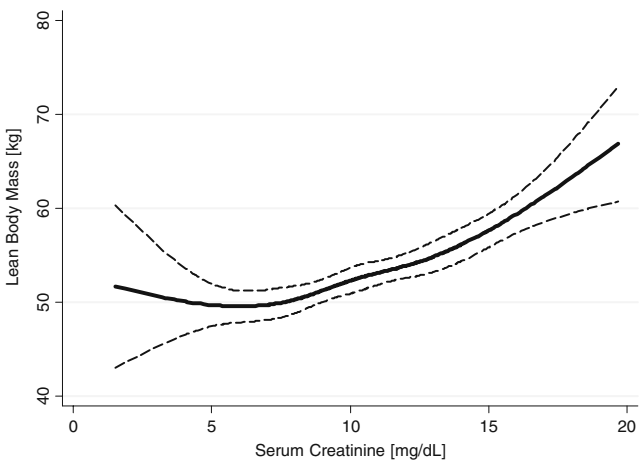
Background: In long-term hemodialysis (HD) patients who receive an adequate dose of dialysis treatment, serum creatinine that is measured immediately prior to an HD treatment session maybe a measure of nutritional status including total body muscle mass and probably striated meat intake in food, but this notion has not been well studied.
Methods: We measured body composition including total body fat percentage and lean body mass (LBM) using a portable near-infrared interactance (NIR) technology in 747 HD patients from eight DaVita dialysis clinic and compared them to 3-month averaged pre-HD serum creatinine.
Results: Patients were 53.6±14.8 years old and included 48% women, 32% Blacks, and 51% diabetics. Three-month averaged serum creatinine was 10.2±3.1 mg/dL. The correlation coefficient between serum creatinine and LBM was 0.26 and 0.19 without any adjustment and adjustment for case-mix (age, gender, race, diabetes, vintage, Charlson comorbidity index, and Kt/V). After dividing the LBM into five a priori selected groups, pre-HD serum creatinine was incrementally higher across higher LBM quintiles. Spline models showed an almost linear association between LBM and serum creatinine even after multivariate adjustment (see Figure).



Conclusions: In HD, serum creatinine is a correlate of LBM, among others. Hence, studies that indicate a positive association between higher serum creatinine and greater survival may indicate the salutary impact of higher LBM.

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Diagnostic markers of cancer-associated skeletal muscle wasting obtained using 1H-NMR profiling of urinary metabolites analyzed by a machine learning approach

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Background and aim: Sensitive detection of the loss of muscle in cachexia requires image-based methods such as computed tomography (CT) which are costly and inconvenient. We assessed a non-invasive metabolomic approach to detect early or slowly evolving muscle wasting.
Methods: We identified and quantified 63 metabolites in 1H-NMR spectra from urine of 91 cancer patients. Rate of dynamic change of total skeletal muscle area (cm^2) at the third lumbar vertebra was assessed from serial CT images. A common statistical technique (partial least squares discriminant analysis (PLS-DA)) and a novel machine-learning technique were used to identify metabolite patterns that discriminated patients with muscle loss rate >0.75%/100 days versus patients maintaining or gaining muscle.
Results: By viewing known biochemical pathways as a Gaussian Markov random field, we built a classifier "pathway-informed analysis" that achieved a prediction accuracy of 76.5% for muscle loss, which outperformed PLS-DA (prediction accuracy, 68.1%). Urine metabolites related to muscle loss included creatin(in)e, 3-hydroxyisovalerate, branched chain and other amino acids, TCA cycle intermediates, and glucose.
Conclusions: We present a robust classifier based on metabolite profiles of single spot urine samples. The accuracy of this already promising diagnostic test may potentially be strengthened by assessing additional metabolites by using other metabolomic methods (such as MS-based methods) and by accounting for some of the presently unexplained sources of variation. In this test, some metabolites correlated with muscle loss appear to originate in skeletal muscle, including creatinine and branched chain amino acids.

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Potential role of defective insulin signaling in skeletal muscle wasting of cancer cachexia

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Cancer cachexia syndrome (CCS) is a highly debilitating paraneoplastic disease observed in more than 50% patients with advanced cancers and directly contributes to 20–30% of cancer deaths. Atrophy of skeletal muscle is a defining and often fatal characteristic of cancer cachexia. Pathological mechanisms of CCS are complex and multi-factorial, which limits the development of an effective means of predicting, preventing, or treating cachexia. To date, research into the disorder was done primarily on various catabolic pathways in skeletal muscle. In the present study, we focus on the dysregulation of glucose utilization in muscle mediated by the impact of aberrant cytokine signaling on components of the insulin responsive pathway together with nuclear receptors. The colon-26 (C26) adenocarcinoma model of cachexia has been established to characterize the morphological and molecular changes which occur during muscle wasting. Molecular analysis revealed altered protein levels of key molecules in the insulin-signaling pathways. Immunohistochemical staining for mouse myosin heavy chain variants also suggests more prominent atrophy in glycolytic type II myofibers in the soleus of cachectic mice. These results suggest that the susceptibility to atrophy in type 2 myofibers may potentially be an adaptive response for alternative cellular energy source due to dysregulated glucose utilization.

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Muscle insulin-like growth factor (IGF) 1 signaling during the progression of cancer cachexia in the ApcMin/+ mouse

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In healthy individuals, muscle mass is maintained by the regulation of protein synthesis and degradation. Wasting can induce an imbalance between these two important processes and lead to the loss of muscle mass. The induction of muscle protein degradation and mechanisms

related to this process are critical mediators of muscle loss in many wasting conditions. The evidence for a regulatory role of muscle protein synthesis and associated signaling that can contribute to muscle wasting is equivocal. The purpose of this study was to determine if IGF-1/mTOR signaling was repressed during the progression of cachexia in ApcMin/+ mice. Male ApcMin/+ mice, an established cachexia model related to intestinal tumors, were sacrificed at peak body weight (12 weeks) and after the onset of cachexia (20 weeks). The gastrocnemius muscles were examined for gene expression and signaling related to protein synthesis. Mice were given an injection of D5Phe 30 min prior to sacrifice to quantify myofibrillar protein synthesis. Cachectic muscle had a 57% reduction in muscle IGF-1 mRNA. mTOR and P70S6 kinase phosphorylation was reduced 53% and 29%, respectively, with the progression of cachexia. The phosphorylation of the eukaryotic initiation factor 4-BP1 was decreased by 88% by cachexia. During the progression of cachexia, the myofibrillar protein synthesis rate was reduced 46%. In summary, muscle from cachectic ApcMin/+ mice has a reduction in IGF-1 gene expression and associated signaling. This corresponds to a reduction in myofibrillar protein synthesis. Future work needs to examine if the induction of protein synthesis during cachexia can reduce muscle mass loss.

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Macrophages protect against muscle atrophy and promote muscle recovery in vivo and in vitro: a mechanism partly dependent on insulin-like growth factor (IGF) 1 signaling

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Macrophages can play pro- and anti-inflammatory functions during muscle injury and recovery. In the present study, in vivo and in vitro models were used to determine the role of macrophages in muscle atrophy and regrowth. In the in vivo model, contractile properties of soleus muscles were measured in mice depleted in macrophages (etoposide, 15 mg/Kg) and submitted to a hindlimb unloading and reloading protocol. Ambulatory mice were used as control. In the in vitro model, large C2C12 myotubes were incubated for 2 days in low serum medium (DMEM 2% horse serum) and co-cultured with macrophages that contained apoptotic neutrophils (anti-inflammatory macrophage) and/or insulin growth factor-1 antibody (anti-IGF-1, 5 μL/mL). Myotube diameter was measured in light microscopy, and protein content was quantified with BCA assay. In vivo experiments showed that mice depleted in macrophages had roughly a 30% decrease in maximal muscle force compared to matched placebo mice at 7 and 14 days post-reloading. Alternatively, in vitro experiments showed that the presence of anti-inflammatory macrophages completely prevented the loss of protein content as well as the myotube atrophy after 2 days in low serum medium. Interestingly, the addition of anti-IGF-1 to the co-culture significantly decreased the ability of anti-inflammatory macrophages to protect against the myotube atrophy. These results clearly indicated that macrophages with anti-inflammatory phenotype prevent muscle atrophy while promoting muscle recovery. The protective effect of this macrophage phenotype is partly mediated by the release of IGF-1.

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Smad2 and 3 transcription factors control muscle mass in adulthood

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Background and aims: Loss of muscle mass occurs in a variety of diseases, including cancer, chronic heart failure, acquired immunodeficiency syndrome, diabetes, and renal failure, often aggravating pathological progression. Preventing muscle wasting by promoting muscle growth has been proposed as a possible therapeutic approach. Myostatin is an important negative modulator of muscle growth during myogenesis, and myostatin inhibitors are attractive drug targets. However, the role of the myostatin pathway in adulthood and the transcription factors involved in the signaling are unclear. Moreover, recent results confirm that other transforming growth factor- β (TGF-β) members control muscle mass.
Methods: Using genetic tools, we perturbed this pathway in adult myofibers, in vivo, to characterize the downstream targets and their ability to control muscle mass.
Results: Smad2 and Smad3 are the transcription factors downstream of myostatin/TGF-β and induce an atrophy program that is muscle RING-finger protein 1 (MuRF1) independent. Furthermore, Smad2/3 inhibition promotes muscle hypertrophy independent of satellite cells but partially dependent of mammalian target of rapamycin (mTOR) signaling. Thus, myostatin and Akt pathways cross-talk at different levels.
Conclusions: These findings point to myostatin inhibitors as good drugs to promote muscle growth during rehabilitation, especially when they are combined with IGF-1-Akt activators.

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Role of insulin-like growth factor (IGF) 1 in follistatin-induced skeletal muscle hypertrophy

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Follistatin (FS) exerts its muscle anabolic action by inhibiting several members of the TGF-β superfamily, including myostatin. We have previously shown that FS-induced muscle hypertrophy is associated with increased levels of insulin-like growth factor (IGF)-II mRNA, a potent inducer of myogenesis. The aim of this study was to investigate the role of IGF-II in FS-induced muscle hypertrophy. To test this hypothesis, FS was overexpressed in muscle of IGF-II KO mice. The tibialis anterior (TA) muscles of KO IGF-II as well as wild-type (WT) mice were electroporated with the plasmid pM1-FS288 (left leg) and the control plasmid pM1 (right leg). Seventeen days after electroporation, TA muscles were removed, weighted, and frozen for biochemical analysis or embedded in paraffin for morphological analysis. Our results show that FS overexpression in TA muscle of WT mice caused muscle hypertrophy characterized by increased muscle mass (+36%, 50.2±2.9 vs 37.1±1.3 mg, $P<0.01$; $n=5$), fiber cross sectional area (CSA; 2.3-fold, 4,081±225 vs 1,812±114 μm^2, $P<0.001$; $n=5$), and IGF-II mRNA (2.3-fold, $P<0.05$; $n=5$). In IGF-II KO mice, FS induced the same degree of muscle hypertrophy. Indeed, muscle mass was increased by 33% (23.1±1.6 vs 17.4±1.0, $P<0.05$; $n=6$) and CSA by twofold (3,973±387 vs 1,970±113, $P<0.01$; $n=6$). In conclusion, our data suggest that IGF-II is not mandatory for muscle hypertrophy induced by FS. So, IGF-II must be considered as a marker more than a crucial player in FS-induced muscle hypertrophy.

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Myostatin signalling in lung and gastric cancer patients

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Background and aims: Myostatin (MSTN) is a negative regulator of muscle mass. We recently demonstrated that MSTN expression is upregulated in an experimental model of cancer cachexia, suggesting that modulations of this signaling might have a pathogenic role in cancer-related muscle wasting. The present study was aimed at investigating whether MSTN signaling is modulated in the muscle of lung (LC) and gastric cancer patients (GC).
Methods: Rectus abdominis and dentatus anterior biopsies were obtained intraoperatively in 16 GC and in 17 LC patients, respectively. Control biopsies (C) were obtained among patients undergoing thoracic or abdominal surgery for non-neoplastic diseases. MSTN, phosphorilated-Smad (p-Smad), and phosphorylated-GSK-3β (p-GSK-3β) protein levels were assayed in muscle biopsies by Western blot. Nutritional assessment was performed for each patient.
Results: Percentage of body weight loss was 3.71±4.58 in LC and 5.59±5.87 in GC. MSTN protein levels were significantly increased in the muscle GC ($p<0.001$), while no differences were observed in the muscle of LC even when stratified for disease stage. P-smad did not change with respect to C in GC. By contrast, in LC, p-smad progressively increased, and p-GSK-3β is significantly increased in GC vs C ($p<0.05$). In LC, only a tendency to increase could be observed in stage I/II of the diseases. By contrast, a decrease was observed in stage III/IV subjects.
Conclusions: The present results support the role of MSTN signaling in the pathogenesis of cancer-related muscle wasting and suggest that this pathway may be considered a target for future strategies aimed at preventing or reducing cancer cachexia.

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Mechanisms involved in cAMP-mediated inhibition of the ubiquiting-proteasome system in skeletal muscle

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Although it is well known that catecholamines inhibit skeletal muscle protein degradation, the molecular underlying mechanism remains unclear. This study was undertaken to investigate the role of beta 2-adrenoceptors (AR) and cAMP in regulating the ubiquitin-proteasome system (UPS) in skeletal muscle. We report that increased levels of cAMP in isolated muscles, promoted by the cAMP phosphodiesterase inhibitor, isobutylmethylxanthine (IBMX), were accompanied by decreased activity of the UPS, levels of ubiquitin-protein conjugates,

and expression of atrogin-1, a key ubiquitin-protein ligase involved in muscle atrophy. In cultured myotubes, atrogin-1 induction following dexamethasone treatment was completely prevented by IBMX. Furthermore, administration of clenbuterol, a selective beta 2-agonist, to mice increased muscle cAMP levels and suppressed the fasting induced expression of atrogin-1 and MuRF-1, atrogin-1 mRNA being much more responsive to clenbuterol. Moreover, clenbuterol increased the phosphorylation of muscle Akt and Foxo3a in fasted rats. Similar responses were observed in muscles exposed to dibutyryl-cAMP. The stimulatory effect of clenbuterol on cAMP and Akt was abolished in muscles from beta 2-AR knockout (KO) mice. The suppressive effect of beta 2-agonist on atrogin-1 was not mediated by PGC-1-alpha (peroxisome proliferator-activated receptor gamma coactivator 1-alpha known to be induced by beta 2-agonists and previously shown to inhibit atrogin-1 expression), since food-deprived PGC-1-alpha KO mice were still sensitive to clenbuterol. These findings suggest that the cAMP increase induced by stimulation of beta 2-AR in skeletal muscles from fasted mice is possibly the mechanism by which catecholamines suppress atrogin-1 and the UPS, this effect being mediated via phosphorylation of Akt and thus inactivation of Foxo3.

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Extracellular zinc signaling in the activation of the ubiquiting proteasome pathway

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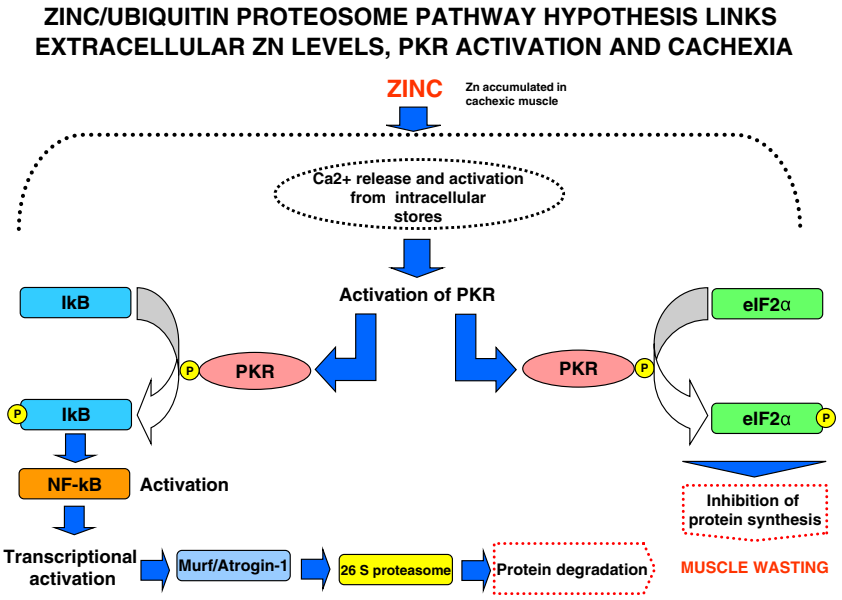
Background and aims: Decreased protein synthesis and increased protein degradation cause muscle wasting. The ubiquitin proteasome pathway is central in regulating these processes. Zn has been shown to accumulate in cachexic muscle. Zn chelating compounds have been demonstrated to both increase protein synthesis and decrease protein degradation. To explain these results on a molecular level, we have formulated a novel hypothesis regarding zinc's role in the ubiquitin proteasome pathway.
Methods: The hypothesis is based on measurements of Ca release from thapsigargin-sensitive intracellular pools, in vitro mechanistic studies in murine myotubes, effect of the zinc chelating compound D-myo-inositol 1,2,6-triphosphate (alpha trinositol, AT) in the cachexia inducing MAC-16 model, data on the fluctuation and regulation of Zn metabolism, and data on the activation of the ubiquitin proteasome pathway.
Results: Extracellular Zn is a powerful signaling ion, and in normal physiological conditions, virtually all Zn is intracellular. Micromolar concentrations of extracellular Zn can activate and mobilize intracellular Ca stores. This intracellular Ca activation can be attenuated by extracellular Zn chelation. The Ca release is zinc specific and cannot be triggered by other heavy metals. PKR is activated by Ca mobilizing agents. PKR activation attenuates protein synthesis through the phosphorylation of eIF2α and increases protein degradation through the activation of NF-kB by phosphorylating IkB.
Conclusion: Extracellular Zn activates PKR by releasing intracellular Ca stores from the ER. The PKR-mediated pathways lead to both the depression of protein synthesis and increase of protein degradation which together result in muscle wasting (see Figure).



