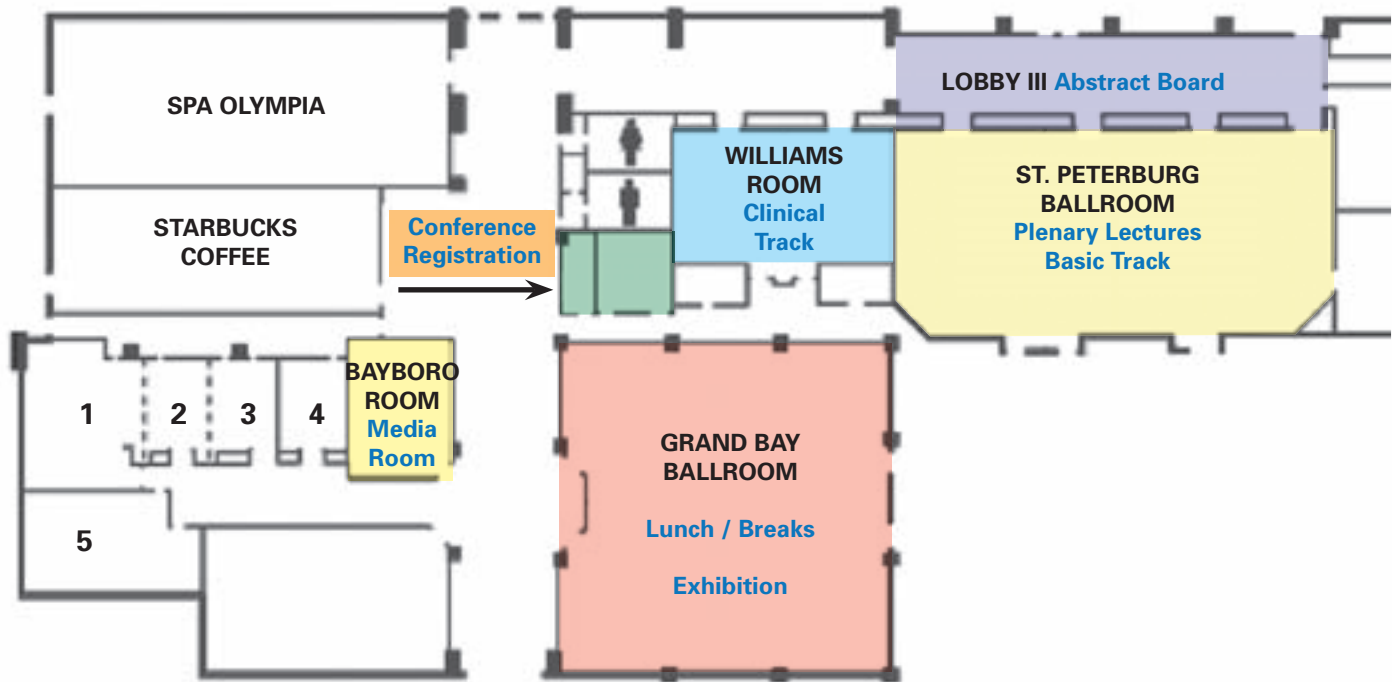


4th CACHEXIA
C O N F E R E N C E
Tampa 6 - 9 December 2007

**FINAL PROGRAM AND
ABSTRACT BOOK**

www.cachexia.org

CONFERENCE VENUE



- 1 Harbor View
- 2 Pier Room
- 3 Skyway Room
- 4 Board Room
- 5 Pinellas Room

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Faculty 95

Chairmen

- John E. Morley, St. Louis, MO, USA
- William J. Evans, Little Rock, AR, USA
- Stefan D. Anker, Berlin, Germany

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Opening Hours of the On-site Registration Desk

Thursday, December 6, 2007	14:30-18:30 hrs
Friday, December 7, 2007	8:00-18:00 hrs
Saturday, December 8, 2007	8:00-18:00 hrs
Sunday, December 9, 2007	8:00-11:30 hrs

Technical Equipment

The following technical equipment will be available in the lecture hall:

- Laptop
- Beamer/LCD Projector
- Laser Pointer
- Wireless Lavalier microphone

The preferred presentation form is Power Point, Version 97 or higher.

The following data files are accepted for presentation:

- CD
- USB drive

To prevent technical difficulties speakers are requested to bring slides to the media room to be uploaded to presentation computers. Slides should be handed in to the technical staff the day prior to the respective lecture.

Media Room

The Media Room is located in the Bayboro Room.

Official Social Program

Anti-cachexia dinner - beach party
 Saturday, December 8, 2007
 20:00 hrs

Trade Winds Island Resort
 5500 Gulf Boulevard
 St. Pete Beach, Florida 33706

Breaks

Refreshments will be available during the breaks in the Grand Bay Ballroom.

Registration Fees

Medical professionals (physicians, scientists, industry)	US\$600
Nurses	US\$350
Abstract presenters	US\$300
Medical or PhD students (written confirmation of status required)	US\$200

A 16:30 – 18:30

ST. PETERSBURG ROOM

OPENING SESSION

Chaired by: William Evans, Little Rock / USA
Giovanni Mantovani, Cagliari / IT

Welcome: John Morley, Stefan Anker, William Evans

1. **Cachexia: prevalence and impact in medicine**
Ken Fearon, Edinburgh / UK
2. **Definition and diagnosis of cachexia / wasting disease**
John Morley, St. Louis / USA
3. **Similarities and differences of wasting diseases**
Stefan Anker, Berlin / GER
4. **The cost of cachexia**
Wayne Levy, Seattle / USA

B 08:30 – 10:00

ST. PETERSBURG ROOM

PLENARY SESSION: Diagnostic criteria of cachexia / wasting disease and their assessment

Chaired by: John Morley, St. Louis / USA
Stefan Anker, Berlin / GER

5. **Anorexia**
Ian Chapman, Adelaide / AUS
6. **Decreased muscle strength and fatigue**
Florian Strasser, St. Gallen / SUI
7. **Assessment of body composition**
Annemie Schols, Maastricht / NL
8. **Anemia**
Gerasimos Filippatos, Athens / GR
9. **Biochemical alterations: inflammation, low albumin**
Kamyar Kalantar-Zadeh, Torrance / USA

C 10:30 – 12:00

WILLIAMS ROOM

CLINICAL TRACK: Cachexia trials

Chaired by: Aminah Jatoi, Rochester / USA
William Mitch, Houston / USA

10. **Ghrelin and ghrelin analogs for wasting disease in CHF and COPD**
Piotr Ponikowski, Wroclaw / PL
 11. **Update on trials in HIV/AIDS-wasting**
Morris Schambelan, San Francisco / USA
 12. **Growth hormone treatment in severe kidney disease**
Viatcheslav Rakov, Zurich / SUI
- Updates on clinical trials – round table discussion**
William Evans, John Morley, Stefan Anker

D 10:30 – 12:00

ST. PETERSBURG ROOM

BASIC TRACK: Ubiquitin-proteasome system

Chaired by: Alfred Goldberg, Boston / USA
HQ Han, Thousand Oaks / USA

13. **Skeletal muscle hypertrophy and atrophy pathways: overview**
David Glass, Cambridge / USA
14. **Myostatin and ubiquitin**
Ravi Kambadur, Singapore
15. **Identification of polyubiquitinated substrates of the skeletal muscle proteasome**
Didier Attaix, Clermond-Ferrand / FR
16. **Thinking outside the box: the example of rho- and rac-kinase inhibitors**
Stephan von Haehling, Berlin / GER
17. **Short plenary poster presentation**
Milan Holecek, Hradec Kralove / CZ

12:00-14:00

Lunch

12:15 – 13:45

LOBBY III

Poster Sessions**PO 1. Cachexia mechanisms 1 (posters 1.01 - 1.20)**

Chair: Didier Attaix, Alfred Goldberg, Daniel Marks

PO 2. Cachexia in cancer (posters 2.21 – 2.39)

Chair: Vickie Baracos, Giovanni Mantovani

PO 3. Methodology studies (posters 3.40 – 3.48)

Chair: Luigi Ferrucci, Florian Strasser

E 14:00 – 15:30 WILLIAMS ROOM F 14:00 – 15:30 ST. PETERSBURG ROOM

CLINICAL TRACK: Cancer cachexiaChaired by: K. Kalantar-Zadeh, Torrance / USA
Marc Hellerstein, Berkeley / USA**18. The clinical problem: epidemiology & pathophysiology**

Aminah Jatoi, Rochester / USA

19. The effects of cancer on skeletal musculature

Vickie Baracos, Edmonton / CAN

20. Causes of death and cardiac problems

Mathias Rauchhaus, Berlin / GER

21. Treatment on cancer cachexia: clinical trials update

Glenn Lesser, Winston-Salem / USA

22. Short plenary poster presentation

Jochen Springer, Berlin / GER

BASIC TRACK: Muscle metabolismChaired by: Herbert Lochs, Berlin / GER
Annemie Schols, Maastricht / NL**23. Is it possible to separate the metabolic effects of cachexia from inactivity?**

William Evans, Little Rock / USA

24. Myostatin

HQ Han, Thousand Oaks / USA

25. Biomarkers for muscle wasting from tissue

William Mitch, Houston / USA

26. Biomarkers for muscle wasting from blood

Stephan von Haehling, Berlin / GER

G 16:00 – 17:40 ST. PETERSBURG ROOM

PLENARY SESSION: Pathophysiology of tissue wastingChaired by: Luigi Ferrucci, Baltimore / USA
Fillippo Rossi Fanelli, Rome / IT**27. "Prometheus" basic science in cachexia lecture: past, present and future trends in cancer cachexia research**

Giovanni Mantovani, Cagliari / IT

28. Muscle events contributing to and limiting muscle wasting

Alfred Goldberg, Boston / USA

29. Stem cells and muscle

Nadia Rosenthal, Rome / IT

30. Of bears, frogs, meat, mice and men: insights into muscle loss and fat deposition

Miranda Grounds, Perth / AUS

17:45 – 19:15

LOBBY III

GENERAL POSTER DISCUSSION

H 08:30 – 10:00

ST. PETERSBURG ROOM

PLENARY SESSION: Wasting disease in chronic illness – common pathways

Chaired by: David Thomas, St. Louis / USA
 Connie Bales, Durham / USA

- 31. Chronic kidney disease**
Joel Kopple, Los Angeles / USA
- 32. Role of intestinal functioning wasting disease**
Herbert Lochs, Berlin / GER
- 33. Chronic heart failure**
Piotr Ponikowski, Wroclaw / PL
- 34. Chronic obstructive pulmonary disease**
Matthias John, Prerow / GER