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FOXO3a mediates signaling crosstalk to coordinate expression of ubiquitin with Atrogin-1/muscle atrophy F-box (MAFbx) and Muscle-specific RING finger protein 1 (MuRF1) during muscle atrophy

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Muscle atrophy is a consequence of disuse and chronic diseases like cancer and diabetes. It has been proposed that atrophy is a result of coordinated increases in expression of genes encoding various components of the ubiquitin-proteasome system including ubiquitin and the muscle-specific E3 ligases Atrogin-1(AT-1)/MAFbx and MuRF1; however, different signaling pathways (e.g., PI3K/Akt/FOXO, IKK/NF-kB, and MEK/ERK/Sp1) regulate these components. For example, insulin deficiency (i.e., diabetes) accelerates proteolysis and increases AT-1/MuRF1 expression via downregulation of PI3K/Akt and subsequent FOXO activation. In contrast, ubiquitin (UbC) transcription is increased by the MEK/ERK/Sp1 pathway, which is activated acutely by insulin. These seemingly paradoxical observations raise questions about how the transcription of these various genes is coordinated. In this study, we tested whether chronic inhibition of the PI3K/Akt pathway by glucocorticoids in muscle cells indirectly activates the MEK/ERK pathway. L6 myotubes were treated with dexamethasone (Dex) for up to 48 h. After 24 h, Dex inhibited PI3K/Akt signaling and increased AT-1 mRNA. MEK/ERK/Sp1 signaling and UbC transcription were simultaneously activated. Interestingly, Dex decreased IRS-1 protein while increasing IRS-2 protein. This suggested that downregulation of IRS-1/PI3K/Akt and FOXO activation leads to a reciprocal increase in IRS-2 expression and activation of MEK/ERK/Sp1 signaling. To test this possibility, we knocked down IRS-1 protein using siRNA or expressed constitutively activated FOXO3a in myotubes. IRS-2 protein, MEK/ERK signaling, and UbC expression were increased. We conclude that FOXO3a mediates a reciprocal crosstalk between the PI3K/Akt and MEK/ERK pathways that coordinately increases both AT-1/MuRF1 and ubiquitin expression, thereby supporting an increased rate of proteolysis.

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Elucidating the role of the ubiquitin E3 ligases and autophagy pathway in human cancer cachexia

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Background and aims: Skeletal muscle loss is a key feature of cancer cachexia, but understanding of the process in humans is limited. We aimed to determine the role of the ubiquitin proteasome E3 ligases and autophagy pathways in skeletal muscle of cancer cachexia patients.
Methods: Fifty-nine patients were recruited (seven weight-stable controls and 52 patients with upper gastrointestinal cancer). Rectus abdominis muscle biopsy was taken during open surgery. FOXO1 and FOXO3a protein levels were measured by Western blot, and expression of ubiquitin E3 ligases and autophagy-related BNIP3 and GABARAPL1 determined using qRT-PCR weight loss of ≥5% was used to define weight-losing (WL) compared to weight-stable (WS) patients, and systemic inflammation (SI) was defined as plasma CRP ≥5 mg/l. Results were analyzed using SPSS ver. 15.
Results: FOXO protein levels and expression of the E3 ligases MuRF1/MAFBx did not significantly differ between the groups. BNIP3 mRNA expression was increased in WL patients compared to WS or control patients (fold change1.5 vs 1.2 vs 1.1, respectively; $p=0.047$). Gabarapl1 showed a trend towards elevation ($p=0.063$) with WS patients showing the greatest fold change (1.7) compared with controls (1.1) or WL (1.5) patients. Both BNIP3 ($p=0.003$) and Gabarapl1 ($p=0.018$) expression increased in patients with SI compared to those without SI and control patients.
Conclusions: The individual prominence of proteolytic pathways appear to differ between preclinical models and human muscle wasting. Whilst this study did not demonstrate increased expression of ubiquitin E3 ligases, there was evidence for activation of the autophagy pathway in patients at an early stage in their cancer/cachexia journey.

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Signalling pathways involved in muscle proteolysis in a cachectic and non-cachectic colon-26 tumour-bearing mice

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Background and aims: Muscle breakdown is a key component of cancer cachexia. The ubiquitin ligases MuRF1 and MAFBx have been shown to be involved in a number of models of cachexia including cancer. We aim to elucidate the upstream signalling pathways involved in muscle loss in the quadriceps muscle in cachectic and non-cachectic tumour-bearing mice.
Methods: Three groups of DBA/Balb-c mice were injected s.c. with C-26 carcinoma cells (cachectic), C-26 carcinoma

cells (non-cachectic) and vehicle, respectively. Mice were sacrificed at day 14 at two different time points (2 a.m. and 2 p.m.), and quadriceps muscle and tumour were harvested and stored at −80°C. Plasma was collected for ELISAs (2 p.m. time point). RNA and protein were extracted for qRT-PCR and Western blot analysis, respectively.
Results: Gene expression of IL-6 was increased 30-fold in cachectic vs non-cachectic tumours. Plasma IL-6 levels were increased 465-fold in cachectic vs control mice and 20-fold in non-cachectic vs control mice. MAFBx and MuRF1 expression was increased 23- and 26-fold in cachectic muscle vs control, respectively, with no significant difference between control and non-cachectic. Fox01 and NFkB gene expression was increased seven- and twofold, respectively, relative to control. The ratio of phosphorylated to total STAT3 protein was elevated 18-fold in cachectic mice vs control and ninefold in non-cachectic mice vs control.
Conclusions: There is evidence of signalling through the Forkhead, NFkB, and IL-6 signalling pathways in this model that are more pronounced in cachectic mice. Selective blocking of components of these pathways will assist in identifying the predominant mechanism of muscle proteolysis.

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Activation of the central melanocortin system induces proteolytic pathways in skeletal muscle

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Background: Activation of the central melanocortin system by cytokines recapitulates many key features of cachexia. Melanocortin blockade has been shown to prevent the loss of lean mass in experimental cachexia and increase lean mass in healthy animals. We hypothesize that melanocortin activation potentiates the loss of muscle mass in cachexia.
Methods: The melanocortin agonist MTII and antagonist AgRP were administered via acute injection or chronic infusion through a lateral ventricle cannula. Gene expression was measured using real-time PCR. Analysis of fiber area and fiber composition was performed by immunohistochemical analysis using specific antibodies. Experimental cachexia was induced in rats by either implantation of a syngenic sarcoma or five-sixths nephrectomy.
Results: Acute and chronic administration of MTII results in the upregulation of the E3 ubiquitin ligases MAFbx and MuRF-1 in skeletal muscle. Furthermore, some of this effect is independent of the decrease in food intake associated with melanocortin activation. Melanocortin blockade is associated with the retention of skeletal muscle weight in experimental cachexia.
Conclusions: Melanocortin activation has been implicated in the pathogenesis of alterations in food intake in cachexia. Melanocortin signaling also has effects that are independent of food intake, including increasing the mRNA levels of the catabolic mediators MAFbx and MuRF-1 in skeletal muscle. Furthermore, melanocortin blockade can reverse myofibrillar atrophy in experimental cachexia. These findings suggest an important role for the central melanocortin system in the pathogenesis of muscle catabolism in cachexia, and further support the use of melanocortin antagonists in its treatment.

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Increased activities of proteasome and lysosomal cathepsins B and L in skeletal muscle from intensive care patients with sepsis

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Background and aims: Intensive care patients suffering from sepsis are characterised by muscle wasting due to increased net protein breakdown. Myofibrillar proteins are mainly degraded via the ubiquitin proteasome pathway. Lysosomes are implicated in the degradation of extracellular and cytoplasmic constituents and of organelles like mitochondria. Research on muscle-wasting conditions previously focused on the importance of the proteasome pathway. Recent reports have indicated that also the lysosomal pathway is activated in atrophying muscle cells. Thus, we have measured the proteolytic activity of both proteasomes and lysosomes in leg muscle biopsies from sepsis patients with multiple organ failure.
Methods: Patients (n=8) admitted with septic shock to the intensive care unit and metabolically healthy controls (n=7) undergoing elective surgery were studied. Muscle biopsies were obtained from the vastus lateralis muscle. The biopsies were homogenised; fractions for analyses of proteasome and lysosome proteolytic activities were obtained by differential centrifugation. Proteasome and lysosomal cathepsin B and L activities were determined using fluorogenic peptide substrates.
Results: In the patients, proteasome activity was 44% higher (p<0.05) as compared to controls. Also, lysosomal

proteolytic activities were higher, cathepsin B by 200% ($p<0.001$) and cathepsin L by 150% ($p<0.001$), compared to controls. The activities of both cathepsin B and L correlated significantly ($p<0.01$) with the proteasome activity in the patients, but not in the controls.
Conclusions: These results give evidence that during catabolic conditions, not only the proteasomal pathway is activated in skeletal muscle but also the lysosomal pathway, possibly even to a greater extent.

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Autophagy-lysosome system and the control of muscle mass

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Background: The size of skeletal muscle is determined by a balance between protein synthesis and protein degradation. In mammalian cells, half-life of proteins is controlled by two proteolytic systems: the ubiquitin-proteasome and the autophagy-lysosome. Autophagy is an evolutionarily conserved mechanism that allows cell survival during starvation through the bulk degradation of proteins and organelles by lysosomal enzymes. In skeletal muscle, both systems are under FoxO regulation, and their excessive activation induces severe muscle loss. Conversely, altered autophagy has been observed in various myopathies. However, the role of autophagy in skeletal muscle has not been determined by loss-of-function approaches.
Methods: Atg7 floxed mice were crossed with transgenic mice expressing cre-recombinase under a muscle specific promoter. Autophagy knockout muscles were characterized by morphological, physiological, biochemical, and molecular approaches and compared to control wild-type muscles.
Results: Here, we report that muscle-specific deletion of Atg7 gene resulted in profound muscle atrophy and age-dependent decrease in force. Atg7 null muscles showed accumulation of abnormal mitochondria, sarcoplasmic reticulum distension, disorganization of sarcomere, and formation of aberrant concentric membranous structures. Atg7 null muscle showed an increase of oxidative stress and an activation of unfolded protein response, which contribute to muscle weakness. Importantly, autophagy inhibition exacerbated muscle loss during denervation and fasting.
Conclusions: In conclusion, autophagy flux is important to preserve muscle mass and to maintain myofiber integrity. Our results suggest that inhibition/alteration of autophagy can contribute to myofiber degeneration and weakness in muscle disorders characterized by accumulation of abnormal mitochondria and inclusions.

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Physical activity level as an outcome measure for use in cancer cachexia trials: a feasibility study

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Background and aims: Cancer cachexia impacts on treatment options, quality of life and survival. New treatments are emerging but need to be assessed using outcomes which patients find meaningful. One approach is the measurement of physical activity levels (PAL) by small lightweight monitors, but experience is limited in cancer patients. The primary aim of this study was to formally assess the acceptability of the ActivPAL™ monitor. We also explored the optimal period of monitoring and the added value of the monitor's estimate of energy expenditure over a step count.
Methods: Acceptability was assessed by compliance using analysis of movement data. Mean values of daily step count and energy expenditure (EE) for 2 or 4 and 6 days of monitoring were compared. Relationships between step count, stepping EE and non-stepping EE were explored.
Results: Sixty patients (mean age, 68 years; ECOG performance status 0–2) with lung or upper-gastrointestinal cancer took part. All but one found the monitor acceptable, and mean (95% CI) compliance was 98% (94–100%). Median daily step counts and EE scores over 2 or 4 days were significantly higher than those from 6 days ($p\leq0.01$). Step count was strongly related to stepping and non-stepping EE ($r=-0.911$, $p<0.01$).
Conclusions: The ActivPAL™ activity monitor is acceptable to patients with outcomes obtained over 6 days recommended for use in future studies.

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Age-dependent anti-catabolic effects of exercise training in the skeletal muscle of chronic heart failure patients: results from the Leipzig exercise intervention in chronic heart failure and ageing study (LEICA)

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Purpose: Muscle wasting in chronic heart failure (CHF) is an independent predictor of mortality. However, the molecular mechanisms that mediate muscle catabolism are largely unknown, and no specific pharmacological agents are available to antagonize the loss of muscle mass. We therefore analysed two different protein degradation pathways in skeletal muscle biopsies of CHF patients and tested the age-dependent effects of exercise training to prevent muscle wasting.
Methods: Sixty CHF patients and 60 healthy subjects (HS) were randomized to 4 weeks of bicycle ergometer training at 70% of the heart rate reserve 4×20 min/day or to a control group (C). Before and after the intervention, a spiroergometry, echocardiography and a muscle biopsy of the vastus lateralis muscle were performed. Expression of the E3 ligase Murf-1 as part of the ubiquitin proteasome system was quantified by real-time PCR standardized for 18S-rRNA and Western blot. As a marker of lysosomal proteolysis, cathepsin-L was measured by real-time PCR.
Results: (1) Clinical training effects: In younger CHF patients (n=15; age, 45±3 years; BMI, 26.8±2.7; LV-EF, 26.8±2.6%), training improved VO_2 max by 36% from 13.3±1.6 to 18.1±1.5 mL/minkg (p=0.008 vs. control). In elderly CHF patients (n=15; age, 68±4 years; BMI, 25.3±2.9; LV-EF, 27.4±3.0%), training increased VO_2 max by 33% from 12.9±1.4 to 17.1±1.1 mL/minkg (p=0.01 versus control). (2) Molecular training effects: At baseline, Murf-1 mRNA expression CHF patients were significantly elevated versus HS at 593±68 versus 410±27 rel. units (p=0.013) and protein expression at 0.90±0.08 versus 0.62±0.05 rel. units (p=0.018). Cathepsin-L was not different between both groups. Training induced a reduction of Murf-1 expression by 34.3% (p=0.02) in younger CHF patients and a reduction of 24.3% (p<0.05) in elderly. Cathepsin-L expression remained unchanged.
Conclusions: Muscle wasting in CHF is mediated via the ubiquitin proteasome system and not the lysosomal system in the skeletal muscle. Exercise training significantly improves VO_2 max in both younger and older patients with CHF. Both younger and older patients with heart failure derive a similar anticatabolic benefit from training interventions. These data underline the clinical role of exercise-based rehabilitation programs to prevent CHF-related muscle wasting in the elderly.

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Phase II non-randomized study of the efficacy and safety of cyclooxygenase (COX) 2 inhibitor celecoxib on patients with cancer cachexia

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Background: Chronic inflammation is one of the main features of cancer cachexia. Experimental and clinical studies showed that cyclooxygenase-2 inhibitors, such as celecoxib, may be beneficial in counteracting major symptoms of this devastating syndrome.
Patients and methods: We carried out a prospective, phase II clinical trial to test the safety and effectiveness of an intervention with the COX-2 inhibitor celecoxib (300 mg/day for 4 months) on key variables of cachexia (lean body mass, resting energy expenditure, serum levels of proinflammatory cytokines, and fatigue) in patients with advanced cancer at different sites.
Results: A sample of 24 patients was enrolled from January to December 2008 and was deemed assessable. A significant increase of lean body mass and a significant decrease of TNF-alpha were observed. Moreover, an improvement of grip strength, quality of life, performance status, and Glasgow prognostic score was shown. There were no grade 3/4 toxicities. Patient compliance was very good: no patient had to reduce the celecoxib dosage nor interrupt treatment.
Conclusion: Our results showed that the COX-2 selective inhibitor celecoxib is an effective single agent for the treatment of cancer cachexia. Although the treatment of cancer cachexia, a multifactorial syndrome, is more likely to yield success with a multitargeted approach, in the present study, we were able to show that a treatment, such as celecoxib, addressing a single target, albeit very important as chronic inflammation, could have positive effects. Therefore, phase III clinical trials are warranted to test the efficacy and safety of celecoxib.

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A phase I, pharmacokinetic (PK), and preliminary efficacy assessment of ALD518, a humanized anti-interleukin (IL) 6 antibody, in patients with advanced cancer

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Background: ALD518 is a humanized anti-IL-6 antibody being developed for the treatment of cancer cachexia and fatigue. The primary objective of the study was to determine the safety of ALD518.
Methods: Nine patients (pts) with advanced cancer, ECOG 0-2, and C-reactive protein (CRP) >10 mg/L were enrolled. Pts were assigned to one of three dose-escalating cohorts (n=3/cohort). ALD518 was given as a single i.v. infusion of 80, 160, or 320 mg. Pts were followed up for 12 weeks. Data included lab safety tests (LSTs), vital signs, ECGs, adverse events, handgrip strength (HGS), FACIT-F, PK, and C-reactive protein (CRP).
Results: Nine pts were evaluable for dose limiting toxicity (DLT) assessment at week 4, and five of nine pts completed all visits. Of the four pts who failed to complete every visit, three were withdrawn due to progressive disease and one to be treated with chemotherapy. There were no DLTs or infusion reactions. There were four SAEs: three disease progressions and one sepsis secondary to a blocked biliary stent. There were no grade 3/4 toxicities. Changes in LSTs, CRP, HGS, and FACIT-F fatigue subscale (pooled ITT analysis) are shown below:

Parameter (median, IQ range)	Pre-dose (n=8)	Post-dose (n=8)		
		Week 2	Week 4	Week 12
Hemoglobin (g/l)	12.3 (11.2–14.0)	13.2 (12.3–14.1)*	13.7 (12.4–15.1)*	14.0 (12.4–15.8)*
Albumin (g/l)	36.5 (33.5–39.5)	42.0 (38.0–42.5)*	41.5 (41.0–45.5)*	42.5 (38.5–46.0)*
Platelets ($\times 10^9$/l)	298 (268–362)	180 (117–234)*	142 (126–204)*	150 (109–203)*
Neutrophils ($\times 10^9$/l)	5.9 (5.8–7.5)	4.8 (3.7–5.9)*	4.3 (3.4–5.0)*	5.5 (4.5–5.6)*
CRP (mg/L)	44.0 (17.6–131.6)	0.9 (0.5–3.9)	0.8 (0.2–3.7)	1.0 (0.2–4.5)
HGS (kg)	26.0 (9.7–35.4)	26.8 (10.7–36.7)*	30.5 (17.3–36.3)	31.7 (19.5–35.9)
FACIT-F	21.5 (14.0–28.0)	30.5(20.5–32.5)*	30.5 (24.5–33.5)*	29.5 (20.0–33.5)*

ALD518 PK elimination $t_{1/2}$ was 31 days (range, 19–39 days)
*p<0.05 (Wilcoxon test)

Conclusion: ALD518 given to pts with advanced cancer was safe and well tolerated. ALD518 reversed fatigue, increased hemoglobin, and albumin, and there was a trend to increased HGS. There was a mild decrease in platelet count that remained stable throughout the study. There was a mild transient fall in neutrophil count during the study.

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Phase II study of the novel peptide-nucleic acid AVR118 in the management of cancer-related anorexia/cachexia

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Background: AVR118 represents a new class of immunomodulatory and cytoprotective drugs in managing anorexia cachexia. Previously in patients with advanced HIV-AIDS, increases in appetite, strength, and alertness were noted.
Aim: The aim of this study is to determine the effect of AVR118 on appetite, early satiety, and nutritional intake in patients with advanced cancer. Secondary endpoints include changes in performance status, lean muscle mass, and quality of life (QOL).
Methods: Eligible patients received 4.0 ml of AVR118 subcutaneous daily injections. Patients underwent bi-monthly evaluations during the 28-day initial treatment (phase A). Evaluations included Karnofsky performance status, Edmonton symptoms assessment scale (ESAS), patient-generated subjective global assessment (PG-SGA), Simmonds functional assessment (SFA), dyspepsia symptom severity index (DSSI), weight, lean body mass, skin fold thickness, and grip strength. Patients who benefited

from phase A could elect to continue with therapy (phase B).

Results: Of 21 enrolled patients, 11 completed phase A. Eight patients choose to continue with AVR118 treatment (phase B). Weight stabilization or gain was observed in seven of 11 patients. Total PG-SGA scores improved significantly ($p \leq 0.01$). Appetite ($p \leq 0.01$) and depression ($p=0.05$) scores improved on ESAS. Frequent burping/belching ($p=0.02$), feeling full ($p=0.04$), and stomach distention ($p=0.03$) improved on the DSSI. The SFA tests showed improvement on time to sit and stand ($p=0.01$). AVR118 was well tolerated with no serious side effects reported.

Conclusions: AVR118 again mediated statistically significant improvements in anorexia, dyspepsia, strength, and depression in this trial, consonant with the previous AIDS trial results.

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Development of early biomarkers for muscle anabolism with randomized, single-blind, placebo-controlled studies involving 7 days of testosterone exposure

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Background and aims: Identifying early biomarkers for muscle anabolism may facilitate development of therapies to treat sarcopenia and cachexia. We investigated N-terminal propeptide of type III collagen (P3NP), RNA and proteomic profiling, urinary 3-methyl-histidine/creatinine (3MH/Cr) ratio, and muscle protein fractional synthetic rate (FSR) as potential biomarkers for testosterone-induced muscle anabolism.

Design: Healthy men aged 60–75 years ($n=22$; study 1) and 18–40 years ($n=27$; study 2) were given one intramuscular injection after randomization to low-dose (study 1, 100 mg; study 2, 200 mg) or high-dose (study 1, 300 mg or study 2, 600 mg) testosterone or placebo. Blood and vastus lateralis muscle samples were analyzed at baseline and 7 days after injection.

Results: Testosterone decreased urinary 3MH/Cr only in elderly subjects. Blood and muscle proteomic profiles did not reveal robust treatment-related changes. In study 1, FSR tended to increase (+0.006%/h) in the 100-mg testosterone group and (+0.011%/h) in the 300-mg testosterone group, but was not statistically different from placebo (+0.005%/h). Plasma P3NP increased in a testosterone dose-dependent manner. A significant correlation between change from baseline P3NP and serum total testosterone was noted ($r=0.43$, $p=0.047$ in study 1; $r=0.69$, $p<0.001$ in study 2). We identified alterations in muscle gene expression that correlated with change in serum testosterone.

Conclusions: One testosterone injection increased plasma P3NP levels in a dose-dependent manner. Skeletal muscle RNA profiling analysis identified genes associated with testosterone treatment. P3NP and RNA profiling are potentially useful early biomarkers for muscle anabolic therapy.

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Oral amino acids and testosterone therapy to improve muscle mass and quality of life during chemotherapy in recurrent cervical cancer: a case study

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Background and aims: Muscle wasting is by far the most important phenotypic feature of cancer cachexia, though little success has been achieved toward reversing or preventing this catabolic process. Anabolic interventions including testosterone and amino acid supplements may be beneficial in reducing and/or reversing muscle wasting in these patient populations.

Methods: A 48-year-old woman diagnosed with recurrent cervical cancer was scheduled to receive three 21-day cycles of cisplatin and topotecan chemotherapy. She consented to a 10-week blinded interventional pilot study where she received daily amino acid supplements (whey protein with meals three times per day) and weekly injections of testosterone enanthate (100 mg i.m.) before and during the weeks of chemotherapy treatment. Blood, dual energy x-ray absorptiometry (DEXA) scans, and functional assessment of cancer therapy-general (FACT-G) questionnaire data were collected before and after the 10-week period.

Results: Total serum testosterone concentrations were raised from 15.6 to 1,210 ng/dL. Body weight increased from 40.2 to 42.7 kg after 10 weeks. Total lean body mass (LMB) increased from 25.5 to 28.5 kg. Trunk LBM increased from 12.6 to 14 kg. Total fat mass decreased from 13.6 to 13.1 kg, and trunk fat mass decreased from 5.5 to 5.2 kg. FACT-G scores improved from 55.4 to 78.1.

Conclusions: Concomitant treatment of oral amino acids and testosterone may be a viable therapeutic option for fighting cachexia and improving quality of life during chemotherapeutic treatment of recurrent cervical cancer. A

double-blinded, placebo-controlled interventional study is in progress (NCT00878995).
This project is funded through NIH/NCI RO1 CA127971 (Sheffield-Moore). Dr. Dillon is funded through NIH T32 CA117834 to UTMB.

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Performance improvement in chronic obstructive pulmonary disease (COPD) cachexia with SUN11031 (a synthetic human ghrelin) in a placebo-controlled trial

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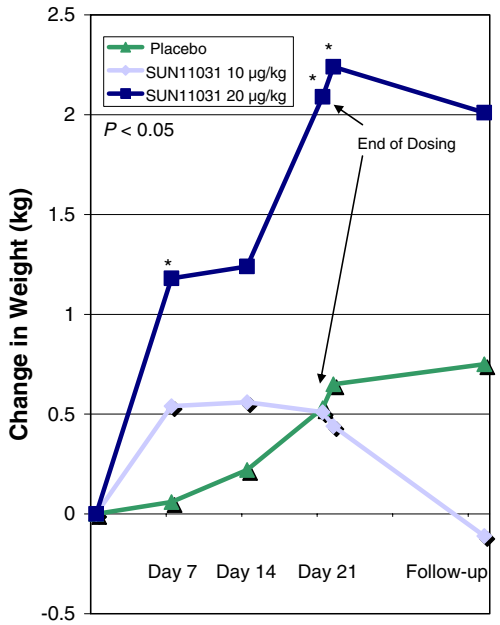
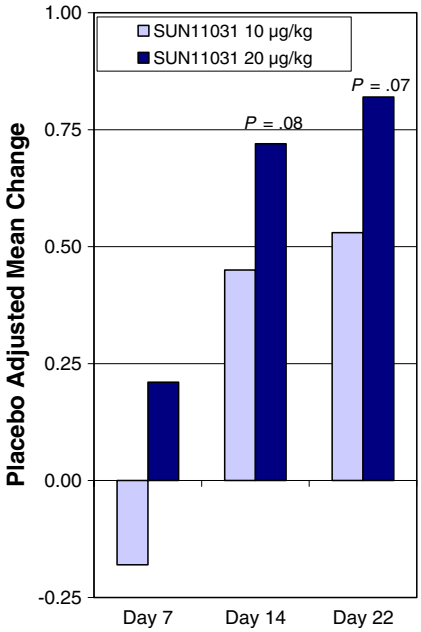
Background: Cachexia occurs in 25% of COPD patients and is associated with increased mortality. SUN11031 is thought to improve appetite, energy balance, and functional outcomes and is under investigation for the treatment of COPD cachexia. Study objectives were to assess appetite, body weight/composition, physical performance, and safety in COPD patients with cachexia.
Methods: Twenty-five patients were randomized to SUN11031 (10 µg/kg (N=9), 20 µg/kg (N=8), and placebo (N=8)) by subcutaneous (SC) twice daily (bid) injections for 21 days. Mean age was 65.3 years, and mean BMI was 19.48 kg/m^2.
Results: GH secretion increased similarly in both SUN11031 groups. Compared to placebo, 20 µg/kg significantly increased mean (±SD) fasting morning body weight by 2.24±1.16 kg (P<0.05; Fig. 1), while lean mass trended toward significance (2.19±1.87 kg; P=0.128). Twenty micrograms per kilogram also improved appetite compared to baseline (P<0.001). The pharmacokinetics of SUN11031in COPD patients was comparable to those in healthy subjects.





SUN11031 showed a dose proportional trend toward improvement in chair stand score (P=0.07, P=0.08 vs placebo; Fig. 2). SUN11031 was well tolerated, and most adverse events were mild.
Conclusion: SC bid SUN11031 was safe, and increased appetite, GH secretion, and body weight with a dose proportional trend towards increase in physical performance.

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Increases in lean body mass observed with a single dose of ACE-031, a form of the soluble activin receptor type IIB

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Background and aims: ACE-031 is a soluble fusion protein derived from a form of the extracellular domain of the human activin receptor type IIB (ActRIIB) linked to the Fc portion of human IgG1. Treatment with ACE-031 increases lean body mass (LBM) in numerous normal and disease preclinical models, including cachexia. We conducted a first-in-human, randomized, double-blind phase 1 study to evaluate the safety and tolerability and pharmacokinetic and pharmacodynamic properties of single, escalating doses of ACE-031 in healthy postmenopausal women.

Methods: Forty-eight subjects received single subcutaneous injections of placebo or ACE-031 at dose levels ranging from 0.02 to 3 mg/kg. Safety, PK, and biomarkers were assessed throughout the study. Effects on LBM and thigh muscle volume were measured by dual energy X-ray absorptiometry (DXA) and MRI, respectively.
Results: ACE-031 was well tolerated at all dose levels and demonstrated a linear pharmacokinetic profile with an average half-life of 10–15 days. ACE-031 at 1 and 3 mg/kg produced dose-dependent increases (2.4% and 2.6%) in LBM measured by DXA as early as day 15 that were sustained through day 57. Subjects given placebo had a 0.2% decrease in muscle volume assessed by MRI at day 29 compared to a 5% increase in subjects receiving 3 mg/kg ACE-031. ACE-031 favorably affected biomarkers of fat mass and bone formation and resorption at doses of 1 and 3 mg/kg.
Conclusions: These data provide clinical proof of concept that blocking ActRIIB signaling has rapid, potent, and durable effects on LBM and support further development of ACE-031.

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Improved insulin sensitivity after therapy with the selective peroxisome proliferator-activated receptor-γ (PPAR-γ) modulating angiotensin receptor blocker irbesartan in patients with chronic heart failure—IRIS-HF: a placebo-controlled, double blinded, randomized study

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Background: Impaired insulin sensitivity (Si) is common in chronic heart failure (CHF), contributes to symptomatic status, and independently predicts prognosis. It has been suggested that the angiotensin II-receptor antagonist irbesartan can improve insulin sensitivity via selective activation of the peroxisome proliferator-activated receptor gamma (PPAR-gamma). We aimed to assess the effect of irbesartan on impaired insulin sensitivity in patients with CHF.
Methods: In a prospective, placebo-controlled, double-blinded, randomized single center study, we included 36 non-diabetic patients with stable ischemic CHF (age, 63±9 years; BMI, 28.2±3.9 kg/m^2; peak VO_2, 16.6±4.8 mL/kg/min, all mean±SD). Irbesartan (target dose 300 mg/day) or placebo was given on top of standard optimum CHF therapy including ACE inhibitor and beta-blockers for 16 weeks. Change of insulin sensitivity from baseline to week 16 (primary endpoint) was assessed using the minimal modeling technique from glucose and insulin profiles of a frequently sampled intravenous glucose tolerance test.
Results: At baseline, both groups were similar for age, NYHA class, peak VO_2, BMI, body composition (DEXA scan), Si ($p=0.2$), and main clinical characteristics. Si was 2.51 ± 1.58 $\text{min}^{-1}\,\mu\text{U}\,\text{mL}^{-1}10^4$ in the study population, which is 30% lower than in healthy controls of similar age ($p<0.05$). In the irbesartan-treated group, S_I increased by 26% ($p<0.001$ within group), but it decreased by 15% in the placebo group ($p=0.17$ within group). Change in S_I from baseline was significantly different between groups (mean difference, 1.044 $\text{min}^{-1}\,\mu\text{U}\,\text{mL}^{-1}10^4$; 95% CI, 0.45 to 1.64; $p=0.001$). Treatment with irbesartan was well tolerated. NYHA class, peak VO_2, and body composition did not change, but blood pressure significantly decreased on irbesartan (−5±2 mmHg, $p<0.002$).
Conclusion: Our study shows that 16 weeks of added therapy with irbesartan compared to placebo significantly improves impaired insulin sensitivity in non-diabetic patients with chronic heart failure. Whether this metabolic effect of irbesartan translates into additional clinical benefits for heart failure patients should be tested in larger studies.

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Use of PORT-A-CATH® for total parenteral nutrition in end-of-life patients

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Background and aim: Vascular access port implantation represents a valid method for long-term chemotherapy. The aim of the present study was to confirm usefulness of ports for TPN.
Method: In the period 1 January 2008 to 30 September 2009 in PMD, in our department, 175 ports were implanted. Twenty of them were used for TPN. We compared the rate of complications in group I patients receiving chemotherapy and group II receiving TPN through port. The catheter and vascular access patency was controlled by fluoroscopy before TPN was started and maintained by flushing with normal saline after each infusion. Chemotherapy group lines were flushed once a month with heparin and saline.
Results: Several complications were encountered in group I: catheter total occlusion 1.14%, partial occlusion 5%, skin infection 1.71%, leakage 2.28%, skin atrophy 1.14%, and no blood back flow after implantation 0.57%. There was no line complication in TPN group.
Conclusion: Lack of complications in group II can be accidental, but the aim of the study was to prove that TPN does not increase the rate of port-a-caths complications,

particularly occlusion of the catheter. Vascular access port is suitable for TPN and can improve the well-being of end-of-live patient. The experience of the medical staff is probably the most important factor in decreasing the total rate of complications.

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Evaluation of visceral lean and fat body mass with CT-scan image analysis in oncological patients with anorexia and cachexia symptoms before and after an anti-cachexia pharmaconutritional treatment

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Background and aims: Body composition evaluation is one of the most important malnutrition indicators, either in physiological or pathological states. Oncological patient with anorexia/cachexia syndrome shows typical symptoms characterized by loss of lean body mass with or without loss of fat mass. Precise assessment of this tissue waste is important to evaluate patient's cachexia characteristics and for the appropriated therapy. CT-scan image analysis technology is an innovative, sensible, and precise new tool in evaluation of body composition, positively described by many authors in different pathologies. The aim of this study is to determine the variations of lean and fat body mass in cachectics oncologic patients treated with anticachexia pharmaconutritional therapy.
Methods: Twenty-five patients with cancer-related anorexia and cachexia were enrolled. Region L2–L3 vertebra abdominal CT was performed at enrollment date and before the start of treatment. The same CT was performed at the end of treatment, after 16 weeks. DICOM radiological images were processed with specific images analysis software Slice-O-Matic (Tomovision, Montreal, QC, Canada) to obtain areas of abdominal tissues.
Results: The results shows a significant increase of muscle mass at L3 vertebra level as well as no significant decrease of mass in the same region in a subset of this patient, confirming the improvement shown in clinical and functional parameters after treatment. The methodology seems to be very good and fit with other data.
Conclusion: This preliminary study confirms the quality of CT image analyses data in oncological cachectic patients, either for first assessment or for follow-up body composition evaluation.

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Randomised controlled pilot study of neuromuscular electrical stimulation of the quadriceps in patients with non-small cell lung cancer

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Background and aims: Patients with lung cancer experience muscle wasting and weakness. Therapeutic exercise may be beneficial but is not always practical. An alternative approach may be neuromuscular electrical stimulation (NMES) of the quadriceps muscles, but this has not been formally examined in patients with cancer. Thus, we have undertaken this pilot study to assess feasibility and inform the design of future studies.
Methods: Sixteen patients were randomised to receive usual care (control group) or usual care plus NMES for 4 weeks. NMES consisted of daily stimulation to both thighs for up to 30 min (frequency 50 Hz, \'on\' cycle 11 to 25%). Adherence was assessed by a self-report diary and semi-structured evaluation form. Quadriceps muscle strength, exercise endurance and free-living physical activity were assessed using a Cybex NORM dynamometer, an endurance shuttle walk test, and an ActivPAL accelerometer (mean daily step count), respectively. Changes in outcome from baseline were compared between groups by mean differences and their 95% confidence intervals using independent t test (P=0.05).
Results: Median (range) adherence to the programme was 80% (69f{100). All patients found the NMES device easy to use. Changes in outcome favoured the NMES group with mean differences of 9.4 Nm (21%) in quadriceps muscle strength, 768 steps (15%) in free-living activity and 138 m (8%) in exercise endurance, but none of the differences was statistically significant.
Conclusion: NMES appear to be acceptable and warrants further study in patients with lung cancer.

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Twenty weeks resistance training in older people is associated with rejuvenation of leg blood flow responses to exercise and feeding which are not attributable to changes in endothelial markers

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Background and aims: Sarcopenia is associated with blunted anabolic responses to feeding and exercise, perhaps partly as a consequence of reduced leg blood flow (LBF) responses. We tested the hypothesis that resistance-training (RT) would improve age-associated blunting in LBF in the resting state and responses to prior exercise and feeding.

Methods: We studied participants of three ages (young, 25 ±3 years, n=12; middle-aged, 50±3 years, n=12; and old, 70±4 years, n=8) before and after 20 weeks of whole-body RT. LBF was measured, and muscle biopsies were taken at rest, postabsorptively, and 120 (LBF) or 150 (biopsies)min after oral feeding began, with one leg rested and the other recovering from 6×8 repetitions of leg extensions (75%, one RM).

Results: Postabsorptive basal LBF was identical in middle-aged and old subjects (0.32±0.03 vs. 0.33±0.03 $l min^{-1}$), but tended to be higher in the young (0.51±0.1 $l min^{-1}$) and was unaffected by RT. Before RT, only the young and middle-aged subjects showed increased LBF after exercise-plus-feeding, whereas after RT, the middle-aged subjects' responses to exercise-plus-feeding were significantly enhanced (+101±16% vs. +147±32%, P<0.01) and the old, rejuvenated (+59±15% vs. +115±47%, P<0.001). There were no differences in endothelial marker proteins (e.g., CD31) or adrenergic receptor proteins (i.e., α-1 or β-2) after RT, nor in eNOS or PKCα phosphorylation after acute exercise-plus-feeding before or after RT.

Conclusions: LBF responses to exercise-plus-food are diminished in older subjects but are restored to the pattern of young subjects by RT without changes in markers previously identified as likely to be involved in microvascular adaptation/vasodilatation.

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Importance of cancer-related inflammatory state (CIS) measured by a prognostic index (PI) for clinical trial completion?

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Background: High dropout rates limit interpretation of cancer chemotherapy trials. Biologic characteristics of dropouts are uncommonly reported. We hypothesized that CIS as measured by PI associates with high dropout rate, and that dropout is not a random event.