I 10:30 – 12:00

ST. PETERSBURG ROOM

K

10:30 – 12:00

WILLIAMS ROOM

BASIC TRACK: New treatment approaches

Chaired by: Robert Wolfe, Little Rock / USA
 Daniel Marks, Portland / USA

- 35. ACE inhibitors**
Marco Pahor, Gainesville / USA
- 36. Nitric oxide: friend or foe in cachexia**
Thomas Thum, Würzburg / GER
- 37. Ghrelin and ghrelin analogs**
Rakesh Datta, Boston / USA
- 38. Immune modulation**
Anthony Cerami, Ossining, USA

CLINICAL TRACK: Sarcopenia

Chaired by: John Morley, St. Louis / USA
 Jochen Springer, Berlin / GER

- 39. Epidemiology**
Richard Baumgartner, Louisville/ USA
- 40. General pathophysiology**
Luigi Ferrucci, Baltimore/ USA
- 41. Cell death regulation and ageing**
Volker Adams, Leipzig / GER
- 42. Clinical insights**
Cornel Sieber, Nuremberg / GER
- 43. Short plenary poster presentation**
Maurits Vandewoude, Antwerpen / BE

12:00-14:00

Lunch

12:15 – 13:45

LOBBY III

Poster Sessions

- PO 4. Intervention studies (posters 4.49 – 4.71)**
Chair: Kenneth Fearon, David Glass, William Mitch
- PO 5. Human cachexia – various (posters 5.72 – 5.87)**
Chair: Morris Schambelan, Annemie Schols
- PO 6. Cachexia mechanisms 2 (posters 6.88 – 6.97)**
Chair: Josep Argiles, Robert Wolfe

L 14:00 – 15:30	WILLIAMS ROOM	M 14:00 – 15:30	ST. PETERSBURG ROOM
CLINICAL TRACK: Nutraceuticals and orexigenics Chaired by: William Evans, Little Rock / USA William Mitch, Houston / USA		BASIC TRACK: Growth factors Chaired by: HQ Han, Thousand Oaks / USA Joel Kopple, Los Angeles / USA	
44. Feeding and wasting disease: the role of nutraceuticals Connie Bales, Durham / USA		48. SARMs Shalender Bhasin, Los Angeles / USA	
45. Eicosopentanoic acid, BCAA and carnitine Fillippo Rossi Fanelli, Rome / IT		49. Mechanogrowth factor/ IGF-I Geoff Goldspink, London / UK	
46. Orexigenics David Thomas, St. Louis/ USA		50. Ghrelin Akio Inui, Kagoshima / JP	
47. Regulation of feeding Daniel Marks, Portland / USA		51. Measuring the response to anabolic therapies by use of kinetic biomarkers Marc Hellerstein, Berkeley / USA	

N 16:00 – 18:00	ST. PETERSBURG ROOM		
PLENARY SESSION: Muscle			
Chaired by: Nadia Rosenthal, Rome / IT Michael Schuster, New York / USA			
52. "Hippocrates" clinical research in cachexia lecture: the metabolic basis of muscle loss Robert Wolfe, Little Rock / USA			
53. Muscle wasting in cancer and ageing: cachexia versus sarcopenia Josep Argiles, Barcelona / SP			
54. Mitochondria and muscle Christiaan Leeuwenburgh, Gainesville / USA			
55. Muscle and fat tissue interaction Liat Mintz, East Brunswick / USA			

From 20:00 hrs

Anti-cachexia dinner – beach party

O 08:30 – 10:30

ST. PETERSBURG ROOM

PLENARY SESSION: Debates

Chaired by: Stefan Anker, Berlin / GER
John Morley, St. Louis / USA

DEBATE 1: Are cytokines always involved in the pathogenesis of cachexia?**56. PRO**

Maurizio Muscaritoli, Rome / IT

57. CON

Michael Schuster, New York / USA

DEBATE 2: What needs to be preserved in acute cachexia – fat or muscle tissue?**58. FAT**

Wolfram Doehner, Berlin / GER

59. MUSCLE

William Evans, Little Rock / USA

DEBATE 3: Is nutritional support essential for anabolics to work in cachexia?**60. PRO**

Arny Ferrando, Little Rock / USA

61. CON

Khursheed N. Jeejeebhoy, Ontario / CAN

P 11:00 – 12:30

ST. PETERSBURG ROOM

PLENARY SESSION: Meeting highlights and closing

Chaired by: John Morley, St. Louis / USA
Josep Argiles, Barcelona / SP

62. Meeting highlights: basic science

Josep Argiles, Barcelona / SP

63. Meeting highlights: pathophysiology

William Evans, Little Rock / USA

64. Meeting highlights: treatment studies

Stefan Anker, Berlin / GER

Awards ceremony:**Young investigator award****Poster award**

A1

Cachexia: prevalence and impact in medicine**Kenneth CH Fearon**

University of Edinburgh, School of Clinical and Surgical Sciences (Surgery), Edinburgh, UK

Cachexia is a debilitating and life-threatening syndrome whereby patients lose weight, muscle and fat and have an impaired response to nutritional support. It significantly impairs quality of life and response to treatment, increasing the morbidity and mortality of chronic diseases such as cancer, COPD, renal failure, and heart failure. To date there has not been a consensus on the definition of cachexia and therefore it is difficult to be precise about its prevalence. Moreover, cachexia is a spectrum and any estimate of prevalence will vary according to the severity of cachexia selected.

Given these caveats it is important to recognise the enormous scale of unmet medical need in relation to cachexia. Approximately one quarter of all deaths in Western Society are due to cancer and the majority of patients with advanced solid epithelial malignancy (except breast) will develop weight loss. Approximately 10-20% of cancer deaths are thought to be related directly or indirectly to the presence of cachexia. Coronary heart disease is the commonest cause of death in developed countries and is the commonest cause of chronic heart failure (CHF). CHF has a prevalence of approximately 3%, with 50% dead at 3 years following diagnosis. The prevalence of cachexia in CHF varies between 15-35% according to the definition of cachexia. In patients with COPD, weight loss and particularly loss of fat free mass has long been recognised to be associated with increased mortality independent of disease severity. COPD is estimated to become the third most common cause of death by 2020. In moderate or severe COPD the prevalence of cachexia is approximately 20%. Finally, end-stage renal failure (ESRD) is present in about 1.5% of the population and is rising at 3% per year. Estimates of cachexia vary considerably (approx 10-20%) and is thought to contribute to death in about 5% of cases.

With regard to quality of life, the impact of cachexia is most easily detected in domains where there is a direct influence from nutritional status (e.g. physical activity) and is less easily detected in global domains. Whereas weight-loss alone may not define patients with reduced functional aspects of self-reported quality of life, a more complete characterisation of the cachectic patient (including reduced food intake and the presence of systemic inflammation) can identify not only patients with reduced subjective and objective physical function but also those patients with adverse prognosis¹.

Perhaps one of the most dramatic measures of the impact of cachexia on patients is provided by documenting free-living physical activity level (using stable-isotope methodology or with simple activity meters). Recent evidence suggests that aspects of physical activity in cachectic cancer patients attending the clinic may be reduced by more than 40%². Such measures do not readily correlate with self-reported quality of life. Therefore it is important to recognise the need for objective measures of function not only to provide natural history data but also to serve as a target for therapeutic intervention.

References

1. Fearon, KCH, Voss, AC, Husted, DS on behalf of the Cancer Cachexia Study Group. Definition of cancer cachexia: effect of weight loss, reduced food intake and systemic inflammation on functional status and prognosis. *American Journal of Clinical Nutrition*, 2006; 83, 1345-50
2. Dahele, M, Skipworth, R, Wall, L, Voss, A, Preston, T, Fearon, K Objective physical activity and self-reported quality of life in patients receiving palliative chemotherapy. *Journal of Pain and Symptom Management*, 2007; 33: 676-85

Notes:

A2

Definition and diagnosis of cachexia / wasting disease**John E Morley, William Evans, Stefan Anker**

Saint Louis University School of Medicine, St Louis, MO, USA

Objective: A conference was held in Washington DC on December 13 and 14, 2006 to arrive at a consensus definition of cachexia.**Participants:** Participants included clinicians, clinical investigators, and basic scientists with expertise in muscle wasting and/or the treatment of diseases associated with accelerated loss of skeletal muscle. This included nephrologists, cardiologists, pulmonologists, oncologists, geriatricians, clinicians who treat HIV associated muscle wasting, and representatives of the National Institutes of Health. The meeting was open and funded by gifts from industry to the University of Arkansas for Medical Sciences Department of Continuing Education.**Evidence:** Participants presented data describing cachexia in clinical settings, the molecular basis of muscle wasting with underlying illness, and age-associated loss of skeletal muscle. In addition, clinicians presented clinical experience in the treatment of cachexia associated with illness. Published data was used to arrive at a consensus definition.**Consensus Process:** After presentation of evidence, an initial draft was composed by committee. Each statement (sentence) of the definition was discussed, edited, and voted on by all participants.**Conclusion:** The consensus definition is as follows and is unchanged from that approved by the participants of this meeting. In addition, the following manuscript was read edited and approved by the committee. "Cachexia is a complex metabolic syndrome associated with underlying illness and characterised by loss of muscle with or without loss of fat mass. The prominent clinical feature of cachexia is weight loss in adults and growth failure in children. 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."**References**

1. Anker SD, Negassa A, Coats AJ, Afzal R, Poole-Wilson PA, Cohn JN, Yusuf S 2003 Prognostic importance of weight loss in chronic heart failure Cachexia definition 13 and the effect of treatment with angiotensin-converting-enzyme inhibitors: an observational study. *Lancet* 361:1077-1083
2. Evans W 1995 What is sarcopenia? *J Gerontol* 50A(special issue):5-8 24. Thomas DR, Ashmen W, Morley JE, Evans WJ 2000 Nutritional management in long-term care: development of a clinical guideline. *J Gerontol MED SCI* 55A:M725-M734
3. Morley JE, Thomas DR, Wilson MM 2006 Cachexia: pathophysiology and clinical relevance. *American Journal of Clinical Nutrition* 83:735-743

Notes:

A3

Similarities and differences of wasting diseases**Stefan D Anker**

Center for Cachexia Therapy, Dept of Cardiology, Charité Berlin, Campus Virchow-Klinikum, Berlin, Germany

Cachexia (i.e. wasting disease) in chronic illness is frequent and causes major morbidity and mortality. Treatments for cachexia are scarce, but many different research efforts are ongoing. Many assume that there are common mechanisms for cachexia development in chronic illness, but published evidence for this suggestion is altogether limited and often only circumstantial.