Methodology: This is a retrospective analysis of stage 3B/4 NSCLC participants of first line platinum-based chemotherapy trials. Patients with available pre-treatment CRP and WBC were included. They were categorized as screen failures, dropouts, or completers and assigned a PI score as an indicator of CIS: 0 if CRP and WBC were normal, 1 if one of them elevated; or 2 if both were elevated (ASCO 2005, 17035).

Results: One hundred nineteen pts (65 male, 54 female) aged 63 (38–80)years were studied. There were 13/119 pts screen failures, 18/119 dropouts, and 88/119 completers. There was no difference in CRP and WBC level between screen failures and those who started the treatment. However, baseline mean CRP level was more than threefold higher in dropouts compared to those who completed the study (105.4 vs. 31.4 mg/L; p<0.001). Baseline WBC was also higher in dropouts when compared to completers (11.4 vs 8.8; p=0.01). Weight loss was significantly higher in dropouts when compared to completers (6.4% vs 3.6%, p=0.05).

Significantly higher proportion of PI 0 and 1 were among completers and PI 2 among dropouts.

PI score	Trial completion		Total	p value
	Completers	Dropouts		
0	36 (41%)	2 (11%)	38	<0.0001
1	37 (42%)	10 (56%)	47	<0.0001
2	15 (17%)	6 (33%)	21	0.006
Total	88 (100%)	18 (100%)	106	

Conclusion: Patients with high PI are more likely to drop out of a trial. Thus, PI marks a patient subset to whom phase III trial outcomes may not apply. PI should be considered as a separate prospectively defined stratum.

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Complex treatment of anorexia–cachexia syndrome

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Background and aims: Decrease in total body mass is one of the major symptoms of presence or development of tumor. In cancer pts, it is mandatory to note that tumor cachexia combines with anorexia and, as a result, develops anorexia–cachexia syndrome.
Materials and methods: History of 23 pts with locally advanced and metastatic tumor process, undergoing ambulatory chemotherapy (CT) for various tumor localizations, was analyzed: a median decrease of 13–15 kg, anorexia stage 1–2, asthenia, low-grade fever, depression, alimentary failure, nausea, foul taste, anemia, hypoprotenemia, pain syndrome (two to three points). Additionally, PG-SGA, QLQ C-30, VAS, and "ABC" (in-house design) scale were used. The treatment scheme included (1) pain control, (2) nutrition support, (3) prokinetics, (4) pro- and prebiotics, (5) correction of anemia, and (6) COX-2 and MOA inhibitors. The pts were divided into two groups according to the duration of complex treatment use: in the first group ($n=12$), the treatment was conducted for a period of 14 days before every cycle of CT, whereas in the second group ($n=11$), the treatment was additionally resumed on the seventh to eighth day after administration of CT for a period of 20 days. Evaluation was performed after two cycles of CT.
Results: As a result of complex treatment use, stabilized body mass, increased physical activity, and increased appetite were observed in 72% of pts from the second group. Similar results were observed only in 18% of pts from the first group (Student's criteria 4, 3; $p \leq 0.001$).
Conclusion: The obtained results allow us to suggest that treatment of anorexia–cachexia syndrome should be complex and prolonged.

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To which extent is cancer cachexia reversible under successful anticancer treatment? An analysis of data in advanced non-small cell lung cancer

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Introduction: Cancer cachexia (CC) causes high burden of morbidity and mortality in advanced solid tumour patients; it is an independent prognostic factor. CC can improve under successful anticancer treatment, as a key pathophysiologic mechanism of CC is catabolic drive caused by the cancer itself. We systematically investigate the evidence for the reversibility of CCS under successful anticancer treatment in advanced (stage III/IV) non-small cell lung cancer.
Methods: Systematic literature review of phase-III trials investigating chemo- or chemo-radiotherapies in advanced NSCLC, published 2000–2009 in major (oncology) journals (JCO, EJC, Ann Oncol, JAMA, Lancet and NEJM). Data extraction includes presence of weight assessment at baseline and overtime, weight as trial endpoint, extent of weight response associated with anticancer treatment and mentioning of CC assessment.
Results: Of 101 trials, 89 met inclusion criteria; mentioning weight assessment, 50 trials; weight in patient characteristics/stratification parameters, 30; weight as prognostic factor, 15; weight loss mentioned in eligibility criteria, 12; weight (loss) in adverse events tables, 5; composite endpoint clinical benefit including weight, 2; direct monitoring of weight change, 3; improvement of weight under study treatment, none; and mentioning the word cachexia in publication, none.
Discussion: This is the first systematic review exploring development of weight associated with anticancer treatment in advanced NSCLC patients. Not one single trial reported weight gain under successful anticancer treatment. Cachexia is commonly an exclusion criteria for study participation and is rather noticed as an adverse factor for successful treatment, but data about weight development were not reported. Prospective trials with weight as endpoint are required.

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Improving care for patients with cancer and anorexia–cachexia syndrome: the Macmillan Durham cachexia pack

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Background and aims: Patients, their caregivers and healthcare professionals describe a sense of hopelessness and helplessness when confronted with the consequences of

cancer-induced anorexia–cachexia syndrome (ACS) [1]. Yet, it is a significant cause of both physical and psychosocial morbidity. A 3-year project, funded by Macmillan Cancer Support, was undertaken in order to define strategies to improve the experience of ACS for patients with cancer. This led to the development of a professional resource pack.

Methods: Baseline projects addressed the following key aspects of care: (a) professionals' awareness and approaches to cachexia, (b) symptom burden for patients with ACS and (c) impact of standardised assessment and management tools on patient symptom scores.

Results: Key findings are as follows: (a) awareness and approach to ACS is highly variable, leading to inconsistent care [2]; (b) symptom burden associated with ACS is significant [3]; and (c) simple interventions can make a significant difference to the experience of ACS [4]. These results led to the development of a set of guidelines on the assessment and management of ACS, the Macmillan Durham cachexia pack (MDCP). This is intended for use by any healthcare professional. The pack includes a short assessment tool, algorithms for management of dietary and exercise needs according to patient's fitness, symptom management advice and a number of patient/carer information sheets addressing the practical and psychological issues they face.

Conclusions: There is room for significant improvement in the experience of ACS, but simple measures make a difference. The MDCP provides a framework for improved assessment and management of common problems seen in ACS.

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The need for qualitative methodologies in cancer cachexia research

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Background: Cancer cachexia is not well understood or managed in clinical practice (Delmore 2000; Poole and Froggatt 2002). Whilst a dedicated effort has been made towards understanding the biological processes of the syndrome, little attention has been paid to its multidimensional impact. This is despite previous qualitative research, enriching our understanding of the holistic impact of the syndrome which traditional quantitative methods could not have uncovered (Reid 2007).

Aim: The aim of this study is to determine the adequacy of the existing clinical knowledge base of cancer cachexia management.

Methods: A systematic critical review of the literature on cancer cachexia was undertaken.

Results: There is a need to develop protocols for care delivery, which move beyond a purely biological approach to care towards a more holistic approach. This can only be achieved by gaining the perspectives of those who are involved in care delivery to advanced cancer patients with cachexia and their families using qualitative methodologies.

Conclusions: Cancer cachexia is a complex, challenging syndrome, which must be understood from a holistic bio-psychosocial model of care in order to meet the multidimensional needs of this client population. The perspectives of those involved in care delivery is required in order to contribute to a knowledge base which will inform the development of interventions directed at empowering patients and their families to understand cancer cachexia and recognise it as part of the disease process.

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Profiling nutritional and performance characteristics in patients undergoing surgery for liver and pancreatic cancer: a pilot study

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Background and aims: Malnutrition in hepatopancreatobiliary cancer (HPBC) patients has been associated with higher postoperative morbidity and mortality. Our aim was to estimate rates of consent and compliance to specialized presurgical assessments (SPA) in patients with HPBC and to identify potential variation in nutritional and performance characteristics related to the time of assessment.

Methods: Consenting subjects with resectable HPBC were required to undergo testing for CBC and differentials, CRP, albumin, DXA, 3-day food recall, upper and lower limb muscle strength, and 6-min walk distance during two separate visits (≥4 weeks (visit 1) and ≤1 week (visit 2) pre-surgery).
Results: Of the 160 newly diagnosed HPBC, 55 (34%) were eligible and 31 (56%) consented. Of the consented patients, 23 (74.2%) completed visit 1 and 16 (51.6%) completed visit 2. Difficulties with transportation, hospitalization, or participation in other studies were the main reasons for not completing visit 2. No differences were found between patients completed only visit 1 or those completed the study. However, patients completed visit 2 had a lower WBC count (5.7 vs. 7.3, $p=0.045$) and covered longer distances (499 vs. 478 m, $p=0.045$), as compared with visit 1. Nutritional and strength parameters did not change significantly between the two visits.
Conclusions: This study reveals that the recruitment of HPBC patients into SPA is feasible and well received. Pts who completed both visits had objective improvement in inflammatory and functional measures. Current results will guide future clinical trials evaluating the effectiveness of preoperative rehabilitation programs in decreasing postoperative complications.

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A standardized approach to identifying functional impairment in cancer cachexia

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Background: The ongoing efforts to agree upon an international definition of cachexia recognize the multidimensional nature of its underlying mechanisms and clinical presentations. As one of the commonly encountered features of cancer cachexia is reduced physical functioning, there is a need to identify and define the spectrum of impairments, activity limitations, and participation restrictions experienced by individuals with cancer cachexia. Such an exercise would be an important step in cataloguing the range of functional impairments and would help direct use of appropriate measurement tools and thus help assess the functional impact of anticachexia treatment programs. The International Classification of Functioning, Disability, and Health (ICF) was developed by the World Health Organization (WHO) to provide a common language to describe health-related states. The ICF adopts a bi-directional bio-psychosocial framework representing an integrated model of human functioning.
Objectives: The objective of this study is to establish standardized functional status indicators (FSIs) that represent all components of human functioning and environmental factors in individuals with cancer cachexia.
Methods: A literature review followed by a Delphi process involving known experts in the field will establish an international consensus on indicators of all functional limitations in patients with cancer cachexia. These FSIs will be validated using data obtained from patient focus groups. FSIs will be coded and linked to items on appropriate outcome measures using a standardized ICF mapping protocol.
Contribution: Standardized cancer cachexia FSIs will promote communication among researchers and healthcare providers, and will help guide choices of appropriate outcome measures and preventative and intervention strategies for patients.

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Association between quality of life and directly monitored activity in patients with upper gastrointestinal (GI) cancer

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Background and aims: Cachexia/anorexia is associated with both reduced quality of life and spontaneous physical activity. We monitored directly spontaneous physical activity (PA) in $n=53$ patients with upper GI cancer to verify associations between quality of life scores in (1) the symptom-focused anorexia/cachexia subscale (ACS) of the Functional Assessment of Anorexia/Cachexia Treatment Questionnaire (FAACT) and (2) the broader quality of life score represented by the Trial Outcome Index (TOI, association of the ACS plus physical wellbeing (PWB) and functional wellbeing (FWB) scores) of the same questionnaire.
Methods: Physical activity, including daily time sitting/lying, standing, walking, number of steps taken, and step cadence, was monitored using an accelerometry-based system (ActivPAL™, UK). Anorexia/cachexia severity was assessed by the application of the FAACT questionnaire. The spearman coefficient was employed to verify associations between the variables.

Results: TOI was strongly associated with time sitting/lying (rs=−0.435, p=0.002) and time standing (rs=0.423, p=0.002), and was also associated with time stepping (rs=0.385, p=0.005) and number of steps taken (rs=0.427, p=0.002). Anorexia/cachexia score was associated (albeit to a lesser degree than TOI) with the time sitting/lying (rs=−0.281, p=0.05) and the time standing (rs=0.289, p=0.040).
Conclusions: Spontaneous physical activity is strongly associated with the broader physical and functional aspects of quality of life. Specific symptoms of anorexia/cachexia are associated to a lesser degree. PA monitoring provides data that are meaningful in terms of patient-reported quality of life and can be used to document objectively the variable impact of cancer and its treatment throughout the cancer journey.

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Do short-term changes in weight influence quality of life and symptoms in advanced cancer?

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Background: The McGill Cancer Nutrition-Rehabilitation Program (CNRP) is a multi-disciplinary team that addresses weight loss, nutritional symptoms, and impaired functional status in patients with advanced cancer. There is evidence of associations between reported weight loss, performance status, and symptoms at a single time point, but our experience suggests that the inter-relationships between these variables are complex and may alter over short time scales. In this study, we investigated the relationships between changes in weight, patient-reported performance status, quality of life and strength between consecutive clinic visits.
Methods: A retrospective study was conducted using the CNRP database. Weight, self-assessed performance status (PS-pt) taken from box 4 of the patient-generated subjective global assessment, and patient-rated strength and QoL from the Edmonton symptom assessment scale were recorded at first and second visits.
Results: Three hundred twenty-three patients (male/female, 169/154; mean age, 64.5±12.0) were included. Between assessments (mean, 6.2 weeks, SD 4.1), 142 patients lost weight (WL, mean −3.71%), 132 gained weight (WG, mean 3.33%), and 49 remained stable. Between the clinic visits, changes in weight, PS-pt, QoL, and strength were significantly correlated. Interestingly, correlations were strongest in the WL group: weight change vs change in PS-pt (R^2=0.23, p=0.01), change in PS-pt vs change in QoL (R^2=0.20, p=0.02), and change in QoL vs change strength (R^2=0.30, p=0.01).
Conclusions: The associations between weight change, PS-pt, strength, and QoL hold true over short timeframes. This highlights the potential for influencing PS and QoL using targeted rehabilitation interventions in advanced cancer.

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Cancer-related fatigue: the impact of skeletal muscle mass and strength in newly diagnosed patients with advanced cancer

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Background and aims: Little is known about skeletal muscle mass and strength development and their association with cancer-related fatigue (CRF). The purpose of this study was to determine if CRF is associated with measures of muscle mass and strength in advanced cancer patients (ACP).
Methods: Eighty-four patients (48 men, 36 women, aged 61.6±13.2 years) newly diagnosed (≤6 months) with inoperable (stages III–IV) gastrointestinal or non-small cell lung cancer participated in this study. All patients completed the brief fatigue inventory (BFI). Handgrip (HGS) and quadriceps (QS) strength was assessed using isometric and isokinetic dynamometry, respectively. Skeletal muscle mass index (SMMI) was calculated from the appendicular lean mass measured via dual-energy X-ray absorptiometry divided by body height squared.
Results: When compared to women, men had significantly greater amounts of total lean body mass, as well as arm and leg mass. Furthermore, men displayed greater handgrip and quadriceps strength. BFI scores (−1, 33), 95% CI (−2.05:−0.61), and QS (−019; β) were negatively associated with HGS (−0.34:−0.04) and SMMI (−9.25; −14.15:−4.34) when

adjusting for age, diagnosis, and survival. Multivariate analyses revealed that, in general, women had lower BFI scores than men. There was a significant sex × SMMI interaction (13.49; 2.52:24.46), where BFI decreased with increasing SMMI in men, but did not change with SMMI in women.
Conclusion: These results suggest that in ACP, CRF is related to muscle mass and strength, which may provide a target for future intervention.

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Changes in circulating leptin levels and leptin gene expression in patients with cancer cachexia

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Background: Leptin, a well-known adipokine, plays an important role in the regulation of energy balance; however, its role in human cancer cachexia is still unknown. We investigated serum leptin levels and leptin gene expression in subcutaneous adipose tissue (SAT) of cancer patients and explored its crosstalk with adipokine zinc-α2-glycoprotein (ZAG), a potential cachexia mediator.
Methods: Thirty-nine patients with oesophageal (n=8), gastric (n=16), or pancreatic (n=15) cancer, with (n=24) or without (n=15) cachexia (weight loss ≥5%), were recruited. Circulating serum leptin levels were quantified by ELISA. Leptin and ZAG mRNA levels in SAT were determined using quantitative real-time PCR. Human SGBS adipocytes were treated with recombinant leptin.
Results: Both serum leptin levels (8.3±1.62 vs 15.1±2.7 ng/mL, P=0.03) and SAT leptin mRNA expression (ΔCt=−4.3 vs −3.01, P=0.03) were lower in cachectic compared with weight-stable cancer patients. Significant correlations were demonstrated between serum leptin and BMI (r=0.68, P=0.0001) and weight loss (r=−0.36, P=0.02), and also between leptin mRNA levels in SAT and BMI (r=0.71, P=0.0001) and weight loss (r=−0.48, P=0.008). Serum leptin positively correlated with leptin mRNA in SAT (r=0.45, P=0.02) and, furthermore, negatively correlated with ZAG mRNA in SAT (r=−0.57, P=0.004). In vitro, leptin treatment led to a decrease in ZAG mRNA in human adipocytes.
Conclusions: In cancer patients, both circulating leptin levels and SAT leptin gene expression decrease with weight loss. Lowered circulating leptin levels may result from the reduction in leptin synthesis by adipocytes. Finally, reduced production of leptin may permit a rise in ZAG expression in SAT in cancer cachexia.

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Systemic zinc redistribution in cancer cachexia

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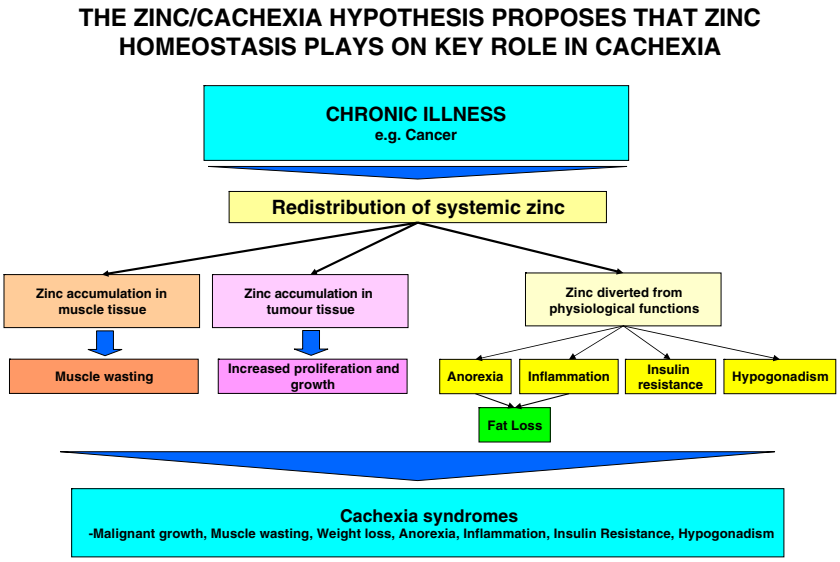
Background and aims: Cachexia is characterized by loss of muscle with or without loss of fat mass. Its manifestations commonly include anorexia, growth failure in children, inflammation, insulin resistance, and hypogonadism. We have formulated a novel hypothesis addressing the links between these seemingly unrelated syndromes.
Methods: We identified a possible common unifying factor behind some of cachexia's clinical manifestations based on mechanistic studies in murine myotubes, effects of the Zn chelating compound D-myo-inositol 1,2,6-triphosphate (alpha trinositol) in the cachexia inducing MAC-16 model, and pre-clinical and clinical data on cachexia and Zn deficiency.
Results: Zn is a critical trace element in physiology. It has been recognized that many tumors draw Zn, and systemic Zn levels often decline in cancer. However, it has not been recognized that in cachexia, skeletal muscle tissue, that constitutes a large portion of total BM (~40% in humans), also significantly and progressively accumulates Zn. Extracellular Zn activates PKR-mediated pathways leading to muscle wasting by mobilizing intracellular Ca. The continuous redistribution of Zn restricts its availability for critical physiological functions. A large body of research demonstrates that Zn depletion and/or deficiency frequently and predictably results in anorexia, growth failure in children, increased inflammation, insulin resistance, and hypogonadism.
Conclusion: In cancer cachexia, Zn is continuously diverted from its normal physiological functions. The clinical manifestations of Zn deficiency are strikingly similar to the symptoms characterizing cancer cachexia. Several conditions are known to cause systemic Zn depletion and deficiency. We suggest that cancer cachexia is one of them (see Figure).



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Zinc-alpha2-glycoprotein mRNA expression is increased in omental and subcutaneous adipose tissue of pancreatic cancer patients

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Background and aims: Cachexia involves loss of adipose tissue and is a major cause of death in patients with advanced pancreatic cancer. Elevated plasma levels of zinc-alpha2-glycoprotein (ZAG) are associated with several types of cancer and may affect lipolysis. ZAG is expressed and secreted by adipocytes, and its expression in adipose tissue is elevated in mice with cancer cachexia. However, the clinical relevance of these findings for loss of adipose tissue mass in patients with pancreatic cancer prior to clinical manifestations of advanced cachexia remains to be established.

Methods: Biopsies of omental and subcutaneous adipose tissue were sampled from non-cachectic pancreatic cancer patients undergoing tumor resection (n=22) and control patients without cancer (n=11). mRNA expression levels of ZAG and other genes were analyzed by qRT-PCR relative to GAPDH. Alterations in subcutaneous and omental adipose tissue mass were monitored by volumetric CT analysis (9 cm from L4/L5 in cranial direction) at inclusion and during follow-up.

Results: Pancreatic cancer patients displayed 3.1- and 1.7-fold higher median levels of ZAG mRNA in omental and subcutaneous adipose tissue, respectively, as compared to controls (P<0.01). Investigations at the mRNA level of 27 other genes involved in fatty acid and triacylglyceride metabolism revealed that 17 were enhanced in omental adipose tissue, whereas three were upregulated in subcutaneous adipose tissue. Preliminary volumetric results comparing adipose tissue mass before surgery and at follow-up suggest that omental adipose tissue is selectively lost as compared to subcutaneous adipose tissue.

Conclusions: These data suggest that ZAG mRNA expression is increased in both omental and subcutaneous adipose tissue in pancreatic cancer patients without clinical symptoms of advanced cachexia. Altered expression levels of genes involved in lipid metabolism might be predictive of selective loss of omental adipose tissue mass in the early stages of cachectic development.

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Anorexia, energy expenditure, and heart rate variability (HRV) in cancer patients: a pilot study

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Background and aims: The hypothalamus is involved in the control of food intake and energy expenditure via the autonomous nervous system. In cancer patients, anorexia and cachexia frequently occur, which can be related to deranged sympatovagal balance. HRV is a reliable index of the autonomous nervous system activity. Thus, in cancer patients, a relationship may exist between anorexia, HRV, and energy expenditure.
Methods: Anorexia and body cell mass (BCM) were measured in four lung cancer patients, using a visual analogue scale (VAS; +100, hunger; −100, satiety) and bioimpedance analysis, respectively. Resting energy expenditure (REE) was calculated based on BCM. HRV was measured using Holter EKG recordings via a computerized software, and the time domain of pNN50 was obtained. Pearson's correlation test was used.
Results: VAS significantly correlates with pNN50 (r= 0.994; p<0.01); negative correlations exist between pNN50 and BCM and REE (r=−0.967, p<0.05; r=−0.966, p< 0.05; respectively).
Conclusions: Data obtained support the hypothalamic involvement in anorexia and cachexia. If confirmed by larger studies, HRV may also represent a useful, non-invasive tool to detect and predict the development of nutritional complications in cancer patients.

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Exercise capacity, body composition, blood flow, and autonomic function in cachectic and non-cachectic patients with pancreatic cancer

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Background: In pancreatic cancer (PCA), cachexia, fatigue, and dyspnea are frequently seen. We hypothesize that the development of cancer fatigue syndrome is at least in part due to cardiovascular pertubation with the consequence of decreased exercise capacity and reduced quality of life.
Methods: To investigate the cardiovascular parameters, we prospectively studied 96 patients with PCA (age, 58.9± 0.9 years; mean±SE, 60 male; body mass index (BMI), 23.2±0.4 kg/m^2; hemoglobin (Hb), 11.3±0.2 g/dl) and 74 healthy controls (CON, 59.7±1.3 years, 40 male; BMI, 26.2±0.5 kg/m^2; Hb, 13.9±0.2 g/dl) in exercise capacity using symptom-limited exercise test, cardiac function using echocardiography, body composition using dual energy X-ray absorptiometry, heart rate variability using 24-h ECG, and peripheral blood flow using venous occlusion plethymography in patients and controls. In a subgroup of 42 PCA patients and 22 CON, TNF-α, interleukin-6, and TNF-receptor 1/2 (TNF-R1/2) were analyzed. Cachectic and non-cachectic patients were compared.
Results: No significant difference was detected between patients and CON in terms of sex, age, and systolic function. Patients with PCA compared to CON displayed impaired exercise capacity (peak VO_2, 21.1±0.6 vs. 27.2± 1.0 mL/min/kg), less total lean mass (68.6±1.3 vs. 78.4± 1.6 kg), increased resting blood flow in the leg (4.3±0.4 vs. 2.8.3±0.2 mL/100 mL/min), impaired autonomic function (SDNN24h, 100.3±30.8 vs. 134±37.5 ms), and elevated pro-inflammatory markers (IL-6, 5.2±0.8 vs. 1.8±0.2 pg/mL; TNF-R1, 1828±132 vs. 1245±69 pg/mL; TNF-R2, 2811±143 vs. 2018±120 pg/mL; all p<0.05). Autonomic dysfunction in cachectic patients was even worse than in non-cachectic patients (SDNN24h, 94.6±4.9 vs. 109.9± 4.5 ms, p=0.03).
Conclusions: Reduced exercise capacity, less lean mass, autonomic dysfunction, increased resting blood flow, and elevated proinflammatory markers are present in patients with PCA. Further studies are required.

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microRNA-199, a potential key modulator of tumor induced cardiac cachexia

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Background: The assembly and maintenance of the cardiac sarcomere, which contains the basic contractile components of actin and myosin, are essential for cardiac function. While often described as a static structure, the sarcomere is actually dynamic and undergoes constant turnover, allowing it to adapt to physiological changes while still maintaining function.
Objective: We evaluated the role of miR199a-5p (miR199) for the sarcomere disorganization in tumor rats with cardiac atrophy.
Methods and results: Quantitative real-time PCR analysis revealed robust expression of miR199 in normal adult rat hearts, while its expression was markedly lower (−40%, $P<0.05$) in tumor-bearing rats with cardiac atrophy. Immunoblotting revealed a marked reduction in cardiac myosin heavy chain protein (α- and β-MHC) in hearts from tumor-bearing rats compared to normal rats, while cardiac troponin T and tropomyosin were not affected. Cultured neonatal rat cardiomyocyte (NRCM) express high levels of miR-199. Lipofectamin-based transfection of anti-miR199 in NRCM reduced miR199 levels by 50- to 100-fold, which was associated with growth retention of cells. Liquid chromatography tandem mass spectrometry revealed enhanced ubiquitination and degradation of α- and β-MHC and Titin, but not of Troponin T and Tropomyosin in anti-miR199-treated NRCM. Furthermore, electron microscopy revealed disappearance of the Z-disc in anti-miR199-treated NRCM. Three ubiquitin-conjugating enzymes, Ube2i, Ube2g1, and Ube2o, emerged as direct targets of miR-199a in NRCM, which was confirmed by immunoblotting and with a reporter plasmids (psiCheck) containing the potential miRNA binding sites of Ube2i, Ube2g1, and Ube2o at the 3′-position of the luciferase reporter gene. In turn, overexpression of miR199 by pre-miR199 transfection prevented MHC degradation and significantly reduced protein levels of Ube2i (−74%, $P<0.01$ vs control pre-miR), Ube2g1 (−69%, $P<0.01$ vs control pre-miR), and Ube2o (−55%, $P<0.01$ vs control pre-miR). Similarly, transfection of cardiomyocytes with siRNA directed against Ube2i, Ube2g1, or Ube2o prevented MHC degradation, an effect that was not observed with control siRNA or siRNA directed against Ube2j, a ubiquitin conjugating enzyme that is not a target of miR-199. Furthermore, HIF1-alpha, a pro-angiogenic protein mainly regulated by the UPS, was not affected by modulations of miR199 and or Ube2i, Ube2g1, and Ube2o expression.
Conclusion: Our observations suggest a key role of miR199 for the homeostasis of sarcomeric protein turnover in postnatal cardiomyocytes and adult hearts. Reduction of miR199 as we report here in tumor-bearing rats promotes cardiac cachexia by increasing UPS-mediated degradation of sarcomeric proteins, i.e., MHC and Titin. miR199 targets hereby a specific subset of ubiquitin conjugating enzymes, i.e., Ube2i, Ube2g1, and Ube2o that seem to be responsible for degrading specific sarcomeric proteins, i.e., MHC and Titin.

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Severe progressive heart failure in experimental cancer cachexia only transiently elevates B-type natriuretic peptide (BNP)

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Background: Cachexia is a common co-morbidity in patients with cancer, which drastically reduces quality of life and survival. Cancer patients also develop shortness of breath of unknown reason, particularly when cachectic. Whether cancer cachexia causes heart failure is not known.
Methods: Juvenile Wistar rats (weight approximately 200 g) were inoculated intra-peritoneally with AH-130 hepatoma cells or saline (sham). Heart function was measured before inoculation and on the day of sacrifice, when invasive hemodynamic assessment and ECG were also performed. Weight and body composition (NMR scan) were analyzed on day 0 and after sacrifice (without tumor).
Results: Animals of all groups showed similar baseline values for weight, body composition, and heart function. Rats with cancer reached a cachectic state on day 7. Animals lost only fat mass at this stage. With progressing disease and cachexia deterioration, cardiac function became severely impaired (see Table). At the end of the study, animals displayed severe cachexia (136±3 g (day 13) vs sham, 239±4 g, $p<0.0001$). Cancer rats lost fat (−11.7±0.6 g (day 13) vs sham, 6.3±0.9 g, $p<0.0001$) and lean tissue (−35.6±1.5 g (day 13) vs sham, 32.3±2.1 g, $p<0.0001$).

	Tumor					Sham
	Day 5	Day 7	Day 9	Day 11	Day 13	Combined
N	6	10	9	7	12	15
Delta BW (g)	8.0±1.1***	−9.0±2.7***	−10.3±3.2***	−40.3±10.0***	−42.4±1.7***	45.2±3.9
LVEF (%)	85.8±0.6	74.1±1.9	80.1±1.5	53.4±1.6***	50.5±1.4***	79.7±1.4
LVFS (%)	52.7±1.5	43.0±1.5**	47.9±2.3	26.5±0.7***	30.3±0.9***	50.5±1.4
LVEDD (mm)	7.02±0.17	6.52±0.19	6.04±0.35	4.94±0.48*	4.05±0.32***	6.50±0.13
LVEDV (μl)	257.5±9.0	197.4±10.9**	186.2±10.7**	102.2±13.5***	68.55±3.96***	251.9±12.6
LVSV (μl)	221.1±8.4	146.2±9.0**	150.5±9.3*	55.0±8.3***	34.4±1.8***	202.1±12.5
LV-mass (mg)	473±25***	495±18***	447±48**	481±76*	220±29***	642±23
LVEDP (mm/Hg)	8.1±3.0	14.2±2.7	−2.9±0.4***	1.5±1.6 *	−2.3±0.4***	19.2±9.0
LVESP (mm/Hg)	108.4±3.9	117.1±4.5	103.9±4.5	82.9±6.2*	66.9±3.7***	110.6±5.4
QRS (ms)	16.1±1.3	17.9±1.2	19.93±2.0	28.1±2.9**	31.6±1,4***	17.2±1.1
BNP (pg/mL)	303±48	1728±737*	774±265	191±58	142±25	435.4±69

BW body weight, *LV* left ventricular, *EF* ejection fraction, *FS* fractional shortening, *EDD* enddiastolic diameter, *EDV* enddiastolic volume, *SV* stroke volume, *EDP* enddiastolic pressure, *ESP* endsystolic pressure
*$p>0.05$; **$p<0.01$; ***$p<0.001$ tumor vs sham

Conclusion: In this model system, severe cancer cachexia causes severe impairment of cardiac function. The nature of these impairments is only partially comparable to processes in chronic heart failure. However, heart failure therapies may be beneficial in this clinical setting.

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A soluble activin receptor type IIB increases muscle mass in a mouse model of cancer cachexia

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Background: Cachexia is characterized by undesired weight loss resulting from loss of both muscle and adipose tissue. Myostatin, a known negative regulator of muscle mass, is upregulated in the colon-26 rodent model of cachexia. Myostatin and other negative muscle mass regulators signal via the activin receptor type IIB (ActRIIB), and treatment of normal mice with a non-signaling, decoy ActRIIB results in a robust increase in lean tissue. This study evaluates the potential beneficial effects of ACE-435, a fusion protein comprised of an optimized extracellular domain of ActRIIB linked to a human Fc, in cachectic mice.
Materials and methods: Eight-week-old BALB/c mice were injected subcutaneously with 1×106 colon-26 adenocarcinoma cells. Two weeks post-implantation, mice were randomly assigned to receive vehicle or 10 mg/kg ACE-435 two times a week. Body weight and in vivo body composition were tracked throughout the study.
Results: ACE-435 treatment resulted in a significant body weight increase that was maintained across the study ($p<0.05$). Seven weeks post-tumor implantation vehicle-treated mice had a 7% loss of baseline lean mass, whereas the ACE-435 group had a 27% increase in baseline lean mass ($p<0.001$). Importantly, fat mass was not different between groups, illustrating that the ACE-435 anabolic effect did not exacerbate fat loss. Also, although not reaching significance ($p<0.076$), the ACE-435 group had an 8-day mean increase in survival.
Conclusions: These data demonstrate that ACE-435 has beneficial effects in alleviating cachexia in tumor-bearing mice. Thus, a soluble ActIIB-Fc fusion protein could be an effective therapy for treating cancer-induced cachexia.

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STAT3 in skeletal muscle links the acute phase response and muscle wasting in colon-26 cancer cachexia

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Background and aims: Cancer cachexia is associated with increased serum cytokines, particularly interleukin-6 (IL-6), and the acute phase response (APR). We hypothesized these observations are mechanistically linked through IL-6/STAT3 actions in skeletal muscle.
Methods: Quadriceps gene expression from mice with colon-26 cachexia, known to be mediated largely by IL-6, was profiled using Illumina BeadChips. GeneSpring was used for data normalization, statistical analysis, and hierarchical clustering. Metacore and NextBio were used for gene and pathway analyses. Selected profiles were validated by qPCR and Western blotting.
Results: Gene expression profiles in early and late cachexia were significantly correlated. The top canonical pathways upregulated in both were inflammation/IL-6/STAT3 signaling, proteolysis, and MAPK signaling. The top downregulated pathways were the PGC1A pathway, calcium regulation/signaling, ECM, focal adhesion, skeletal myogenesis, and insulin signaling. Increased pSTAT3 was observed in cachexia both in liver and skeletal muscle. STAT3 target genes, including SOCS3, fibrinogen, serum amyloid A, lipid binding protein, and haptoglobin were coordinately induced in both tissues. By Western blotting, fibrinogen was induced 80-fold over normal in quadriceps and tenfold in liver. Quantitation of muscle versus liver fibrinogen demonstrated that up to 40% of serum fibrinogen could be muscle derived.
Conclusions: The IL-6/STAT3 signaling pathway is highly induced in liver and skeletal muscle in colon-26 cachexia. APR genes were highly expressed not only in liver but also in skeletal muscle. These results indicate that skeletal muscle contributes to the APR in cancer and that STAT3 activation in muscle provides a mechanistic link between muscle wasting and the APR.

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Male hypogonadism in advanced cancer patients: nutritional, functional, and quality of life correlates

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Background: Male hypogonadism often occurs in advanced cancer patients (ACP), but its clinical impact remains unclear. Our aim was to explore the relationships between low free testosterone (LFT) and nutritional, functional, and quality of life (QOL) characteristics in ACP.
Methods: Free testosterone levels were measured in 100 males (median age, 64 years) newly diagnosed with advanced non-small cell lung (38.8%) and gastrointestinal (61.2%) cancers. The Edmonton symptom assessment scale (ESAS), brief fatigue inventory (BFI), patient-generated subjective global assessment (PG-SGA), McGill quality of life questionnaire (MQOL), handgrip strength test (Jamar®), DXA for body composition, albumin, CRP, and WBC were evaluated. Multiple regression analyses (controlling for age, diagnosis (lung/GI), treatment (radio/chemo), survival, and concurrent medications) were performed.
Results: Seventy-six patients (76%) presented with LFT (<31.1 pmol/L). As compared to eugonadic patients, patients with LFT had similar QOL, weakness, dyspnea, and appetite according to the ESAS and similar CRP and WBC levels, worse performance status by PG-SGA Box 4 (beta, 0.59; 95% CI, 0.07, 1.11), worse BFI total score (16.68; 2.03, 31.32), worse MQOL physical score (−2.20; −4.01, −0.39) and total score (−1.42; −2.54, −0.30), lower handgrip strength (−11.67 lbs; −20.39, −2.95) and percentile (−11.23; −20.56, −1.89), lower lean mass for the upper limbs (−0.76 kg; −1.44, −0.09), and lower albumin (−3.77; −7.21, −0.77).
Conclusion: Our data show relationships between male hypogonadism and worse nutritional, functional, and QOL characteristics in ACP. Clinical trials for the treatment of hypogonadism in this patient population are warranted to confirm a cause–effect nature of these associations.