The common pathophysiologic factors for cachexia in chronic illness include

- neurohormonal activation,
- inflammation & immune activation,
- hormone resistance syndromes (like growth hormone and insulin resistance),
- lack or insufficiency of anabolic (counter) responses,
- activation of specific muscle wasting processes, and
- activation of lipolysis pathways.

Together these pathophysiologic changes can be termed catabolic/anabolic imbalance.

We also need to consider that symptoms are similar in different cachexia syndromes. They include:

- shortness of breath,
- fatigue and muscle weakness, and
- likely also (mostly minor) depression.

In summary, I suggest that cachexia in different chronic illnesses similarly show a complex and multi-factorial pathophysiology resulting in a similar set of debiliating symptoms and adverse clinical outcomes. In conclusion:

- 1) treatments that are successful in one cachexia patient subgroup likely may also be useful in other cachexia indications,
- 2) more than one specific treatment approach will be necessary to optimally treat patients with cachexia, and
- 3) if one treatment is successful in one cachexia subgroup other treatment approaches still have a chance to be successful in that particular subgroup (even if used/tested on top of the first treatment).

Notes:

A4

The cost of cachexia**Wayne C Levy**

University of Washington, Department of Cardiology, Seattle, WA, USA

Elevated BMI is associated with an increased risk of developing heart failure. For each unit increase in BMI, the risk of developing heart failure increases by 5% in men and 7% in women. However, in heart failure patients, a lower BMI is associated with a ~4% higher risk of death for each unit increase. Cardiac cachexia, unintentional weight loss, is associated with markedly increased risk of death. In the general population in the only publication that I could find which evaluated healthcare costs and BMI, patients with lower and higher higher BMI (lowest risk BMI 26-27) had roughly doubled costs at age 20 and ~25% higher cost at age 60 in nonsmokers. Cytokines are thought to be a major component of cardiac cachexia. Medications that are used like in cardiovascular disease like ACEI, ARBs, beta blockers, omega 3 fatty acids, and statins may also be beneficial in cardiac cachexia. The Seattle Heart Failure Model is a multivariate risk model to estimate survival in patients with heart failure (SeattleHeartFailureModel.org). The Kaplan-Meier 1 year survival for BMI groups of <21, 21-25, 25-30 and >30 is 82.3%, 85.6%, 89.0%, and 90.5% respectively. In Val-HeFT, patients who had a BMI>30 spent 1/2 as many hospital days/year as subjects with a BMI <22. Using an average baseline 1 year survival of 75% for the BMI group <21 on no medications, the routine medications we use for heart failure like ACEI, beta blockers, aldosterone blockers are very cost effective with generic versions being well below \$1,000/life year saved in heart failure patients. In conclusion, I would urge all cachectic heart failure patients to be treated with an ACEI, beta blocker, aldosterone blocker, and potentially statin therapy. Agents like omega 3 fatty acids and cardiac resynchronization therapy may have additional benefits.

References

1. Kenchaiah S, Evans JC, Levy D, et. al. Obesity and the risk of heart failure. *N Engl J Med.* 2002;347:305-313.
2. Davos CH, Doehner W, Rauchhaus M, et. al. Body mass and survival in patients with chronic heart failure without cachexia: the importance of obesity. *J Card Fail.* 2003;9:29-35.
3. Levy WC, Mozaffarian D, Linker DT, et. al. The Seattle Heart Failure Model: prediction of survival in heart failure. *Circulation.*2006;113:1424-1433.

Notes:

B5

Anorexia**Ian M Chapman**

The University of Adelaide, School of Medicine, Adelaide, Australia

Appetite and food intake decrease with normal ageing, the so-called anorexia of ageing. Elderly people eat up to 30% less on average than young adults. The age-related decrease in energy intake is greater than that of energy expenditure, so on average body weight decreases after about age 60 years. Weight loss *per se*, particularly if unintentional, is associated with increased mortality rates in older people. Furthermore, the weight lost with aging is disproportionately of skeletal muscle, which predisposes to the development of sarcopenia, which has additional adverse effects. Together the reductions in food intake, body weight and muscle mass predispose to the development of under-nutrition in older people, which is harmful and surprisingly common. A variety of methods have been used to assess feeding behaviour in older people, including measurement of acute and chronic food intake (eg food diaries, direct measures in acute studies), visual analogue questionnaires and biomarkers such as blood concentrations of hormones including cholecystokinin (CCK) and ghrelin. They show that healthy older people feel less hungry and more full before meals than young adults, consume smaller meals more slowly, become satiated more easily and eat smaller snacks less often between meals. Some insight has been obtained into the mechanisms responsible for this age-related anorexia (1). There is evidence for a role for increased circulating levels of the satiating hormone CCK, increased sensitivity to CCK (2), impaired homeostatic responses to food deprivation, increased activity of satiating cytokines, altered gut motility and possibly reduced androgen activity, particularly in men. An understanding of the factors which contribute to the physiological and pathological declines in food intake in older people may aid the development of effective forms of prevention and treatment.

References

1. Chapman IM. The anorexia of aging. In: Clinics in Geriatric Medicine: Gastroenterology Editors: John E Morley and Syed H Tariq, Published by Elsevier Saunders Inc 2007, 23 (4):735-756.
2. MacIntosh CG et al. Effect of exogenous cholecystokinin (CCK)-8 on food intake and circulating leptin, insulin, and CCK concentrations in older and young adults; evidence for increased CCK activity as a cause of the anorexia of aging. J Clin Endocrinol Metab. 2001 86:8530-8537.

Notes:

B6

Decreased muscle strength and fatigue**Florian Strasser**

Kantonsspital St. Gallen, Department Internal Medicine, Section Oncology/Haematology and Palliative Medicine, St. Gallen, Switzerland

Both decreased muscle strength and fatigue are proposed (in addition to decreased muscle mass, anorexia, and biochemical alterations) as diagnostic criteria for cachexia/wasting, defined as involuntary weight loss. Decreased muscle strength can be seen as a key feature – of a least subtypes – of cachexia (muscle phenotype). In contrast, fatigue is – in advanced cancer – a multi-factorial phenomenon with insecure independent diagnostic value for cachexia even though it is associated with decreased muscle strength, but representing an important “global outcome” of cachexia.

In advanced cancer, fatigue can be defined as a subjective feeling of tiredness, weakness or lack of energy. Various groups (NCCN, Oncology Nursing Society, Fatigue-Coalition [ICD-10]) proposed definitions for cancer-related fatigue (CRF). A persistent debate is whether CRF has a qualitative difference to fatigue in everyday life of a general population or to the Chronic Fatigue Syndrome, or represents one end of a continuum of intensity, as a behavioural concept ranging from tiredness to fatigue and then to exhaustion. Fatigue is also defined, by a spectrum of approaches, as a multi-dimensional syndrome of physical (easy tiring and decreased capacity to maintain performance), cognitive (impaired mental concentration, loss of memory), and emotional/affective (reduced motivation, vigour, mood) components; but also an anticipatory sensation of difficulty in initiating a certain activity (generalized weakness). No clear consensus exists for the use of terms, often weakness or asthenia or lassitude is used for physical and tiredness for cognitive/mental fatigue.

The pathophysiology of CRF may be conceptualized into primary fatigue, related to the tumor itself, either through energy depletion (peripheral mechanism) or dysregulated hypothalamic-pituitary-adrenal axis or serotonin metabolism (central), these mechanisms may be significantly related to (pro-inflammatory) cytokines. Cancer-related concurrent syndromes, comorbidities and medications may cause secondary fatigue (anaemia, cachexia, infections, metabolic [Ca, H₂O] and endocrine disorders, depression, delirium, sedating drugs for symptom control, prolonged physical inactivity, other).

Various instruments (patient-reported outcomes) are available for screening of CRF (single symptom) or its assessment as symptom cluster or clinical syndrome. In addition to patients' perceived experiences (symptoms), semi-objective measures are used (cognitive dimension: psychomotor tests, structured interviews [DSM-IV]; physical dimension: physical activity meters, muscle strength tests, activities' questionnaires; emotional dimension: structured interviews [DSM-IV]).

Muscle strength does roughly correlate with muscle mass, but other factors such as patients' motivation or characteristics of the neuromuscular junction, may substantially influence measured muscle strength. The diagnosis of decreased muscle strength can be based on relatively simple bedside instruments, such as grip strength measurement, raise-up-and-go time, or bench press; however, advanced assessment requires special machines (Cybex) and measures of walking or stairs depend on other factors too.

In conclusion, to diagnose cachexia, muscle strength should be measured directly, taking motivational influences in mind, whereas perceived fatigue may be seen as global outcome, and alternatively physical activity may be measured by body-worn sensors.

References

1. Lukes Radbruch & Florian Strasser, et al. Fatigue in Palliative Care Patients – an EAPC approach. Palliative Medicine, in press.
2. Strasser F. Palliative care: evaluation instruments in daily clinical practice. Ann Oncol 2006;17 (Supplement 10): x299-303.

Notes:

B7

Assessment of body composition

Annemie Schols

University Hospital Maastricht, Department of Pulmonology, Maastricht, The Netherlands

Objectives:

1. The rationale for body composition in the diagnosis of cachexia/wasting disease
2. The use of body composition in outcome assessment
3. Clinical and laboratory methods to assess body composition (including reference values)

References

1. Schols AM, Broekhuizen R, Weling-Scheepers CA, Wouters EF. Body composition and mortality in chronic obstructive pulmonary disease. *Am J Clin Nutr* 2005;82:53-9.
2. Schutz-Y, Kyle U, Pichard C. Fat-free mass index and fat mass index percentiles in Caucasians aged 18-98 y. *Int J Obes Relat Metab Disord*. 2002
3. ESPEN Working group. Bioelectrical impedance analysis - part I: review of principles and methods. *Clin Nutr* 2004; 23: 1226-1243.
4. ESPEN Working group. Bioelectrical impedance analysis—part II: utilization in clinical practice. *Clin Nutr* 2004;23: 1430-1453.

Notes:

B8

Anemia and cachexia**Gerasimos Filippatos**

Department of Cardiology, University of Athens, Greece

Cachexia is frequently encountered in a number of chronic diseases, being associated with poor prognosis and survival. Besides weight loss, cachexia is commonly followed by several additional abnormalities, including altered hepatic glucose and lipid metabolism and anemia. Anemia is encountered in one fourth to half of patients with chronic heart failure, depending on age and clinical severity. Similarly, its prevalence in chronic obstructive pulmonary disease was found to be 23%, while patients with renal insufficiency and cancer present even higher anemia frequencies.

Besides a number of well-defined etiologies, including iron deficiency, chronic kidney disease and therapeutic procedures, such as chemotherapy, anemia is believed to result at least in part from a chronic inflammatory process that interferes both with the erythropoietin production and its interaction with bone marrow receptors and the release of stored iron in the reticuloendothelial system. Thus, a systemic inflammation, leading to the dysregulation of the expression of several cytokines could be a common pathogenetic mechanism underlying the disease-related impairment of hematopoiesis and the development of cachexia.

A growing body of evidence links the presence of anemia with increased mortality as well as a wide range of complications, longer hospitalization and poor quality of life. In addition to their prognostic role and their pathogenetic implications, hemoglobin levels along with cachexia-related proteins may serve as diagnostic and prognostic biomarkers in patients with chronic diseases, such as heart failure.

Furthermore, anemia and cachexia constitute currently two quite appealing therapeutic targets, attracting much of the research effort in chronic diseases.

References

1. Argilés JM, Busquets S, Felipe A, López-Soriano FJ. Muscle wasting in cancer and ageing: cachexia versus sarcopenia. *Adv Gerontol.* 2006;**18**:39-54.
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Notes:

B9

Biochemical alterations: inflammation, low albumin**Kamyar Kalantar-Zadeh**

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In patients with cachexia who do not have nephritic syndrome or significant liver disease, a low serum albumin can occur as a result of low protein intake or inflammation. Whereas some reports indicate that serum albumin synthesis can be significantly altered by alterations of dietary protein,[1] it has been suggested that hypoalbuminemia is a pathognomonic feature of wasting syndrome (cachexia), since it is usually not present during starvation without other features of wasting syndrome such as inflammation or hypercatabolism. In otherwise healthy individuals, only small decline in serum albumin is observed with starvation as shown in the Minnesota starvation experiment by Keys et al [2] or in cases of anorexia nervosa.[3] In chronic disease states such as chronic heart failure, chronic kidney disease or cancer cachexia, hypoalbuminemia appears more strongly associated with inflammatory markers and pro-inflammatory cytokines including C-reactive protein, interleukin-6 and tumor necrosis factor alpha.[4] In renal failure, for instance, even relative hypoalbuminemia (i.e., lowered serum albumin below 4.0 g/dL but still above 3.5 g/dL) is the strongest predictor of mortality, so that a serum albumin level below 4.0 g/dL is associated with 2 to 5-fold increase in death risk in both end-stage renal disease patients [5] and moderate stages of chronic kidney disease.[6] Similar outcome data have been emerging in chronic heart failure. Indeed hypoalbuminemia has been associated with atherosclerotic cardiovascular disease in dialysis patients,[7] in whom a unique presentation of protein-energy wasting described as "cachexia in slow motion" can be observed. The etiology and relative contribution of inflammation vs. protein-energy wasting to the development of hypoalbuminemia of wasting is not clear. During the acute phase response, protein synthesis may be shifted towards production of inflammatory molecules leading to less anabolic reserve for albumin synthesis, a hypothesis that has been criticized by some opinion leader as oversimplification of the complex interrelation between hypoalbuminemia, inflammation and cachexia.

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Notes:

C10

Ghrelin and ghrelin analogs for wasting disease in CHF and COPD**Piotr Ponikowski**

Cardiology Department, Centre for Heart Disease, Military Hospital, Wroclaw, Poland

Ghrelin, a growth hormone (GH) – releasing peptide produced by the stomach, is an endogenous ligand for the GH secretagogue-1a (GHS-1a) receptor. Ghrelin increases GH secretion, stimulates food intake, increases gastric emptying and food assimilation. Additionally, recent studies have suggested that this hormone may also be anti-inflammatory, may act on the central nervous system to decrease sympathetic nerve activity and have direct cardio-protective effects. All these physiological properties suggest that ghrelin may become a candidate for the treatment of wasting in chronic disease.

Up to now, clinical application of ghrelin has been evaluated only in a few, small, observational studies with patients suffering from cardiac cachexia in the course of chronic heart failure (CHF) and pulmonary cachexia in the course of chronic obstructive pulmonary disease (COPD). In CHF, administration of ghrelin improved muscle wasting as indicated by an increase in muscle strength and lean body mass which further resulted in a significant improvement in exercise tolerance. Additionally ghrelin exerted beneficial haemodynamic effects and improved left ventricle function. Treatment with ghrelin in COPD patients improved food intake and resulted in an increase in body weight, lean body mass, peripheral and respiratory muscle strength. Larger, controlled studies are now needed to validate an intriguing concept that supplementation of ghrelin may become a therapeutic approach in patients with cardiac or pulmonary cachexia.

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Notes:

C11

Update on trials in HIV/AIDS-wasting

Morris Schambelan

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Megestrol acetate (MA) is approved for the treatment of anorexia, cachexia or an unexplained, significant weight loss in patients with AIDS. Prior to the advent of highly active antiretroviral therapy (HAART), MA-induced weight gain was primarily fat. This presentation will review two trials of MA therapy in HIV-infected patients done in the HAART era. In the first, Mwamburi et al compared 2-months treatment with MA (800 mg qd) to oxandrolone (OX, 10 mg bid) in a randomized trial in 40 patients with a weight loss of $\geq 5\%$. Mean weight gain in the MA and OX arms were 2.8 and 2.5 kg, respectively. Lean body mass (LBM, measured by bioelectrical impedance analysis, BIA) accounted for 39% and 56% of the weight gain, respectively ($p=0.38$). Both drugs were safe and well tolerated. The second trial, performed in 14 AIDS Clinical Trials Units in the United States, was designed to test the hypothesis that co-administration of testosterone enanthate (TE) with MA could enhance LBM accrual. In addition, the authors (Mulligan et al) evaluated the effects of MA \pm TE on sexual functioning and the hypothalamic-pituitary-adrenal axis. Seventy-nine HIV-positive men with $\geq 5\%$ weight loss or BMI < 20 kg/m² were randomized to receive MA (800 mg qd) plus TE (200 mg; MA/TE; N=41) or placebo (MA/PL; N=38) biweekly for 12 weeks. Both groups experienced robust increases in weight (median 5.3 and 7.3 kg in MA/TE and MA/PL, respectively), LBM (3.3 and 3.3 kg by BIA), and fat (3.0 and 3.8 kg). There were no significant differences between groups in the magnitude or composition of weight gain ($P=0.44$, 0.90, and 0.11 for weight, LBM, and fat, respectively). Trough testosterone concentrations decreased to a greater extent in MA/PL (-12.3 vs. -6.1 nmol/L in MA/TE; $p=0.04$). Cortisol levels became nearly undetectable in subjects with plasma MA levels > 150 ng/mL. Sexual functioning was preserved with MA/TE but worsened in MA/PL. Thus, although through testosterone levels were higher in subjects with TE, there was no significant difference in the magnitude of LBM accrual. The marked increase in LBM seen in both of these studies suggests that the anabolic effects of MA might be enhanced in the HAART era.

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Notes:

C12

Growth hormone treatment in patients with severe kidney disease

Viatcheslav Rakov on behalf of the APCD Study Group
Novo Nordisk Health Care AG, Zurich, Switzerland

Patients with End Stage Renal Disease (ESRD) on chronic haemodialysis experience high rate of mortality (exceeding 20% in the US), morbidity and significant decrease of Quality of Life (QoL). Malnutrition as a result of a chronic catabolic state, inflammation and reduced food intake, is recognised as a condition which negatively influences clinical outcomes in these patients. Low serum albumin and loss of lean body mass (LBM) are considered as important biological markers of malnutrition and chronic catabolic state. Studies have shown a strong link between low serum albumin, decreased LBM and mortality in patients with ESRD on haemodialysis. Growth Hormone (GH) may beneficially influence metabolic processes in these patients by stimulating the protein synthesis, improving cardiovascular risk profile and increasing the QoL.

We performed a 6-month, double-blind, parallel-group, placebo controlled and multinational study. The study population included non-diabetic patients with ESRD who were ≥ 18 yr, were on adequate haemodialysis treatment for at least 3 months, had serum albumin ≤ 40 g/L and had no contraindications for treatment with growth hormone accordingly to labelling. Patients were randomised to treatment with daily subcutaneous injections of growth hormone (Norditropin[®], Novo Nordisk A/S; Denmark) 20, 35, or 50 $\mu\text{g}/\text{kg}/\text{day}$ or placebo. The primary end-points of the study were LBM and serum albumin changes after 6 month of GH treatment comparing to placebo. Body composition (inclusive LBM) was primarily evaluated using standardised DXA scan. Serum albumin was measured centrally in a certified lab.

230 patients were screened, 139 randomised and 55 patients were withdrawn from the study (37 of those due to adverse events). All dosages of GH treatment resulted in significant increase in LBM compared to placebo ($p < 0.001$). A positive trend toward serum albumin levels was observed in the lowest GH group vs. placebo ($p = 0.063$). We observed a significant improvement of QoL in patients treated with GH in relation to their physical activity ($p = 0.042$) evaluated by Kidney Disease Quality of Life Short Form general subscale. We found also significant improvements other biomarkers of mortality – homocystein, HDL, transferrin - and QoL related parameters in patients under active treatment. 596 adverse events were registered during the study; the majority of them (527) were mild or moderate. Frequency of adverse events did not depend on GH dose. No difference was observed compared to placebo. However, adverse events assessed to be possibly/probably related to GH treatment increased with dose of GH. Mortality rate for this study was 11% and 8% in placebo and treatment group respectively. GH treatment was not associated with any serious safety concerns with regard to glucose metabolism; treatment did not affect left ventricular mass size.

GH therapy safely improved LBM, health related QoL and some other important markers of morbidity and mortality risk in adult patients who were on maintenance haemodialysis. A long term study on a bigger patient population is warranted to investigate whether these treatment benefits may be associated with improved clinical outcomes.