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Functional, nutritional, and quality of life correlates of hypogonadism in advanced cancer: a qualitative systematic review of the literature

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Background and aims: Hypogonadism has been associated with a host of secondary symptoms including diminished energy, poor concentration, depression, and decreased physical performance. These symptoms are often present in advanced cancer patients (ACP), and hypogonadism is a key pathophysiologic mechanism for both sarcopenia and cancer cachexia (CC). To our knowledge, no study has yet systematically reviewed available literature on clinical correlates of hypogonadism in ACP. Therefore, we conducted a qualitative systematic review of the literature regarding the

impact of hypogonadism on functional and nutritional status, body composition, symptoms, and quality of life in ACP.
Methods: Through a systematic search of both Pubmed and EMBASE databases, six original articles were selected, and their results were tabulated for comparative qualitative analysis.
Results: No definitive associations between nutritional, functional, or QOL characteristics and hypogonadism were found in the reviewed studies. Possible associations between hypogonadism and weight loss, low albumin, body cell mass, peripheral fat, and muscle mass, inflammation, pain, and opioid consumption, as well as worse emotional and functional well-being, must be confirmed by better-designed studies.
Conclusions: Clear epidemiological data is not yet available to indicate whether hypogonadism is independently correlated with clinical and biological sequaela of CC. Future studies examining hypogonadism in ACP should (a) use standardized kits with sufficient sensitivity and precision in the lower ranges for either free or bio-available testosterone, (b) use multivariate models on adequately sized samples to identify independent clinical correlates of hypogonadism; and (c) include measurements of luteinizing hormone to differentiate primary from secondary hypogonadism.

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Metabolic, nutritional, and inflammatory characteristics in elderly women with newly diagnosed advanced cancer

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Background and aims: Few studies have focused on the metabolic, nutritional, and inflammatory profiling of elderly patients with advanced cancer. The aim of the study was to determine energy expenditure at rest and during activity, body composition, nutrition, and inflammatory characteristics of elderly females with and without advanced gastrointestinal cancer.
Methods: Twenty elderly (74.8±6.7 years) females (nine newly diagnosed advanced gastrointestinal cancer and 11 healthy) were evaluated using indirect calorimetry at rest and throughout a 6-min walk test. Body composition, nutritional intake, and blood markers of nutrition and inflammation (CBC, albumin, and CRP) were also measured in all study subjects.
Results: Compared to healthy controls (H), cancer patients (C) had similar energy expenditures, but significantly lower respiratory quotients at rest (C, 0.74±0.02; H, 0.81±0.07; $p<0.05$). During the 6MWT, the cancer group walked shorter distances (337.2±92.0 m; 480.4±49.0; $p<0.001$) at slower speeds (0.94±0.26 m/s; 1.33±0.14; $p<0.001$) with lower oxygen uptakes (11.7±3.6 mlO_2/kg/min; 15.0±2.4; $p<0.05$). The cancer patients ingested fewer calories (22.4±5.6 kcal/kg; 33.1±2.3; $p<0.01$) and presented with higher levels of CRP (27.1±40.5 mg/L; 2.1±1.4; $p<0.05$). No differences in body composition were observed.
Conclusions: Our preliminary data suggest that elderly women with newly diagnosed advanced cancer have a greater reliance on fat metabolism at rest and walk shorter distances at lower heart rates and levels of oxygen consumption. These metabolic differences, coupled with the elevation in inflammatory markers and reduction in caloric intake, may signal an early anorectic-cachectic condition, which may have important diagnostic, treatment, and prognostic implications.

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Association between hypogonadism, C-reactive protein, symptom burden, and survival in male cancer patients with cachexia: a preliminary report

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Background and aims: In patients without cancer, hypogonadism is associated with lower lean body mass, increased symptom burden, and decreased survival. Hypogonadism in cancer cachexia could exacerbate symptoms, facilitate a pro-inflammatory state, and decrease survival.
Methods: A retrospective chart review of 98 consecutive male patients referred to a cachexia clinic at a cancer center, 57 (58%) had serum C-reactive protein (CRP) and 68 (69%) had total testosterone evaluated. Symptoms were evaluated by the Edmonton symptom assessment scale (ESAS) and bioimpedance by Tanita body composition scale.
Results: All male patients reported weight loss of >5% within the preceding 6 months; median age, 60; CRP, 20 mg/L; and median testosterone, 185 ng/dL (6.42 nmol/L). The degree of weight loss over the preceding 3 months correlated with CRP levels ($r=0.42$, $p<0.05$), but not with testosterone. Fat free mass and body mass index (BMI) showed no correlation with CRP levels or testosterone. Testosterone correlated inversely with CRP levels ($p<0.01$). Survival with testosterone ≤185 ng/dL (6.42 nmol/L) decreased compared to those >185 ng/dL (13 vs. 62 weeks, $p=0.004$). Patients with CRP >10 mg/L had decreased survival compared to ≤10 mg/L (15 vs. 46 weeks, $p=0.01$). Among patients with both testosterone and CRP, the combination of low testosterone

and high CRP levels significantly decreased survival compared to other groups (p=0.039).
Conclusions: Elevated CRP levels were associated with increased symptom burden and decreased survival. Low testosterone was associated with decreased survival and correlated inversely with CRP levels. Our results suggest testosterone and CRP may be additive or synergistic as markers for survival in male patients with cachexia.

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Does interleukin (IL) 6 best reflect cachexia in advanced cancer patients?

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Background and aims: Recent diagnostic criteria of cachexia (Evans et al. 2008) have included higher levels of circulating IL-6. The aim of this study was to compare the clinical relevance of four commonly studied pro-inflammatory cytokines, IL-6, IL-1-beta, IL-8, and TNF-alpha, in advanced cancer patients (ACP).
Methods: Plasma collected from 83 patients (61.8±12.9 years) with newly diagnosed advanced lung and gastrointestinal (GI) cancer was analyzed for the above cytokines. Adjusting for gender, age, diagnosis (lung/GI), treatment (radio/chemo), comorbidity, and medications, we explored differences in albumin and CRP levels, presence of >5% weight loss/ 6 months, sarcopenia, and survival between patients with "low levels" (lowest tertile) versus those with "high levels" (highest tertiles) for the considered cytokines.
Results: Patients with high IL-6 had the lowest levels of albumin (IL-6-beta, −5.02; 95% CI, −7.69 to −2.35; IL-1-beta, −3.90, −6.53 to −1.26; IL-8, −2.91, −5.64 to −0.17; and TNF-alpha, −3.15, −5.89 to −0.40) and highest levels of CRP (IL-6, 44.21, 28.78 to 59.65; IL-1-beta, 27.34, 9.45 to 45.24; IL-8, 26.14, 9.06 to 43.22). Sarcopenia was primarily associated with high TNF-alpha (5.56, 1.47 to 20.97) and IL-1-beta (5.35, 1.37 to 20.97), whereas only IL-1-beta reflected weight loss (7.14, 1.87 to 27.26). Decreased survival was associated with high IL-8 (HR, 7.58, 2.84 to 20.22), IL-6 (3.76, 1.44 to 9.87), IL-1-beta (3.50, 1.42 to 8.63), and TNF-alpha (3.17, 1.26 to 7.89).
Conclusions: While IL-6 remains the best marker of inflammation, other cytokines (in particular IL-1-beta) may better reflect clinical correlates of cachexia in ACP.

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Increased activity of liver 11-beta-hydroxysteroid dehydrogenase type 1 (11-beta-HSD1) in male colon cancer patients: role of the deregulation of growth hormone (GH)—insulin-like growth factor I (IGF-I)

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Background and aims: Liver 11-beta HSD1 converts cortisone to the biologically active hormone cortisol: its increased activity plays a central role in the visceral fat accumulation of the metabolic syndrome and Cushing's syndrome. The activity of the enzyme is under the control of GH-IGF-I axis, with GH playing an inhibitory role. Cancer-cachexia patients often display GH resistance and/ or GH deficiency and enhanced cortisol levels.
Methods: We evaluated the cortisone to cortisol conversion in 20 male colon cancer-cachexia patients (and 20 age-matched controls) by measuring serum cortisol after oral cortisone (Cortone®) administration. We also evaluated by L4 CAT the abdominal visceral and subcutaneous fat.
Results: We found enhanced cortisol peak levels (P<0.01) and larger area-under-the-curve (P<0.01) after oral cortisone administration in cancer patients. There was a correlation (r= 51, P<0.01) between the visceral fat area and cortisol peak. No significant changes were observed in basal ACTH and GH levels. IGF-1 levels were lower in cancer patients (P<0.01).
Conclusions: An enhanced cortisol production by liver may contribute to visceral fat accumulation in colon cancer-cachexia patients so contributing to their cardiovascular derangement and is related to the downregulation of GH-IGF-1 axis.

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Exercise capacity, body composition, cardiac function, and peripheral blood flow in cachectic and non-cachectic patients with non-small cell lung cancer

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Background: In non-small cell lung cancer (NSCLC), cachexia, fatigue, and dyspnea are frequently seen. We

hypothesize that the development of cancer fatigue syndrome is at least in part due to cardiovascular pertubation with the consequence of decreased exercise capacity and reduced quality of life.

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

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

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

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Criterion-based validation of accelerometer-derived energy expenditure in advanced cancer

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Background and aims: Accelerometer-based activity monitoring offers potential for simple, inexpensive, and objective assessment of physical activity and energy expenditure of cachectic patients in practice and clinical trials. This study aimed to assess criterion-based validity of an activity monitor (ActivPAL™) using a stable isotope dilution technique.

Methods: Accelerometer-based measurements were validated against total energy expenditure (TEE) and energy expenditure of activity (EEA) assessment by a 2-week doubly labelled water (DLW) and indirect calorimetry protocol in advanced cancer patients (n=7 outpatients) and healthy controls (n=10 assessments in nine subjects). Agreement was assessed by Bland–Altman plots and within-subject standard deviations (Sw).

Results: Cancer patients were older (mean, 62 versus 29 years; p<0.001) and had lower KPS (mean, 87 versus 100; p<0.001), TEEDLW (mean, 2,321 versus 3,202 kcal/day; p<0.01), and EEADLW (mean, 742 versus 1,609 kcal/day; p<0.01) compared with controls, but did not differ significantly in lean body mass or fat mass. TEEActivPAL (mean, 2,859 kcal/day; SD, 787; range, 2,129–5,045) and TEEDLW (mean, 2,849 kcal/day; SD, 849; range, 2,017–5,309) demonstrated a mean difference of 9 kcal/day (Sw, 198; 95% LOA, −191, 587) or absolute percentage error of 0.4%. EEAActivPAL (mean, 1,244 kcal/day; SD, 825; range, 486–3,334) and EEADLW (mean, 1,262 kcal/day; SD, 807; range, 374–3,424) demonstrated a mean difference of −18 kcal/day (Sw, 168; 95% LOA, −161, 497) or absolute percentage error of 1.4%. In a multivariate model, EEAActivPAL explained 85.1% of the variation in TEEDLW (p<0.001).

Conclusions: Activity monitoring provides valid estimates of EEA and TEE in cancer patients and controls, and should be considered as an endpoint in trials of anti-cachexia therapies.

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Validation of body composition measurements by regional computed tomography in advanced cancer patients: preliminary data

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Background and aim: Predictive equations to estimate body composition in advanced cancer patients (ACP) from computed tomography (CT) images have been recently proposed (Mourtzakis et al. 2008). We evaluated the accuracy of these equations to estimate whole body fat

mass (FM), whole body fat-free mass (FFM), and appendicular skeletal muscle (ASM) in a new inception cohort of ACP who also underwent dual-energy X-ray absorptiometry (DXA) measurements.
Methods: FM, FFM, and ASM estimates from CT and DXA measurements obtained within 2 weeks (n=18) or 1 month (n=25) from one another were compared using Spearman's correlation coefficient and Bland–Altman analysis.
Results: For the 2-week interval, CT and DXA measurements correlated as follows: FM (ρ=0.94; p<0.01), FFM (ρ=0.74; p<0.01), and ASM (ρ=0.68; p<0.01). The limits of agreement between both methods were FM (−2.88±3.86 (−10.45, 4.68)kg), FFM (−0.93±7.51 (−15.64, 13.79)kg), and ASM (0.71±1.02 (−1.29, 2.71)kg).
For the 1-month interval, the following correlations were obtained: FM (ρ=0.88; p<0.01), FFM (ρ=0.80; p<0.01), and ASM (ρ=0.74; p<0.01). The limits of agreement were FM (−3.82±4.07 (−11.79, 4.15)kg), FFM (−2.97±7.47 (−17.61, 11.68)kg), and ASM (0.13±0.97 (−1.78, 2.04)kg).
Conclusions: Despite high to moderate correlation between both methods for the measurements of FM, FFM, and ASM, the CT model does not appear to be a surrogate for DXA. Further studies are needed to clarify both the precision and the clinical value of CT for measuring body composition in ACP.

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Role of bioelectrical impendance analysis in advanced cancer

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A frequent manifestation of advanced cancer is protein-energy malnutrition (PEM), timely identification and treatment of which can lead to improved patient outcomes. Bioelectrical impedance analysis (BIA) is an easy-to-use, non-invasive and reproducible technique to evaluate changes in body composition and nutritional status. Phase angle, derived from by BIA, has been described as a prognostic tool in several chronic diseases and in cancer patients. Thirteen patients (58.4±7.5 years, 58.0±10.6 kg and BMI 20.2±3.5 kg/m^2) with advanced cancer have been consecutively referred to the Clinical Nutrition Unit of Federico II University, in Naples. Primary cancer localization was gastric in five, colorectal in three, esophageal in two, pancreatic in two, and pulmonary in one. Anthropometric (weight, height, body mass index and skinfolds) and haematobiochemical (albumin, cholesterol, lymphocytes, haemoglobin and cholinesterase) parameters have been measured. BIA (STA/BIA, Akern) was performed in all patients, and phase angle (expressed in degrees) calculated. The Kaplan–Meier method was used to calculate survival and Cox model to evaluate the prognostic effect of phase angle independent of other clinical and nutritional variables. Phase angle resulted strictly related (R^2=0.384, p=0.024) with survival time, slightly (p=0.055) related with lymphocyte count, but not related with the other anthropometric and biochemical parameters evaluated. This preliminary study confirms phase angle as a prognostic indicator in patients with advanced cancer. Similar studies on larger sample sizes are needed to further validate the prognostic significance of phase angle in advanced cancer patients.

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Changes in body composition during and after head and neck cancer treatment: preliminary results

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Background and aims: Head and neck cancer patients (HNCP) are at risk for weight loss during treatment, due to oral sequalae of cancer treatment, e.g., mucositis and swallowing problems, dental problems, and trismus. Loss of fat free mass (FFM) reduces immune function and quality of life. To determine whether weight loss during treatment consists of loss of fat mass and/or FFM, we measured body composition prospectively.
Methods: Adult patients with squamous cell carcinoma in the oral cavity, pharynx, or larynx and undergoing surgery plus radiotherapy, radiotherapy, or chemoradiation with curative intent were included. All patients received dietary intervention during treatment. Only patients treated with chemoradiation (n=4) received prophylactic gastrostomy placement. Fat mass and FFM were measured by dual energy X-ray absorptiometry, before treatment (T0) and after treatment (T1). Paired samples t test was used to analyze changes in FFM and fat mass between T0 and T1.

In total, 30 patients will be included. Data of the current 11 patients will be presented here.
Results: Interval between T0 and T1 was 82±21 days. Mean body weight and body mass index at T0 were 75.5± 18.7 kg and 23.3±5.5, respectively. Ten patients lost body weight during treatment. Body weight declined with 2.8± 4.2 kg (p=0.051). FFM significantly declined with 1.8± 2.7 kg (p=0.049). Fat mass declined with 1.0±2.4 kg (p=0.224).
Conclusions: The preliminary results indicate that HNCP lose weight, of which 64% is FFM. Intervention should be adapted to maintain or increase FFM.

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Plasma myoglobin concentration is unrelated to muscle mass in elderly patients with early colorectal cancer

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Background and aims: Progressive muscle wasting is a characteristic feature of cancer cachexia and increases the risk of a poor prognosis and diminished quality of life. It has been suggested that in patients with pancreatic and gastric cancers (Weber et al., J Mol Med 85:887–896, 2007), plasma myoglobin concentration is a good index of muscle mass, enabling wasting to be assessed conveniently. Therefore, we wished to test its reliability for this purpose in patients with early colorectal cancer.
Methods: We studied 11 patients with colorectal cancer (five men, six women; 74±11 years) and eight age and sex-matched healthy controls (four men, four women, 64± 3 years), and 56 previously examined control patients with a normal diagnostic CT. We measured plasma myoglobin concentration, both leg muscle mass (LMM), by dual-energy X-ray absorptiometry (DXA) and psoas muscle cross-sectional area (CSA) by computerized tomography (CT) at the third lumbar vertebra.
Results: Plasma myoglobin was 31.2±9.6 vs. 42.9± 8.71 ng/ml (P>0.05) in patients with cancers and controls, respectively. LMM was 6.49±1.94 vs. 6.42±1.62 kg in patients and controls, respectively; psoas CSA in the patients with colorectal cancer was 3,363±1,631 mm^2, i. e., not significantly different from that in the controls (2,873±1,103 mm^2). There was no significant correlation between myoglobin and psoas CSA (r^2=0.0074; P=0.87) or LMM (r^2=0.4684; P=0.80) in patients who lost LMM of 8 (2–15) g/day.
Conclusion: In early colorectal cancer, the extent of muscle wasting cannot be assessed by measurement of plasma myoglobin concentration.

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A central role for neuronal mTOR in cancer-induced anorexia

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Background and aims: Accumulating evidence indicates that the pathogenesis of cancer anorexia is mediated by persistent anorexigenic signals into the hypothalamus. Several factors are considered to be putative mediators of cancer anorexia, including interleukin 1 and 6 (IL-1, IL-6) and tumor necrosis factor (TNF). Recently, hypothalamic mammalian target of rapamycin (mTOR) signaling has been considered a cellular fuel sensor for regulation of food intake. The aim of this study was to examine the role of hypothalamic mTOR in the control of food intake model of cancer-induced anorexia in rodents.
Methods: Walker-256 tumor and Lewis lung carcinoma (LLC) cells were implanted in rat and mouse, respectively. Intrahypothalamic infusion of rapamycin was performed in tumor-bearing (TB) rats during 4 days before analysis, whereas recombinant IL-1-beta and TNF-alpha were acutely infused into the third ventricle of control rats. Food intake analysis, dissection of hypothalamic regions, and Western blotting were combined to evaluate the hypothalamic mTOR activity.
Results: Here, we show that neuronal mTOR pathway is activated in anorexic TB rat and mouse model. As expected, we found high levels of TNF and IL1 in the hypothalamus of TB rat group. Interestingly, intrahypothalamic infusion of recombinant IL-1 or TNF increases mTOR activity and evokes anorexia in control rats. Moreover, central infusion of specific inhibitor of mTOR rapamycin partially blunts the anorectic effect in TB rats. We also observed that the mTOR expression was most intense in the arcuate nucleus, DMH/VMH and PVN when compared to LH in control rats.