Notes:

D13

Skeletal muscle hypertrophy and atrophy pathways: overview**David J Glass**

Novartis Institutes for Biomedical Research, Cambridge, MA, USA

In the adult mammal, skeletal muscle mass can be modulated through the interplay of protein synthesis and protein degradation pathways. For example, atrophy – the loss of muscle mass of individual muscle fibers – is caused by the simultaneous downregulation of the IGF1/Akt hypertrophy pathway and the upregulation of the E3 ubiquitin ligases MuRF1 & MAFbx, which have been shown to be required for skeletal muscle atrophy. The precise mechanisms by which the E3 ligases cause the breakdown of muscle fibers have not yet been completely elucidated. In this talk, recently identified substrates for MuRF1 will be discussed. Further, a particular method for inducing atrophy – by exposing muscle cells to supraphysiologic levels of glucocorticoid – will be explored to determine how hypercortisol states may bring about atrophy in pathologic clinical settings. Also the relevance of atrophy signaling to the loss of muscle during old age – sarcopenia – will be discussed.

Notes:

D14

Myostatin and ubiquitin

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Nanyang Technological University, School of Biological Sciences, Rm 2n-09, Genomics and Genetics Division, Singapore

Myostatin, a Transforming Growth Factor-Beta (TGF- β) super-family member, has been well characterized as a negative regulator of muscle growth and development. Myostatin has been implicated in several forms of muscle wasting including the severe cachexia observed as a result of conditions such as AIDS and liver cirrhosis. Here we show that Myostatin induces cachexia by a mechanism independent of NF- κ B. Myostatin treatment resulted in a reduction in both myotube number and size *in vitro*, as well as a loss in body mass *in vivo*. Furthermore, the expression of the myogenic genes *myoD* and *pax3* was reduced, while NF- κ B (the p65 subunit) localization and expression remained unchanged. In addition, promoter analysis has confirmed Myostatin inhibition of *myoD* and *pax3*. An increase in the expression of genes involved in ubiquitin-mediated proteolysis is observed during many forms of muscle wasting. Hence we analyzed the effect of Myostatin treatment on proteolytic gene expression. The ubiquitin associated genes *atrogen-1*, *MuRF-1* and *E2_{14k}* were up-regulated following Myostatin treatment. We analyzed how Myostatin may be signaling to induce cachexia. Myostatin signaling reversed the IGF-1/PI3K/AKT hypertrophy pathway by inhibiting AKT phosphorylation thereby increasing the levels of active FoxO1, allowing for increased expression of atrophy-related genes. Therefore our results suggest that Myostatin induces cachexia through an NF- κ B independent mechanism. Furthermore, increased Myostatin levels appear to antagonize hypertrophy signaling through regulation of the AKT-FoxO1 pathway.

Notes:

D15

Identification of polyubiquitinated substrates of the skeletal muscle proteasome**Didier Attaix**, Lydie Combaret, Daniel Taillandier

Human Nutrition Unit, Proteolysis Group, INRA and Human Nutrition Research Center of Clermont-Ferrand, Ceyrat, France

The ubiquitin-proteasome system (UPS) is recognized as the major proteolytic pathway responsible for muscle wasting. The UPS is widely believed to degrade the major contractile proteins such as actin and myosin heavy chain. However, the potential substrates of the UPS are still poorly characterized (Witt et al. 2005; Fielitz et al. 2007). Based on the steps present in cells, *i.e.* recognition of substrates tagged by a poly-ubiquitin(Ub) degradation signal and deubiquitination, we developed an affinity matrix-based purification of poly-Ub conjugates. Ub-conjugates have been purified from MG132-treated C2C12 myotubes using the Ub binding domains of the S5a proteasome subunit bound to an affinity matrix and then deubiquitinated by the catalytic domain of the USP2 enzyme. This two step purification of proteasome substrates involving both protein-protein interactions and enzyme mediated release allows highly specific isolation of poly-ubiquitinated 26 S proteasome substrates, which were then resolved on two-dimensional gels post-deubiquitination (Ventadour et al. 2007). More than 50 potential substrates of the proteasome have been already identified in C2C12 cells. The technique has now been adapted to muscle samples and we provided evidence for polyubiquitination of a subset of proteasome substrates in skeletal muscle.

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Notes:

D16

Thinking outside the box: the example of rho- and rac-kinase inhibitors**Stephan von Haehling**

National Heart & Lung Institute, Imperial College School of Medicine, London, UK

The first human oncogene to be discovered was Ras. It was first discovered in viruses that cause sarcomas in its host, the rat (ras = **rat sarcoma**). The Ras protooncogenes encode guanosine triphosphate (GTP) binding proteins that are involved in cell proliferation, differentiation, and other pleiotropic responses involved in malignant transformation. Ras is only one of five subfamilies of small monomeric GTPases. Rho is another such subfamily. Its first gene was identified as a Ras homologue (rho = **ras homologue**) and cloned in 1985. The Rho family currently comprises several members including RhoA, RhoB, RhoC, Rac1, Rac2, and Cdc42, all of which represent small GTPases. They cycle between the GTP-bound active and the guanosine diphosphate-bound inactive form. Their active forms interact with several effectors. The best characterized Rho effector is Rho-kinase (ROK), which exists in two isoforms: ROK α (also known as ROCK2) and ROK β (ROCK1). Inhibitors of Rho activity have been developed. One of these, fasudil, was approved in 1995 for the treatment cerebral vasospasm in patients with subarachnoid haemorrhage for its vasodilating properties. Rho-kinase inhibitors have other interesting properties that may prove beneficial in patients with cachexia.

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Notes:

D17

Short plenary poster presentation**Proteasome inhibitor MG 132 has different effect on protein metabolism under in vivo and in vitro conditions****Milan Holecek**, Muthny T, Kovarik M, Sispera L

Charles University Medical Faculty, Department of Physiology, Hradec Kralove, Czech Republic

Ubiquitin-proteasome system plays an important role in degradation of myofibrillar proteins in skeletal muscle. Two separate studies were performed using Wistar rats to evaluate the effect of proteasome inhibitor MG 132 on protein metabolism.

In the first study, m. soleus or m. extensor digitorum longus were incubated in medium with 30 $\mu\text{mol/l}$ MG 132 or without inhibitor (control). Changes in proteolysis and protein synthesis were determined according to the rate of the tyrosine release, chymotrypsin-like activity and L-[1- ^{14}C]leucine incorporation into the muscle protein. In the second study, rats were injected with MG 132 (10 mg/kg b.w.) or with solvent. Changes in whole-body protein metabolism were estimated using infusion of L-[1- ^{14}C]leucine. The results were analyzed using unpaired Students' test.

In in vitro study, MG 132 significantly decreased both proteolysis and protein synthesis. In in vivo study MG 132 induced the increase in whole-body proteolysis and protein synthesis. Proteasome-dependent proteolysis was inhibited in skeletal muscle and activated in the liver and kidney. Protein synthesis increased in skeletal muscle, liver and kidney.

We conclude that MG-132 affects both protein anabolic and protein catabolic pathways via the direct effect on proteasome-dependent proteolysis and indirect effect on proteolysis and protein synthesis via unidentified mediators. The results also demonstrate that the observations in vitro may not necessarily reflect the in vivo situation.

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Notes:

E18

The clinical problem: epidemiology & pathophysiology

Aminah Jatoi

Mayo Clinic Rochester, Division of Medical Oncology, Rochester, Minnesota, USA

The cancer anorexia-weight loss syndrome predicts a poor prognosis. This talk will review some of the epidemiologic data to support this statement. It will also attempt to provide an explanation for what accounts for this tight association between weight loss and early demise.

Notes:

E19

The effects of cancer on the skeletal musculature**Vickie E Baracos**

University of Alberta, Cross Cancer Institute, Edmonton, Alberta, Canada

Skeletal muscle wasting is a key constituent of the evolution of human body composition associated with advanced stages of cancer. A critical comparison of available methods suggests that in addition to Dual Energy X-Ray (DXA), CT image analysis is a powerful and practical means to quantify specific muscles, to follow their dynamic changes during the cancer trajectory and to predict whole body muscle mass (1,2). We are assessing muscle mass in a prospective cohort of patients at a regional cancer centre (n=2350). Subjects are evaluated at the time of diagnosis of locally advanced or metastatic solid tumors of the lung or gastrointestinal tract; analysis is based on CT images taken for disease staging. Regional analysis at the 3rd lumbar vertebra (L3) is used, since prior work showed muscle at L3 to be highly related to whole-body muscle tissue (2). Images are analyzed for tissue cross-sectional area (cm²) using Slice-O-Matic software V4.3 (Tomovision, Canada), which is then used to estimate whole body appendicular skeletal muscle (ASM). To establish the prevalence of low muscle mass in the cancer patient population, we used the cutoffs for sarcopenia established by R. Baumgartner et al. for elderly people (♂ <7.26 kg/m²; ♀ <5.45 kg/m²). Analysis of the data to date (n=535) suggest a very high prevalence of low muscle mass in patients newly presenting with advanced cancer. Overall, 57.5% of patients had estimated ASM below the sex-specific cutoffs; with a prevalence of 89% in patients with BMI <20 and 62% between BMI 20 and 25. Also 15.9% of the patients in the overall population were obese, and 19% of these had low ASM (sarcopenic obese). Thus muscle depletion is ubiquitous and already well-advanced at the time of diagnosis. Ongoing work is aimed at determining the further evolution of muscle wasting and its clinical determinants. The underlying biochemical changes resulting in muscle loss remain to be clarified in cancer patients however in related work in animal models (3) we obtained evidence that a common set of transcriptional adaptations underlie the loss of muscle mass in different catabolic states. A set of genes, termed 'atrogins', included many genes involved in protein degradation and several growth-related mRNAs were down-regulated. Genes required for ATP production and glycolysis were down-regulated, as were many transcripts for extra-cellular matrix proteins.

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Notes:

E20

Causes of death and cardiac problems**Mathias Rauchhaus**

Charite, Campus Virchow-Klinikum, Department of Cardiology, Berlin, Germany

Cancer belongs to the leading causes of death. It is commonly recognized that patients with cancer die from cancer related causes predominantly due to the direct destruction of the organ in question. There is very little evidence that patients with cancer die from fatal co-morbidities associated with cancer such as infections, hemorrhage, or organ failure. Moreover, symptom generation in particular (shortness of breath) and quality of life in general (cancer fatigue syndrome) is purely understood. We hypothesize that in patients with cancer, inflammatory parameters such as cytokines produced by the tumor trigger systemic inflammation and infections, thereby causing multi-organ failure and the generation of heart failure-like symptoms, thus culminating in increased rates of cardiac arrhythmias finally leading to sudden cardiac death in cancer.

Notes:

E21

Treatment on cancer cachexia: clinical trials update**Glenn J Lesser**

Wake Forest University School of Medicine, Winston-Salem, NC, USA

Involuntary weight loss is a significant and sometimes life-threatening condition experienced by a variety of cancer patients. This weight loss may be present at diagnosis or result from treatment and its extent varies widely by tumor type and by treatment modality. As a consequence of progressive weight loss, patients with cancer are at an increased risk for infection, poor wound healing, decreased functional status, respiratory failure and death. Furthermore, weight loss in patients with cancer disproportionately represents a loss of muscle mass which may lead to a decline in performance status, limit treatment options and reduce survival. Corticosteroids, progestational agents, prokinetic agents, nonsteroidal agents, cannabinoids, dietary supplements and anabolic steroids have all been used in patients with cancer and anorexia/cachexia. Randomized clinical trials provide support only for the use of corticosteroids and progestational agents in these patients. Megestrol acetate, an orally active synthetic congener of the natural steroid progesterone, is the most widely studied progestational agent. It promotes weight gain predominantly through an increase in adipose tissue. The efficacy and dose response of megestrol acetate has been clearly established in a series of large, randomized, double-blind, placebo-controlled trials performed by the North Central Cancer Treatment Group. Anabolic agents have the potential to improve body composition and promote weight gain in cancer patients by maintaining or enhancing lean body mass. Oxandrolone is a potent oral anabolic steroid exhibiting minimal androgenic effects. When combined with adequate protein intake, oxandrolone has been shown to promote weight gain through an increase in lean body mass. The National Cancer Institute-funded Wake Forest University Community Clinical Oncology Program (CCOP) Research Base has recently completed a Phase III, randomized study comparing the effects of oxandrolone and megestrol acetate on weight and lean body mass in patients with solid tumors and weight loss who were receiving chemotherapy.

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Notes:

E22

Short plenary poster presentation**Bisoprolol, oxypurinol and spironolactone - results on body composition, physical activity, food intake and survival in rats with cancer cachexia****Jochen Springer & Stefan D Anker**

Division of Applied Cachexia Research, Department of Cardiology, Charité Medical School, Berlin, Germany

Background: Cachexia is a common co-morbidity in cancer patients (up to 80% depending on the tumor) and is associated with a drastic reduction in quality of life and survival. High levels of catecholamines, aldosterone and uric acid (a danger signal associated with inflammation) have been described in cachexia. Beta blockers have been shown to reduce the onset of cachexia in chronic heart failure and aldosterone antagonism reduces inflammation as well as oxidative stress.

Methods: Juvenile rats (weight approx. 200 g) were inoculated intra-peritoneally with 10⁸ AH-130 hepatoma cells and treated with the xanthine oxidase (XO) inhibitors allopurinol and oxypurinol, the beta blocker bisoprolol, the aldosterone antagonist spironolactone or placebo. Twenty-four hour food intake and 24 hour locomotor activity, as indicator of quality of life (QoL), 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).

Results: Placebo treated animals showed severe cancer cachexia with a mean body weight loss of more than 25%, with wasting of both fat and lean mass. This translated into a reduced quality of life as indicated by an approx. 60% lower activity and an approx. 77% reduced food intake compared to day 0, as well as a high mortality (73%). Bisoprolol dose-dependently prevented the wasting process to some extent and improved food intake and physical activity, which resulted in a lower mortality. Spironolactone showed a dose-dependent reduction of weight loss, improved QoL and a strong reduction of mortality. Inhibition of XO also resulted in better outcome, with the second generation inhibitor oxypurinol being superior to allopurinol.

Conclusion: In this animal model of cancer cachexia, all drugs tested here improved important aspect of the wasting process. Compared to placebo, drug treatment resulted in reduced mortality, less weight loss with a better body composition and improved quality of life indicators.

Notes:

F23

Is it possible to separate the metabolic effects of cachexia from inactivity?**William J Evans**

University of Arkansas for Medical Sciences, Little Rock, AR, USA

Long-term bedrest result in an accelerated loss of skeletal muscle. It is characterized by increased sensitivity to cortisol, insulin resistance, increased loss of nitrogen, and a substantial decrease in the rate of muscle protein synthesis with no evidence for a change in the rate of muscle protein breakdown. Similarly cachexia is associated with accelerated loss of skeletal muscle and insulin resistance. However, one important hallmark of cachexia is increased muscle protein breakdown that is associated with an increase (above baseline) muscle protein synthesis rate. Clinically, without a kinetic measurement of protein metabolism, it is difficult to separate the combined effects of decreased physical activity from that of cachexia. One important distinction may be responsiveness to a nutritional intervention. Recent studies have demonstrated that bed rest associated muscle atrophy may be ameliorated by a supplement of essential amino acids while there is little evidence that cachexia is responsive a nutritional intervention. Consideration of the metabolic consequences of both bed rest and cachexia may help to prevent loss of muscle mass and function in hospitalized cachectic patients.

Notes:

F24

Myostatin

Jin-Lin Wang, Xiaolan Zhou, Keith Kwak, Yanping Song, Robert Rosenfeld, Ching Chen, Hosung Min, Thomas Boone and **HQ Han**

Department of Metabolic Disorders and Protein Science, Amgen Inc. Thousand Oaks, CA, USA

Insulin plays a critical role in the maintenance of skeletal muscle homeostasis and kidney function. Insulin deficiency or resistance results in muscle wasting and nephropathy. Myostatin, a member of TGF-beta family, is a negative regulator of muscle growth. Here we have examined the effect of a myostatin antagonist, referred to as anti-myostatin peptibody, on muscle growth and kidney function in streptozotocin-induced diabetic mouse model.

Young adult male C57BL/6 mice were injected with multiple low-doses of streptozotocin (STZ) (ip, 40 mg/kg/day for 5 days). The onset of diabetes in the STZ injected mice was determined by blood glucose elevation and insulin deficiency. Four months after STZ injection, the diabetic mice were randomly assigned into two weight-balanced groups (n=9/group) and were subsequently treated with anti-myostatin peptibody (5 mg/kg, 3/wk, SC) or vehicle for 7 weeks. Body weight, food intake, and blood glucose levels were monitored. Body composition was measured by NMR. Towards the end of the 7-week treatment, mice were placed in metabolic cages for measuring kidney function parameters, including 24-hour urine volume, creatinine clearance rate and urinary albumin excretion. Muscle and organ weights were determined during terminal necropsy.

STZ injection resulted in insulin deficiency, hyperglycemia, body weight loss and muscle atrophy as well as kidney function impairment. Anti-myostatin peptibody treatment led to significant increases in body weight, lean body mass and skeletal muscle mass in STZ diabetic animals. The peptibody treatment also significantly attenuated kidney hypertrophy and reduced 24-hour urine volume and urinary albumin excretion in the diabetic animals. No effect on food intake or blood glucose levels was observed.

These results demonstrate that administration of anti-myostatin peptibody is effective in restoring skeletal muscle growth and improving kidney function under insulin-deficiency disease condition, suggesting a potential benefit in treating diabetes-associated muscle atrophy and nephropathy. The mechanisms by which anti-myostatin peptibody achieve these pharmacological effects remain to be further investigated.

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Notes:

F25

Biomarkers from tissue signifying muscle wasting**William E Mitch**

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The muscle wasting that occurs in a variety of diseases from space travel to trauma/immobilization is difficult to document. This shortcoming has impaired the development of strategies directed at preventing muscle wasting. Available measurements include: sequential CT or MRI examinations (per measurement, \$1000 & \$1500 respectively with no discount); measurement of protein turnover by infusing labeled amino acids; and measurement of nitrogen balance). The latter are cumbersome and expensive.

Many conditions causing muscle wasting activate the ubiquitin-proteasome proteolytic system (UPS) in muscle but the UPS minimally degrades actomyosin or myofibrils. In models of accelerated muscle protein degradation, we found activated caspase-3 and demonstrated that it readily degrades actomyosin to provide substrates for the UPS. Caspase-3 activity in muscle leaves behind a "footprint", a characteristic 14 kD fragment of actin in the insoluble fraction of muscle (if present in the soluble fraction, the UPS rapidly degrades it)¹. In rodent models of uremia, acute diabetes and other muscle wasting conditions, the 14 kD actin fragment accumulates². Likewise, in muscle of patients undergoing hip replacement therapy for osteoarthritis, we measured the abundance of the 14 kD actin fragment simultaneously with protein degradation using stable isotope techniques³. The rate of protein degradation was positively correlated with the abundance of the actin fragment ($r = 0.78$). In muscle of patients with burn injury, the fragment was also increased. In muscle biopsies of hemodialysis patients, the actin fragment was increased. Some patients trained in "endurance exercise (i.e., bicycle-like exercise) for 18 weeks had a significant decrease in the amount of the actin fragment in muscle. This did not occur with resistance exercise or in patients who did not undergo exercise training. Thus, a muscle biopsy could be used to detect increased muscle protein degradation as an increase in the 14 kD actin fragment abundance. The abundance of this potential "biomarker" is correlated with measured rates of protein degradation and the abundance does respond to therapy.

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Notes:

F26

Biomarkers for muscle wasting from blood**Stephan von Haehling**

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Biomarkers are widely established in clinical decision-making. The troponins, for example, have become almost indispensable in the diagnosis of acute coronary syndromes. In the field of chronic heart failure, natriuretic peptides such as N-terminal pro B-type natriuretic peptide or, more recently, mid-regional pro-atrial natriuretic peptide, are becoming important tools in establishing the diagnosis of heart failure and estimating the clinical course and the prognosis of the respective patient. Such markers have not been firmly established in cachexia but several candidates have been identified. Using such an approach, it might be possible to identify patients for more specific therapies depending on the predominant type of tissue that is lost.