Conclusion: Taken together, our data demonstrated that hypothalamic mTOR activity is critical to decreases food intake in tumor-bearing (TB) rodent models.

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The role of the 5-Ht2c receptor in tumor-induced anorexia and gastrointestinal motility in rats

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Background and aims: Cancer cachexia is a debilitating and life-threatening syndrome characterized by anorexia and loss of fat and skeletal muscle. It is a major source of increasing the morbidity and mortality of cancer patients. We examined the role of the serotonergic system in the food intake, gastrointestinal (GI) motility, and appetite-regulating neuropeptide gene expression in tumor-bearing rats.
Methods: Tumor-bearing rats received an intraperitoneal inoculum of 108 AH-130 Yoshida ascites hepatoma cells. GI motility was measured in conscious, freely moving rats by a strain gauge force transducer method. Plasma acyl ghrelin levels were determined by EIA. Appetite-regulating neuropeptide gene expressions in the hypothalamus were quantified by real-time RT-PCR. Effects of acyl ghelin and 5-HT2c receptor antagonist (SB242084) on food intake and GI motility in tumor-bearing rats were examined.
Results: Anorexia was induced after day 5 following tumor implantation. At day 7, food intake, body weight, fat, and skeletal muscle were significantly reduced in tumor-bearing rats, while plasma cytokine (IL-1-beta, IL-10, and IFN-gamma) levels were elevated. Plasma acyl ghrelin concentration was significantly higher in tumor-bearing rats than normal rats in freely fed state; however, acyl ghrelin was lower in 24-h food-deprived state. Neuropeptide Y (NPY) and agouti-related peptide (AgRP) mRNA expression in the hypothalamus were upregulated in freely fed tumor-bearing rats. The fasted motor patterns in both the antrum and duodenum were disrupted and replaced by the fed-like pattern in tumor-bearing rats. Acyl ghelin (3 nmol) significantly increased the motor activity in the antrum of tumor-bearing rats, however failed to improve the phase III-like contractions of fasted motility and food intake. 5-HT2c receptor antagonist induced the fasted motor patterns and markedly reversed the decreased food intake in tumor-bearing rats. In addition, hypothalamic NPY mRNA expression was significantly increased by 5-HT2c receptor antagonist.
Conclusions: The present study demonstrated that 5-HT2c receptor plays an important role in tumor-induced anorexia and relieving gastrointestinal motility, and it may be a potential target for the treatment of cancer cachexia.

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Methotrexate-induced anorexia is not associated with the systemic inflammatory response but with a decrease in serum levels of alpha-MSH autoantibodies

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Background and aim: Methotrexate (MTX) chemotherapy is accompanied by intestinal inflammation and anorexia of an unknown mechanism. In this work, we studied if MTX-induced anorexia can be related to a systemic increase in interleukin 1β (IL-1β) or to altered production of autoantibodies (autoAbs) against α-melanocyte-stimulating hormone (α-MSH).
Methods: Sprague–Dawley rats received MTX (2.5 mg/kg s.c. for 3 days, D1–D3), while pair-fed (PF) and ad libitum-fed control rats received saline. PF rats were allowed ad libitum food intake from D9. Blood samples were taken at D1, D5, D19, and D33. Food intake and body weigh were monitored daily.
Results: In MTX rats, D5 was characterized by maximal suppression of food intake and body weight as well as by intestinal inflammation. PF rats showed identical with MTX group body weight loss till D9. Serum levels of IL-1β were not different among the groups at D1, but at D5, both MTX and PF groups showed a decrease in IL-1β vs. controls, which can be attributed to food restriction. At D19, levels of IL-1β recovered in both MTX and PF rats. At D5, levels of both total and free α-MSH IgG and IgM autoAbs were decreased in MTX but increased in PF rats. In contrast, at D19 levels of α-MSH, autoAbs were higher in MTX than in PF or control rats.
Conclusions: These data do not support involvement of systemic proinflammatory factors but suggest that a decrease of autoAbs against α-MSH, an anorexigenic hormone, can play a role in MTX-induced anorexia.

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Changes of affinity of autoantibodies against alpha-melanocyte-stimulating hormone are involved in the mechanisms of anorexia nervosa and bulimia

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Background and aim: Autoantibodies (autoAbs) against α-MSH have been identified in humans, and their serum levels were oppositely correlated with psychological traits in anorexia nervosa and bulimia. In this work, we studied if patients with anorexia and bulimia may display autoAbs with different affinity for α-MSH resulting in their opposite role to modulate α-MSH signaling.
Methods: Total IgG and IgM were isolated from sera of patients with anorexia nervosa restrictive type, bulimia, or healthy controls. Affinity of IgG and IgM autoAbs for α-MSH was measured using BIAcore, and the epitope responsible for IgG binding to α-MSH was determined. Effect of IgG autoAbs on binding of α-MSH-I125 to melanocortin MC4 receptors was studied in transfected cos-7 cells. The effect of α-MSH autoAbs on feeding and anxiety was studied in a rat model of passive transfer of affinity purified IgG α-MSH autoAbs from patients with anorexia or bulimia into the hypothalamus of rats.
Results: An increased affinity of IgG autoAbs for α-MSH was found in subjects with bulimia, while affinity of IgM autoAbs was lower in both anorexia nervosa and bulimia. AutoAbs of higher but not of lower affinity was able to block α-MSH-I125 binding on MC4 receptors. Central injections of high or low affinity α-MSH autoAbs in rats resulted in increased and decreased food intake and body weight, respectively, and increased anxiety in both groups.
Conclusions: These results provide the strong evidence that pathological changes in affinity of α-MSH autoAbs are involved in biological mechanisms of anorexia nervosa and bulimia.

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Leukemia inhibitory factor (LIF) inhibits food intake by directly activating proopiomelanocortin (POMC) neurons

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Background: Cachexia is linked to an increase in circulating inflammatory cytokines, including leukemia inhibitory factor (LIF). Central LIF administration recapitulates the cardinal features of cachexia, which closely resemble central melanocortin activation. We hypothesized that LIF inhibits feeding by directly activating proopiomelanocortin (POMC) neurons.
Methods: LIF was administered by bolus injection or chronic infusion through a lateral ventricle cannula to evaluate effects on food intake. Expression of LIF-R and activation of POMC neurons were evaluated using in situ hybridization and IHC. We measured LIF-induced alpha-MSH release from hypothalami by radioimmunoassay. Mice null for gp130, the signal transducing subunit of the LIF-R complex, in POMC neurons (PomcCre-gp130flox) were generated to confirm that LIF directly activates POMC neurons, and that this activation mediates LIF-induced anorexia.
Results: LIF-R is expressed by 20% of ARC POMC neurons, and LIF significantly increased cFos in POMC neurons compared to vehicle. LIF administration maintained a significant decrease in food intake over 3.5 days. Though control animals reduced food intake post-injection, PomcCre-gp130flox mice were resistant to LIF-induced anorexia. LIF increased MSH release ex vivo compared to a CSF in control hypothalami, but not PomcCre-gp130flox hypothalami.
Conclusions: LIF acts centrally to inhibit food intake by directly activating POMC neurons, thereby increasing melanocortin tone. This effect requires expression of functional LIF-R/gp130 complex in POMC neurons. Further, LIF induces anorexia without evidence of tachyphylaxis, closely resembling the clinical syndrome of cachexia. This research implicates the role of LIF in cachexia and supports the utility of melanocortin antagonism in its treatment.

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Key phosphatase in cancer-induced anorexia

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Background: Cancer anorexia–cachexia syndrome is observed in 80% of patients in the advanced stages of cancer and is a strong independent risk factor for mortality. Accumulating evidence indicates that the pathogenesis of cancer anorexia is mediated by persistent anorexigenic signals into the hypothalamus.

Objective: Here, we investigated the role of neuronal protein tyrosine phosphatase 1B (PTP1B) in the control of food intake and body weight during cancer-induced anorexia.
Methods: Western blot, ELISA, and protein tyrosine phosphatase activity assays were combined to evaluate the role of hypothalamic PTP1B during cancer-induced anorexia in rodents.
Results: A selective transient reduction of PTP1B expression in areas surrounding the third ventricle of hypothalamus drive by antisense oligonucleotide (ASO PTP1B 4.0 nmol) evoked anorexia, severe weight loss, and death in control rats (n=10). Interestingly, hypothalamic PTP1B expression was markedly reduced (~70%) in three different models of cancer-induced anorexia in rodents, contributing to anorexigenic signals mediated by STAT-3 (n=5). Furthermore, we found that classical pro-inflammatory mediators are able to suppress the neuronal PTP1B expression. Acute i.c.v. infusion of recombinant IL-1-beta (5 ng) or TNF-alpha (10^{-6} M) reduced hypothalamic PTP1B expression 45% and 40%, respectively, in control rats (n=5). Similar results were found 6 h after intraperitoneal LPS infusion (n=6). Conversely, i.c.v. infusion of anti-IL-1-beta antibody (50 ng) or specific TNF-alpha inhibitor, Inflixmab (0.6 μg) during 4 days, restored PTP1B activity, increased food intake 42% and 35%, respectively, body weight 32% and 30%, respectively, and survival in tumor-bearing animals (n=10). Whilst forced reduction in the PTP1B expression using i.c.v. infusion of ASOPTP1B blunted the effects of i.c.v. infusion of anti-IL-1-beta antibody or Inflixmab to increased food intake and survival in tumor-bearing rats (n=6).
Conclusion: Collectively, these data showed that hypothalamic PTP1B activity represents a core modulator of food intake and body weight, linking the central inflammation and the anorexigenic signals during cancer-induced anorexia.

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Resting and total energy expenditure in simple and stress starvation

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Protein-energy malnutrition (PEM) derives from the imbalance between the supply of nutrients and the body's demand to ensure vital functions. While in anorexia nervosa (AN) works, a physiological adaptive response defined "simple starvation" in cancer patients acts an inflammatory cascade inducing a metabolic stress ("stress starvation"): amino acids from the protein stores and fatty acids from the adipose tissue are converted into energy to satisfy the metabolic needs. Energy requirements not only depend on the aggressiveness of the disease and level of inactivity it causes but also on the treatment and the presence of prior malnutrition. Resting metabolic rate (RMR) has been measured in 16 young women with restrictive AN, 12 patients with cancer cachexia (CC), and 15 healthy subjects. RMR resulted not significantly different between healthy controls and CC (2,415±1,227 vs 2,475±1,987 kcal/die) patients, but significantly lower in restrictive AN (1,345±726 kcal/die; p=0.001). Physical activity level (PAL), evaluated with a questionnaire, resulted very poor in CC (corresponding to 601±512 kcal/die), due to fatigue and asthenia, but very dynamic in AN patients (1,340±712 kcal/die). Dietary induced thermogenesis (DIT) was comparable in all the three groups. Finally, TEE (total energy expenditure) results slightly low in CC patients than healthy controls. This observation should invite in CC patients to carefully consider the quality than the quantity of the given nutritional support.

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Differences between severe undernutrition in case of cachexia and anorexia nervosa

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Aims: We aim to study the distinctive features between severe malnutrition in case of cachexia and anorexia nervosa.
Methods: Twenty-two patients were divided into two groups. Group 1 (three men, nine women, aged from 27 up to 69 years) included patients with severe organical pathology, which led to development, undernutrition, and cachexia (cancer, stroke, malabsorption, and short bowel syndrome). The second group amounted to ten women aged from 14 up to 45 years, suffering by the anorexia nervosa, cited to development of severe undernutrition, and studied the clinical signs and body composition (bioelectrical impedance and DEXA).
Results: In patients from group 1, body mass index is 15.9–18.0, content of body fat is 4–15%, midarm circumference is 16–24 cm, and total protein of blood is 51–73 g/l. In the process of treatment, the mass of the body has increased only at 25% of ill, up to 1–2 kg. Lethality in this group is 33%. At patients with anorexia nervosa (group 2), body mass index is 9.1–16.5, content of body fat is 2–17%, midarm circumference is 12–22 cm, and total protein of blood is 48–66 g/l. During treatment, the mass of the body increased in all patients, on 4–11 kg. Lethality in this group is 0%.
Conclusions: Despite the fact that the anorexia nervosa results in development considerably more distinct undernu-

trition, it is distinguished by more favorable flow and practically full convertibility than in contrast to cachexia, developing on the background of organical pathology.

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The obesity paradox in cats with spontaneously occurring heart failure

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Background: Although cachexia is associated with impaired survival in heart failure, overweight or obese human and canine heart failure patients appear to have a survival advantage. It is not known if this relationship also exists in other species. The purpose of this study was to determine the effects of body condition and body weight changes in cats with spontaneously occurring heart failure due to cardiomyopathy.
Methods: All cats with congestive heart failure secondary to cardiomyopathies (e.g., hypertrophic (HCM), dilated (DCM), restrictive, and arrhythmogenic right ventricular (ARVC)) were eligible for the study. Medical records were reviewed, and data regarding initial body weight and body condition, subsequent changes in body weight, and treatment were collected. Survival times were determined for cats that were discharged from the hospital and that lived >1 day.
Results: One hundred one cats were enrolled in the study: HCM (n=74), DCM (n=5), ARVC (n=1), and other cardiomyopathies (n=21). Survival was significantly different between cats that gained, lost, or maintained body weight over the course of their disease, with cats that gained weight surviving the longest (P=0.003). There was a trend for initial body condition score to be associated with survival (P=0.051), but medications were not significantly associated with survival time.
Conclusions: These results suggest that changes in body weight are an important consideration in the survival of cats with heart failure, as in people and dogs. Further studies on mechanisms of body composition changes in cats with spontaneously occurring heart failure and potential treatments are warranted.

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Hormonal profile, appetite, and energy/protein homeostasis in response to fasting and re-feeding in hemodialysis patients and control subjects

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Background and aims: Malnutrition is thought to be prevalent in uremia due to either anorexia or heightened catabolism. We aimed to study the pathogenesis of anorexia and catabolism.
Methods: We examined the profile of peptides known to regulate energy homeostasis including leptin, NPY, alphaMSH, acylated ghrelin, PYY, and GLP-1 during baseline, 40 h of fasting and 20 h of re-feeding. Simultaneously, we assessed body composition with DEXA scan, recorded the visual analogue scale (VSA) for appetite assessment, and measured energy and protein intake and output in ten hemodialysis (HD) patients and eight controls (C).
Results: Endocrine assessment showed elevated plasma leptin, PYY, NPY, and reduced acylated ghrelin in HD patients. MSH and GLP-1 were not different from the controls. Fasting suppressed leptin and PYY in both groups; post-prandial rise of PYY and GLP-1 was prominent, and pre-prandial rise of ghrelin was less in HD patients. Kinetics showed that the elevation of leptin and PYY was due to increased secretion and not impaired degradation. Energy intake, expenditure, protein intake, output, and balance were similar in the two groups. The VAS and body fat and lean mass distribution were not different between the C and the HD groups.
Conclusion: In contrast to prevailing view, we found that well-dialyzed HD patients, in the absence of any concomitant illness, were neither anorexic nor catabolic. The endocrine finding of elevated PYY, leptin, and suppressed ghrelin is, however, consistent with an anorexigenic profile. Pathogenesis of these hormonal changes is unknown.

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Neural activation in anorexia lung cancer patients to food images. Functional magnetic resonance imaging study

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Background and aims: Anorexia is a major symptom in cancer patients, is the most common cause of malnutrition, and is associated with a poor response to treatment and may contribute to early death. The etiology of cancer anorexia is

not completely understood, is multifactorial, and involves most of the neural signaling pathways modulating energy intake. We examined cerebral responses to visual stimuli in anorexia lung cancer patients (LCP) to describe neural activations to food stimuli.

Methods: Functional neuroimaging data were collected from anorexia LCP, after four postprandial hours, using magnetic 3.0-T resonance imaging (fMRI) with pleasant and unpleasant visual food images. Thirty slices were positioned in axial orientation with field of view 240×240 mm^2, matrix 64×64, slice thickness 5.0 mm, GAP 0 mm, echo time 40 ms, repetition time 3,000 ms, and flip angle of 90°. Data were analyzed with Brain Wave software AW1.5 on a voxelwise basis using multiple regression generating a *t* test map.

Results: Preliminary results of six anorectic LCP showed significant activation in bilateral visual occipital cortex, regardless of the pleasant or unpleasant stimuli. Anorexia LCP displayed no activation of neural regions in hungry process associated with an increased desire to eat, including prefrontal cortex and amygdala, consistent with previous study with a sample of satiety or even obese subjects, the weak activation with food stimuli point to decreased food-related somatosensory processing stimuli in anorexia LCP.

Conclusions: Preliminary findings support a development model of cancer anorexia-related changes in cerebral functioning develop from lower order sensory processing in anorexia CLP.

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Taste disorders in oncology patients under chemotherapy

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Background and aims: Smell or taste dysfunction can have a significant role in the anorexia development of oncologist patients and can adversely affect food choice and weight loss. We evaluated self-perceived taste function to ascertain whether differences in threshold taste perception exist in patients and control subjects and were associated with global subjective evaluation for malnutrition and nutrients ingestion.

Methods: We enrolled 60 subjects, 30 patients with cancer after the second chemotherapy cycle and 30 control subjects without cancer. Dietary nutrients consumption was obtained with a validate food frequency questionnaire. Three concentrations of three basic flavors: sucrose (sweet), urea (bitter), and umami (glutamate) were dissolved in distilled water to ascertain perception threshold (PT) and recognition threshold (RT).

Results: Cancer patients presented higher PT to sweet flavor (6.4 vs 4.4 mmol/ml, $p=0.03$) and higher RT (100 vs 95 mmol/ml, $p=0.04$) to bitter flavor than the controls. Cancer patients with sweet PT higher than the median showed significant lower ingestion of calories per day (2,043 vs 1,586 kcal, $p=0.02$), proteins (81.4 vs 54 g/day, $p=0.01$), carbohydrates (246 vs 192 g/day, $p=0.05$), and zinc (19 vs 11 mg/day, $p=0.01$). There was no association with PT or RT in any of the three basic flavors and nutrient ingestion in the control group.