Notes:

G27

“Prometheus” basic science in cachexia lecture: past, present and future trends in cancer cachexia research

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Historically, my interest in cancer cachexia dates back to the early 1990s when we observed that patients with advanced cancer exhibiting cachexia as well as patients with no overt signs of cachexia showed high levels of proinflammatory cytokines, not known to have any correlation with cachexia. Additionally, we observed that the level of proinflammatory cytokines increased progressively paralleling the clinical course of the disease and correlated well with stage of disease and ECOG-PS. In 1995 (1) we treated 11 head and neck cachectic cancer patients with megestrol acetate (MA) for three months and observed an increase of body weight, appetite and quality of life accompanied by a significant decrease of IL-6 and TNF-alpha. Soon after, we set out to find out the mechanism of action of progestagens, the only agents clinically available then to counteract cachexia. An experimental laboratory trial followed in which medroxyprogesterone acetate (MPA) was shown to downregulate the release of IL-6 and TNF-alpha, and also serotonin, by PBL, thus explaining the mechanism of action of MPA. Our findings demonstrated: 1) the correlation between cytokines and cachexia, the former being one of the main factors playing a role in its onset; 2) and that drugs effective in cachexia, such as MPA, exert their activity by decreasing cytokine production/release (2). Our interest was then captured by leptin, an hormone released by adipocytes, which could be considered a strong candidate in the pathophysiology of cachexia. We studied circadian leptin levels of cachectic cancer patients which were much lower than normal: this finding fits perfectly with a malnourished body such as a cachectic one and suggests that leptin does not play a causal role in cachexia but simply that the correct signals reaching the central regulatory sites (hypothalamus) are counteracted or misinterpreted by some molecules (cytokines) (3,4). Later, we demonstrated that there is an inverse correlation between leptin (lowest) and proinflammatory cytokines (highest), accompanying the most advanced stage of disease and the worst ECOG PS: moreover, they are predictive of very short survival. (4). Furthermore, we also observed that the majority of cachectic cancer patients exhibit signs of oxidative stress (OS) and thus introduced the novel concept of cachexia/OS as descriptive of the status of most advanced cancer patients. Patients with OS also exhibit high levels of inflammatory cytokines and low levels of leptin (5). In our view, cytokines (IL-6 and TNF-alpha) are the expression of a non-specific inflammatory body response against the tumor which occurs through a chronic activation of the immune system.

Based on our research and clinical experience, we carried out a Phase II clinical trial, according to the Simon's "two-stage" design, with the aim to test the efficacy and safety of an integrated treatment based on a pharmacological support, antioxidants, and drugs, all given orally, in a population of advanced cancer patients with cachexia/OS. 39 patients were included. The endpoint variables body weight, and particularly lean body mass (LBM), increased significantly, as well as appetite, while IL-6 and TNF-alpha decreased and fatigue and quality of life improved. The treatment was shown to be safe and well tolerated without any toxic effect (6,7). The study was followed in April 2005 by a phase III randomised study to establish the most effective and safest treatment able to improve the identified "key" variables of cachexia/OS: LBM, resting energy expenditure, total daily physical activity, IL-6 and TNF-alpha and fatigue. All enrolled patients are scheduled to receive as basic treatment: polyphenols + alpha lipoic acid + carbocysteine + Vitamins E, A and C, oral. Patients are then randomised to one of the following 5 arms: 1) MPA/ MA; 2) Pharmaco-nutritional support containing EPA; 3) L-carnitine; 4) Thalidomide; 5) MPA/MA + Pharmaco-nutritional support + L-carnitine + Thalidomide. The duration of the treatment is 4 months. At September 2007, 180 patients with advanced stage (96% stage IV) tumors at different sites were randomized: the study is still in progress.

Presently, there is not a consolidated treatment for cancer cachexia. As progestagens and corticosteroids are obsolete drugs and considering that anti-TNF-alpha monoclonal antibody (infliximab) was shown to be ineffective, research interest is currently shifting towards the use of different approaches addressing the potential targets involved in the pathophysiology of cachexia. Current new trends include: anti-IL-6 humanized monoclonal antibody; anti-myostatin, inhibiting proliferation and differentiation of myoblasts; ghrelin mimetic with orexigenic and anabolic activity (8); non-steroidal selective androgen receptor modulators (SARMs) (9); insulin treatment (10). In my opinion, as cancer cachexia is multi-factorial, the treatment should be also multi-dimensional/ multi-targeted.

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Notes:

G28

The activation of muscle protein degradation in catabolic states

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Much has been learned in recent years about the rapid atrophy of skeletal muscle occurring upon disuse or denervation of muscle, fasting, excessive glucocorticoids, and systemic diseases, including cancer cachexia, sepsis, and cardiac or renal failure. Our prior work has established that in these diverse conditions, there is a common program of transcriptional changes, in which a set of atrophy-related genes are induced or repressed in a similar manner. Included in this set are a variety of genes that enhance the cell's capacity for protein breakdown by the ubiquitin-proteasome pathway, especially the atrophy-specific ubiquitin ligases, atrogin-1 and MuRF1. MuRF1 appears to play a specific role in ubiquitination and degradation of myofibrillar proteins. Atrogin-1 and many other atrogenes are induced by the FoxO family of transcription factors, and overproduction of FoxO3 by itself causes dramatic muscle atrophy. In addition FoxO3-dependent transcription stimulates the cell's other main proteolytic system, the autophagic/lysosomal pathway. In atrophying muscles, mRNAs for many autophagy-related genes are induced. On the other hand this FoxO-dependent atrophy program is inhibited by the major factors promoting muscle growth, IGF-1 and insulin, which activate the PI3K-Akt pathway. Contractile activity causes production of the transcriptional coactivator, pgc-1 α , which in addition to causing mitochondrial production, can reduce the catabolic actions of FoxO3. Overproduction of pgc-1 α in transgenic animals or by gene electroporation retards muscle atrophy. This action of pgc-1 α appears to account for the sparing of pgc-1 α -rich Type 1 fibers and the selective loss of pgc-1 α -poor Type II fibers in most cachectic states. During atrophy, the cell's two main proteolytic systems are activated coordinately to cause the breakdown of different cellular components; the loss of contractile proteins via the ubiquitin-proteasome pathway and of organelles (e.g. mitochondria) via autophagy.

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G29

Stem cells and muscle

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The resolution of tissue injury involves a complex interaction between local repair mechanisms and cells of the immune system, which participate in the removal of necrotic cells but may also provide support for the action of stem and progenitor cells in the repair process. Macrophages are the major infiltrating cell population in injured skeletal muscle, required for removal of damaged myofibers as well as for subsequent muscle regrowth and differentiation. In a mouse model of enhanced regeneration of skeletal muscle expressing a local IGF-1 isoform, we found that that improved bone marrow contribution to rebuilding damaged muscle could be attributed to the myeloid/macrophage lineage. To test whether this requires the local polarization of macrophages towards an anti-inflammatory, or M2 phenotype, we exploited a genetic blockade to the induction of C/EBP β transcription factor in response to injury, which is essential for macrophage polarization. Persistence of inflammatory M1 macrophages in damaged muscle of these mice was insufficient for effective regeneration, which was impaired in the absence of M2 polarization. These observations highlight the role of infiltrating immune cells in tissue repair and provide the first direct genetic link between the action of M2 macrophage polarisation and muscle regeneration.

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G30

Of bears, frogs, meat, mice and men: insights into muscle loss and fat deposition**Miranda Grounds**, Chai R, Gebiski BL, Shavlakadze T

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Extreme loss of skeletal muscle mass (atrophy) occurs in human muscles that are not used. In striking contrast, skeletal muscles do not waste away in hibernating mammals such as bears, or aestivating frogs, subjected to many months of inactivity and starvation. What mechanisms protect against muscle atrophy in some species? Severe atrophy also occurs with cachexia and ageing. Another clinical muscle-related concern is the increase in obesity and fat deposition in humans that results in disturbed metabolism, impaired response to insulin and diabetes. In the meat industry, a key aim is skeletal muscle growth with a strong interest in fat deposition (marbling) within muscle. Intensive research in these diverse fields provides new insights into the complex molecular interactions that control skeletal muscle growth/hypertrophy/atrophy (myogenesis) and fat deposition (adipogenesis)[1]. In our laboratory, the central role of IGF-1 in muscle hypertrophy is investigated using transgenic mice with skeletal muscle specific over-expression of the novel Class2:Ea IGF-1 isoform: this results in marked hypertrophy of normal and dystrophic (mdx) muscles, yet no increase in specific force and no effects on the classical Akt/mTOR/p70S6K signaling pathway downstream of the IGF-1 receptor [2]. IGF-1 and the pro-inflammatory cytokine TNF have opposing effects with TNF increasing muscle wasting and the breakdown of dystrophic muscles: it is proposed that TNF interferes with IGF-1 signaling [3] via JNK phosphorylation of IRS-1ser307. Using these models, our research focuses on the impact of gender, age, exercise, dietary interventions and inflammation on muscle mass and fat.

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Notes:

H31

Wasting in chronic kidney failure**Joel D Kopple**

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Protein-energy wasting (PEW) occurs commonly in individuals with advanced (stage 5) chronic kidney failure. Approximately 40% of adults undergoing maintenance dialysis treatment have evidence of PEW. This is a problem of great importance because indicators of PEW are strongly associated with morbidity and mortality, particularly mortality from cardiovascular disease. The causes of PEW include the following:

1. Decreased nutrient intake which may be caused by anorexia from uremia, superimposed illnesses, and emotional depression. The inability to procure, prepare or digest foods due to superimposed illnesses, psychiatric disease, debility, dementia or poverty may also decrease nutrient intake.
2. Nutrient losses into dialysate in patients undergoing maintenance hemodialysis (losses of about 1 g protein/dialysis, 3 - 4 g peptides/dialysis and 10 - 12 g amino acids/dialysis) and chronic peritoneal dialysis (losses of about 8 g protein/day, possibly 2 - 3 g peptides/day and about 2.0 - 3.5 g amino acids/day).
3. Increased net catabolic rate (i.e. difference between protein synthesis and degradation) caused by superimposed inflammatory, catabolic illnesses, which can be clinically apparent or clinically inapparent (e.g., no apparent clinical illness, but inflammation associated with renal failure per se, reactions to hemodialysis or peritoneal dialysis access catheters, dialyzer tubing, impure dialysate, transplanted kidneys that have failed, or unknown causes).
4. Uremic milieu which is caused by increased levels of counter-regulatory hormones (e.g. glucagon) or parathyroid hormone and resistance to anabolic hormones (insulin, growth hormone and IGF-1).
5. Increased blood or tissue levels of inflammatory mediators – catabolic cytokines (e.g., C-reactive protein, IL-6, TNF-alpha, IL-1), increased oxidant levels, decreased levels of anti-oxidants (vitamin E, vitamin C, selenium, and GSH), and carbonyl stress.
6. Acidemia.
7. Fecal nitrogen may be slightly increased.
8. PEW anteceding the development of chronic kidney failure.