Conclusions: Changes of sweet PT and bitter RT were found in patients under chemotherapy which were associated with lower ingestion of nutrients and total energy consumption. Taste alterations could be an important factor in malnutrition in cancer patients under chemotherapy.

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Early weight loss is not caused by dysphagia in patients with non-small lung cancer treated with concurrent

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Background: Concurrent chemoradiotherapy (chemo-RT) in locally advanced non-small cell lung cancer (NSCLC) is associated with increased acute oesophageal toxicity and dysphagia.

Aim: The aim of this study is to investigate body weight changes during concurrent chemo-RT in relation to the onset of dysphagia.

Methods: Fifty-one patients with NSCLC treated with chemo-RT were included. Body weight was measured at diagnosis, at start, and at week 1, 2, and 3 of chemo-RT. Toxicity was scored according to the CTCAE system. Associations between body weight loss and grade of dysphagia were analyzed in a linear mixed regression model. Results are expressed as mean±standard deviation.

Results: Between diagnosis and start of chemo-RT, patients lost 0.34±3.4% of body weight ($p=0.57$). During concurrent chemo-RT, patients lost 1.4±2.2% (p 1 at start of chemo-RT, and only 7%, 19%, and 31% of patients had grade >1 dysphagia at week 1, 2, and 3, respectively.

Regression analysis showed no association between body weight loss and grade of dysphagia.
Conclusions: In this study, we show that body weight loss already occurs during the first 3 weeks of concurrent chemo-RT and is not associated with dysphagia. Further research is ongoing to elucidate the mechanisms underlying this observation. Understanding the mechanisms of weight loss would allow for intervention strategies.

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Temporal changes in symptoms, but not in energy intake, among patients with advanced pancreatic cancer

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Aim: Cancer cachexia and weight loss are major contributors to morbidity in advanced pancreatic cancer (PC). Weight loss can be considered as a disturbance of energy balance, but little is known about the importance of symptoms for changes in energy intake (EI). Study objectives were to prospectively assess EI, weight, and symptoms during the disease course and investigate associations between symptoms and EI.
Methods: From 2006 to 2008, 39 patients (females, 18/males, 21) with advanced PC were included and monitored every fourth week until death. EI was measured by the 24-h recall method. The Edmonton symptom assessment scale (ESAS) was used to assess symptoms.
Results: The patients had a median age of 62 years (48, 88), median ECOG status 1, and median survival of 5 months. Seventeen patients (44%) had locally unresectable cancer, 16 (41%) metastatic, and six (15%) recurrent disease. Upon inclusion, 28 (72%) patients reported weight loss ≥10%, and the median daily EI was 7.1 MJ (1.3, 14.8). Negative correlations (Spearman's rho, $p<0.05$) were found between EI and pain (−0.44), fatigue (−0.47), dry mouth (−0.37), depression (−0.35), and wellbeing (−0.41), but not between EI and appetite loss (−0.28, $p>0.05$). After 1 month ($n=28$), median daily EI was 7.6 MJ (0.3, 16.2), and general wellbeing was the only symptom significantly associated with EI (−0.44, $p<0.05$).
Conclusion: Several symptoms seem to contribute to reduce EI at inclusion. Careful monitoring and follow-up of symptoms might therefore be an important intervention to reduce weight loss in advanced PC.

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Influence of poor nutritional status on clinical outcome of elderly women with primary or recurrent ovarian cancer: results of a prospective study

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Background: Global malnutrition is widespread among patients with ovarian cancer. The purpose of this study was to investigate the influence of the nutritional status on the clinical outcome of patients with primary or recurrent ovarian cancer with respect to their age, as age and tumour-free surgery are independent prognostic parameters for overall survival.
Methods: In a 2-year-period (January 2007 to December 2008), 152 consecutive patients were analysed. Seventy-nine (52%) of them had primary and 73 (48%) recurrent disease. At the time of admission for surgical therapy, the body composition was analysed with bioelectrical impedance analysis (BIA) using phase angle alpha and ratio of extra-cellular mass and body cell mass (ECM/BCM). Serum protein parameters were registered. The risk of malnutrition was estimated by nutritional risk score (NRS) and nutritional risk index (NRI). Nutritional risk was defined as NRS ≥3 or NRI ≤100. During operation, a standardised and validated tumour documentation tool (IMO) was performed. Clinical outcome parameters such as length of hospital stay or postoperative complications were documented with regard to the age (</>65 years).
Results: The median age of patients was 56 years (range, 48–66), with median BMI, 24.4 kg/m^2 (range, 21.8–27.3). Almost one third were older than 65 years ($n=43$/29%). The ≥65-year group had a significant higher rate of nutritional risk classified by NRS (14/43 vs. 16/109, $p<0.05$) and NRI (99.6 vs. 105.7, $p<0.001$), whereas the BMI (25.4 vs. 24.1, $p<0.05$) was significantly increased. These patients (≥65 years) had significantly higher BIA-ECM/BCM ratio (1.3 vs. 1.1, $p<0.001$), they needed significantly more often blood transfusions during or after surgery (3 vs. 0.5, $p<0.001$), the length of stay was significantly higher (18 vs. 14 days, $p<0.005$) and the phase angle α was significantly lower (4.5° vs. 5.2°, $p<0.001$) as well as serum albumin (4.0 vs. 4.3, $p<0.005$). There were no differences in weight loss, food intake, post-operative complications and complete resection rate between the ≥65 and <65 group.

Conclusions: Elderly patients with ovarian cancer have an increased risk of malnutrition before oncologic surgery, evaluated by NRS, NRI and BIA, phase angle α and ECM/BCM ratio, although operative and postoperative complications are not increased. Elderly patients with nutritional risk factors should be identified for an individualised nutritive care before surgery and may thereby have an improved outcome.

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Advanced cancer patients and home parenteral nutrition

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Under-nutrition and cachexia occur frequently in cancer patients and are, per se, responsible for excess morbidity and mortality. Since January 2008 to August 2009, all cancer outpatients consecutively referred to our ward, no more eligible for oncologic therapy, have been evaluated. Demographic, anthropometric and oncologic (site of primary tumour, weight loss in the last 6 months, presence of systemic symptoms and Karnofsky performance status (KPS)) data were collected. Patients have been divided according to the previous body weight loss and the class of KPS (56 have KPS $\leq$50 and 44 KPS $\geq$60). Patients were 100 (48 male, 42 female; median, 61 years (38–79)), BMI was 19.05 kg/m^2 (13.6–27.5), weight loss was 18.6% (range, 6–47%; 26 have lost <10% and 74 $\geq$10%) and primary cancer localization was 32 stomach, 24 colon, 12 ovary, 11 esophagus, 11 pancreas, 6 lung, 2 gallbladder and 2 uterus. All patients complained anorexia and fatigue, in 18 cases associated with dysphagia, in 37 nausea/vomiting, in 12 pain and in 5 mild ascites. Median survival was 4 months (2–10): 68 patients survived <4 months and 32 $\geq$4 months. When patients were divided according to the primary cancer localization and the previous weight loss, prognosis resulted not different in the different groups; when divided according to KPS, survival resulted significantly different (3.7$\pm$1.7 months when KPS $\leq$50 and 5.4$\pm$1.4 months in KPS $\geq$60; p=0.0001). Weight loss and anorexia are frequent in an unselected series of cancer terminal outpatients. Karnofsky results related with survival being a prognostic indicator for terminal cancer patients on HPN. This study is part of an ongoing international prospective investigation on the predictability of survival in incurable cancer patients on HPN. The study is sponsored by the European Society of Clinical Nutrition and Metabolism (ESPEN) and is still open to the clinical investigators who are involved in this field.

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Translation initiation of protein synthesis and myosin transcription in human skeletal muscle tissue in response to overnight total parenteral nutrition (TPN) before operation

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Aim: In recent years, it has been a great increase in knowledge of translational control of protein synthesis after feeding or provision of amino acids, but such information in patients is based on labelled tracer kinetics mainly, which is based upon pool uncertainties. Our aim of the present study was therefore to determine if overnight provision of a "standard" total parenteral nutrition (TPN) regimen was enough to activate translation initiation factors for protein synthesis and transcription of myofibrillar proteins in patients scheduled for surgery.
Methods: Patients who underwent surgery in the upper gastrointestinal tract were included in the study and received overnight (12 h) infusions of either saline or TPN as all in one (30 cal/kg/day, 0.16 gN/kg/day) prior to surgery. Infusions lasted until muscle biopsies were taken from the rectus abdominus muscle directly after anaesthesia induction. Initiation factor complexes in the muscle tissue were analysed with immunoprecipitation of proteins followed by Western blot. mRNA expressions of myosin heavy chain 2A (MHC 2A) and actin were measured by real-time PCR.
Results: Standard overnight TPN infusion increased formation of eIF4G·eIF4E complexes and reduced the amount of 4EBP1 found in complex with eIF4E, indicating increased protein synthesis compared to patients who received saline infusions (p<0.05). TPN infusion also increased phosphorylation of 4E-binding protein 1 and p70s6 kinase (p<0.05). MHC 2A mRNA expression was significantly altered by TPN treatment (p<0.05), while similar alterations in actin expression did not reach significant levels in the same tissue specimens.
Conclusions: Provision of standard total parenteral nutrition for 12 h activates initiation factors for translation of skeletal muscle proteins, which was translated into altered tissue content of myosin transcripts, indicating stimulation of synthesis of myofibrillar proteins.

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Reduction of low grade inflammation restores blunting of postprandial muscle anabolism and limits sarcopenia in old rats

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Background and aim: Sarcopenia may result from a defect in muscle protein synthesis stimulation and proteolysis inhibition after food intake. With aging, muscle is less sensitive to the stimulatory effects of amino acids. Aging is also characterized by the development of a low-grade inflammation (LGI) with increased plasma levels of cytokines and acute phase proteins (fibrinogen and alpha2-macroglobulin (alpha2M)). Whether or not blunting LGI may restore the stimulation of post-prandial (PP) muscle protein synthesis during aging has been assessed.
Methods: Eighteen- to 20-month-old rats were divided into a control group (C) and a group treated with a non-steroidal anti-inflammatory drug (ibuprofen (IBU), 30 mg/kg/day). After 5 months of treatment, rats were studied at the post-absorptive (PA) or PP state; muscle proteolysis and protein synthesis were assessed. Signaling pathways (mTOR/S6K1/S6/4EBP1) and FoxO3a were measured.
Results: Body weight, fibrinogen, and alpha2M levels were similar in both groups at the beginning of the experiment. Five months of treatment decreased significantly fibrinogen, alpha2M, and IL6 levels in IBU vs. C. Muscles were significantly heavier in IBU vs. C rats (+6%). As expected, food intake had no effect on muscle protein synthesis and proteolysis in C rats, whereas ibuprofen restored the stimulation of muscle protein synthesis (+25%) and the inhibition of proteolysis (−16%). IBU treatment did not affect the mTOR signaling pathway, whereas it restored muscle FoxO3a phosphorylation.
Conclusion: Prevention of LGI development during aging maintained muscle protein anabolism by food intake and slowed down muscle atrophy in elderly.

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Impaired muscle recovery after immobilization-induced atrophy in old rats is not due to a lack of normalization of the ubiquitin-proteasome-dependant proteolysis or the mitochondria-associated apoptotic pathway

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Background and aim: Sarcopenia might be partially explained by an impaired recovery of skeletal muscle mass after a catabolic state. Associated with this, periods of immobilization increase with age (bed-rest and convalescence) and may contribute by themselves to muscle atrophy during aging. An imbalance of muscle protein metabolism, apoptosis, and cellular regeneration/differentiation is certainly involved. This study aimed to explore the mechanisms involved in muscle atrophy during immobilization and subsequent recovery in old rats.
Methods: Forty-eight-old rats were subjected to unilateral hind limb casting immobilization for 8 days (I8) and allowed to recover for 10 to 40 days (R10–R40; $n=8$ per time). Muscle atrophy, apoptotic (caspase-3 and -9, apoptosome) and proteasome-dependent proteolytic activities, and regeneration/differentiation processes (myogenic factor, Myf5) were assessed in the same rat on both non-immobilized (C) and immobilized (I) gastrocnemius muscles.
Results: At I8, a significant muscle atrophy (−21%, $P<0.05$) occurred in the gastrocnemius muscle, and recovery was absent even at R40. In parallel, we observed at I8 (1) an increase of caspases-3 and -9, apoptosome, proteasome chymotrypsin-like activities (+20% to +80%, $P<0.05$), and amount of apoptotic nuclei; (2) a decrease amount in Myf5 (−50%, $P<0.05$) in I compared to C. These proteolytic and apoptotic activities were rapidly normalized (R10).
Conclusion: Our results showed that a short immobilization period during aging initiated muscle atrophy that was not recovered after 40 days and may then contribute to initiate sarcopenia. The lack of recovery is not due to a defect in proteolysis or apoptosis downregulation during the recovery period and remains to be explored.

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Exercise induced time-dependent transcription in human skeletal muscle

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An extensive long-term, longitudinal assessment of muscle functional and genome-wide transcriptional changes during heavy resistance training in human volunteers has not yet been described. We investigated muscle mass (DEXA) and isometric strength increases and transcriptional changes (Affymetrix Array) in muscle at several time points over the course of a 70-day resistance training program (supervised exercise, three times/week, at 80% of one repetition

maximum). The data show a linear relationship between changes in muscle mass and strength gains with training ($r>|0.6|$, $p<0.01$). There was a rapid and profound change in the muscle transcriptome within 2 days of the first bout of exercise, but many of these changes waned after 35 days of training. The changes clustered into several patterns that range from those genes that changed early, but then returned rapidly to baseline expression levels, to those that changed only after several bouts of exercise and continued to show a change whilst the training program progressed. We have seen that each individual pattern appears to activate different metabolic programs. Thus, the data suggest that muscle undergoes discreet sequential transcriptional changes in response to exercise to affect a series of sequential metabolic changes that ultimately result in the training adaptations observed.

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Muscle quality is associated with longer and less fragmented sedentary time in healthy older men

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Background and aims: Physical activity interventions for sarcopenia promote a decrease in sedentary time and an increase in energy expenditure. However, recent work has shown that programmed physical activity is accompanied by a spontaneous increase in sedentary time both before and after physical activity; muscle quality is improved while maintaining a constant energy cost of daily living. We hypothesized that muscle quality in healthy older people would be associated with increased sedentary behaviour.
Methods: We studied 26 healthy volunteers (14 men, mean age, 79.3 (3.4)years). Spontaneous physical activity was directly monitored (7 days) using the ActivPAL™ accelerometry-based system, which continuously registers time sitting/lying, time standing, time walking, number of steps taken and step cadence. Sedentary behaviour, i.e. total and pattern of sedentary time, was measured with pattern (fragmentation) defined as the number of sedentary bouts/total sedentary time. Body composition was measured using DEXA (Hologic Discovery) and lower limb extensor power (LEP) using the Nottingham Power Rig. Muscle quality (MQ) was defined as LEP/lower limb lean mass (Wkg^{-1}).
Results: MQ was 13.11 (4.90)Wkg^{-1}. Sedentary time was 17.26 (1.79)$h\,day^{-1}$. In men (but not women), muscle quality was significantly associated with not a longer ($r=0.608$, $p=0.021$) and less fragmented ($r=-0.620$, $p=0.018$) pattern of sedentary time, in between longer bouts of activity ($r=0.555$, $p=0.039$).
Conclusions: In men, an increase in sedentary time and a less fragmented activity pattern may be a consequence of maintaining optimum muscle quality in older age. Sex differences in sedentary activity behaviour need to be resolved further.

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Are thigh adipose depots protective for risk of death in older persons with lower lean mass? The Age, Gene/Environment Susceptibility-Reykjavik Study (AGES-Reykjavik)

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Background: Lower lean mass is thought to increase risk of poor outcomes in old age. Cachexia is associated with loss of both lean and adipose. Using computerized tomography (CT) data of the thigh from a large population-based study, we explored whether adipose depots imaged in the thigh might be protective for death risk in persons with lower lean.
Methods: We used mid-thigh CT scan data from 687 men (215 deaths) and 932 women (170 deaths) aged 79±5.5 in the AGES-Reykjavik follow-up (2002–2004) of the randomly chosen Reykjavik Study. Only persons in the lowest tertile of thigh lean cross-sectional area (men <118 cm^2, women <85 cm^2) were included. Logistic models were used to assess risk for thigh subcutaneous fat, intermuscular fat, or fatty infiltration into muscle from Hounsfield Unit (HU). Final models were adjusted for age, BMI, height, smoking, functional status, C-reactive protein, lean CSA, and leg strength.
Results: None of the adipose depots was protective of risk of death. In the men, greater fatty infiltration into muscle increased risk of death ($P=0.04$ men, $P=0.69$ women). In women, smaller lean CSA increased risk of death ($P=0.02$ women), but lean CSA was not associated with risk in men, adjusting for other confounders ($P=0.44$ men).
Conclusions: Within this large population of men and women with lower lean mass, no adipose depot as assessed

from CT improved risk of short-term survival. Increased fatty infiltration into muscle as assessed by HU increased risk of death in men only.

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Sarcopenia is predictive of convalescent care in elderly cancer patients following resection of colorectal cancer

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Background and aims: Skeletal muscle wasting is defining characteristic of cancer cachexia and has been associated with poor physical function. We tested if muscle depletion (sarcopenia) predicts convalescent care requirement after primary colorectal cancer (CRC) resection.

Methods: Stage 2–4 CRC patients (n=237) were studied; surgery dates were identified using the Alberta Cancer Registry. A computed tomography (CT) image taken before surgery was used to compute total muscle surface area (square centimeter) at the third lumbar vertebra (L3). Sarcopenia was defined using reported L3 muscle index (L3 muscle (square centimeter)/height (square centimeter)) cut points (female, 38.5 cm^2/m^2; male, 52.4cm^2/m^2; Prado et al., Lancet Oncology 2008). Inpatient hospitalization data from the provincial ministry of health was searched for ICD-10 diagnostic codes suggesting inpatient convalescent care following surgery.

Results: Overall, 39.2% of patients were sarcopenic, and 9.3% of the patients in the entire population received convalescent care. The mean length of stay in convalescent care was 14.4±10.5 (SD)days. Of patients, 47.7% were ≥65 years, and 93% of all patients needing convalescent care were in this age group. In patients ≥65 years, 49.5% of patients were sarcopenic, and the risk of convalescent care after surgery was significantly higher in individuals with sarcopenia compared to those without (25.0% vs. 10.5%, p=0.0438, χ^2 test).

Conclusions: Sarcopenia is common before CRC primary tumor resection surgery. Convalescent care largely occurred in patients ≥65 years, and sarcopenia is a risk factor for this outcome, suggesting functional impairments. This is potentially associated with higher costs and burden on the healthcare system.