Why is protein energy wasting associated with increased morbidity and mortality? The explanation is not entirely clear but may be due to the fact that measures of PEW in maintenance hemodialysis patients correlate strongly with indicators of inflammation. Indeed, evidence indicates that inflammatory processes may induce PEW by increasing the net catabolic rate and also by preceding anorexia with decreased nutrient intake. Thus, the chronic inflammation that occurs not uncommonly in patients with advanced chronic kidney failure may predispose to both PEW and also vascular disease and high mortality. Clinical trials are currently underway that will examine this question.

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H32

Role of intestinal functioning wasting disease**Herbert Lochs**

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The intestine can play two different roles in wasting-disease:

1. Intestinal functions like absorption, barrier-function and immune-function are disturbed by wasting-diseases as has been demonstrated in cachectic children in developing countries. Mucosal atrophy accompanied by severe malabsorption of carbohydrates, protein and fat develops. This damage is reversible, with recovery of the nutritional status. Similar changes have also been found in critically ill patients. Furthermore, the intestinal barrier-function is disturbed with an increase of permeability. This can lead to the translocation of bacterial toxins, like LPS and consequently to an inflammatory reaction. The degree of intestinal inflammation in this situation is unclear.
2. Disturbances in intestinal function may lead to wasting as it has been demonstrated in several wasting syndromes like AIDS, cardiac cachexia, liver-cirrhosis, inflammatory bowel disease and even malignancies. These diseases primarily lead to a disturbance of the intestinal barrier by different mechanisms. In liver-cirrhosis as well as in chronic heart-failure it seems that the elevated pressure in the portal-venous circulation leads to an edematous thickening of the intestinal mucosa associated with a partial loss of intestinal barrier with pathological permeability-tests, as well as adherence of bacteria to the mucosa. This also leads to an increased inflammatory infiltrate in the mucosa and sub-mucosal compartment. Secondary to these changes bacterial toxins like LPS and bacteria are translocated and initiate a systemic inflammatory reaction with increased TNF α and pro-inflammatory cytokines. As a consequence of this inflammatory reaction a reduction of food intake and an increase of energy expenditure develop, which eventually leads to weight-loss and wasting.

In many wasting diseases it is difficult to differentiate if the intestinal dysfunction is an initiating event, or a consequence of the disease. However, the intestine seems to play a central role in wasting and it would therefore be urgent to develop therapies to improve the intestinal barrier as one strategy to deal with cachexia.

Notes:

H33

Chronic heart failure**Piotr Ponikowski**

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The clinical syndrome of chronic heart failure (CHF) has traditionally been viewed as the disease of the ventricular myocardium. There is now mounting evidence to show that the complex pathophysiology of CHF begins within the heart, but later involves dysfunction of most body organs, including the cardiovascular, musculoskeletal, renal, neuroendocrine, haemostatic, immune, and inflammatory systems. These peripheral abnormalities become independently involved in the progression of the disease and generate symptoms.

Body wasting (i.e. cardiac cachexia) is a serious complication of CHF which may affect 10-15% of CHF patients during natural course of the disease. There is now evidence that cardiac cachexia in CHF is a generalised process that encompasses loss in all body compartments i.e. lean tissue (skeletal muscle), fat tissue (energy reserves) and bone tissue (osteoporosis). Pathophysiology of cachexia in the CHF syndrome still remains unclear and the following underlying mechanisms may be involved: poor nutrition and malabsorption, impaired calorie and protein balance, hormone resistance, proinflammatory immune activation, neurohormonal derangements, depletion in anabolic drive. All of them lead to severe dysregulation in catabolic/anabolic balance (in favour of catabolism), that typically characterises cachectic CHF patients.

Development of cachexia in the natural course of CHF syndrome usually coincides with severe symptoms of dyspnoea and weakness which further results in poor quality of life. Wasting is also related to very poor outcome, independently of the other established prognosticators in CHF. Mortality of cachectic CHF patients is higher than in most malignant disease as nearly half of these subjects are dead within 18 months. It has not yet been established whether prevention and treatment of cachexia complicating CHF syndrome is possible. Two classes of life-saving therapies in CHF – angiotensin converting enzyme inhibitors and beta-blockers, may be able to prevent development of cachexia. There are some promising options that may be considered to treat cardiac cachexia in CHF such as hypercaloric feeding, appetite stimulants, exercise training or anabolic agents. However at the current stage all of them need to be evaluated in the clinical trials and even more importantly cachexia itself should become accepted therapeutic target among clinicians treating patients with CHF.

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Notes:

H34

Chronic obstructive pulmonary disease**Matthias John**

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Chronic obstructive pulmonary disease (COPD) is an inflammatory disease. In the chronic course of the disease, the pathogenesis and clinical manifestations of COPD are not restricted to pulmonary structural and functional impairment but rather have multi-systemic implications including metabolic, hormonal and organ dysfunctions such as in skeletal muscle, heart, brain and skeleton. Weight loss due to tissue wasting is frequent in COPD and may ultimately lead to cachexia as a serious co-morbidity in advanced disease state. Cachexia in COPD - like in other chronic diseases - is associated with a greater susceptibility to exacerbation of clinical symptoms, with severely impaired functional capacity, poor health status and quality of life. The loss of skeletal muscle tissue and, in particular, of respiratory muscle is associated with a loss of power and endurance leading to a further mechanical impairment of lung function with consecutive hypoxia and hypercapnia. Inflammatory cell activation and hormonal derangements may further add to a hypermetabolic state and an overall catabolic/anabolic imbalance leading to depletion of endogenous energy storages and eventually to structural tissue degradation.

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Notes:

I35

ACE inhibitors**Marco Pahor**

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The disablement process is often accompanied by sarcopenia or muscle loss, which is associated with virtually all identified disability risk factors. Clinically, the association between body composition and physical performance has been documented by several studies. However, loss of strength is greater than loss of muscle mass with age implying that the quality of remaining muscle may be reduced. Although there are limited data explaining potential physiological mechanisms that contribute to muscle quality, sarcopenia is frequently associated with fat accumulation, and the percentage of body fat increases with age even if weight does not. However, the relationship between fat and muscle function may not be linear, suggesting that there may be an optimal ratio of lean to fat mass for physical function. There are no definitive pharmacological interventions proven to prevent decline in physical function either by modulating body composition or by other means. One exception may be angiotensin-converting enzyme inhibitors (ACEIs). ACE is an important component of the renin-angiotensin system, the central hormonal regulator of blood pressure. Recent evidence suggests that ACEIs may improve physical function by means of direct effects on body composition in older persons, rather than through its blood-pressure-lowering effects. Clinical and genetic studies in humans and experimental evidence in animals suggest that modulation of the renin-angiotensin system is associated with metabolic and biochemical changes in skeletal muscle and fat, changes that are associated with declining physical function. ACEIs may modulate this process through a variety of molecular mechanisms including their influence on oxidative stress and on metabolic and inflammation pathways. We describe potential biological mechanisms of ACE inhibition and its contribution to declining physical performance and changing body composition. Promising pharmacoepidemiological studies and experimental evidence in animals suggest that there are appropriate models in which to study this effect.

Notes:

I36

Nitric oxide: friend or foe in cachexia**Thomas Thum**

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Cachexia is a consequence of chronic diseases and is associated with high amounts of local and circulating cytokines, such as TNF alpha, IL1beta and gamma interferon. Important involved molecular pathways include nuclear factor kappa B mediated activation of its downstream target gene inducible nitric oxide synthase (iNOS) that leads to a degradation of muscle specific proteins and transcription factors, such as MyoD, finally resulting in muscle wasting (e.g. Di Marco et al., Mol Cell Biol, 2005). Indeed, iNOS expression in muscular tissues of cachectic patients (COPD, heart failure, cancer) is usually induced, whereas specific iNOS inhibitors were shown to prevent onset of cachexia in certain tumour animal models, whereas in other types of cancer overexpression of iNOS resulted in cancer cell apoptosis and tumour regression (for Review see Fukumura et al., 2006, Nature Reviews). In further contrast are other disease models of cachexia such as experimental colitis or chronic heart failure, where NO production by eNOS plays an important role in limiting cellular injury and weight loss.

Various types of cachexia are related to reductions of systemic or local IGF-1 expression (e.g. heart failure). Infusion of IGF-1 or exercise training leading to increased local IGF-1 expression prevent increased protein breakdown and iNOS expression in cachexia, demonstrating a profound anticatabolic effect of IGF-1. In addition, IGF-1 leads to an increase of eNOS expression, overall NO bioavailability and mobilization of endothelial progenitor cells (Thum et al., Circ Res, 2007; Thum et al., J Clin Endocrinol Metab, 2007), which might prevent progression of certain forms of cachexia.

The differences in the effects of NO in cachexia either produced by iNOS or eNOS may be due to differences in NO distribution, enzyme localization, dose and exposure duration to tissues, as well as spatial and temporal activation of NOS isoforms. Increased iNOS activity may produce toxic levels of NO that inhibit key enzymes in muscle catabolism and impair contractile performance of skeletal muscle. Treatment with either specific NO donors or inhibitors may be useful in preventing cachexia, dependent to the cause (e.g. use of iNOS inducers or eNOS inhibitors in cancer cachexia versus eNOS stimulation and iNOS inhibition in chronic heart failure).

Notes:

I37

Ghrelin and ghrelin analogs

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The discovery of ghrelin, the natural ligand for growth hormone secretagogue-1a (GHS-1a) receptor, led to new insights into how this novel hormone connects gut, brain and other organs to regulate not only GH secretion, but also multiple other functions including energy balance and metabolism. Consequently, ghrelin is now viewed, not just as a GHS, but also as a key modulator of energy homeostasis, and, as such, is an attractive target for indications with impaired energy balance. In catabolic conditions such as cachexia associated with various sub-acute and chronic disorders, therapeutic intervention with ghrelin may induce a combination of enhanced food intake, increased gastric emptying and food assimilation, coupled with an increase in GH that would promote nutrient incorporation into muscle and fat reserves. To potentially exploit the anti-cachexic properties of ghrelin for therapeutic utility, we have produced analogs of ghrelin with an emphasis on efficacy in increasing body weight (BW) gain. From these efforts, we have developed a novel ghrelin receptor agonist, BIM-28131. BIM-28131, a conformationally constrained 5-amino acid peptide, is highly potent in binding and activating the GHS-1a receptor. BIM-28131 shows enhanced plasma stability and is observed to have a 10-fold greater circulating half-life than native ghrelin. It increases GH secretion with similar efficacy as natural ghrelin. BIM-28131 is also highly efficacious in increasing weight gain in normal rats, with a unique, biphasic pattern of activity. Beagle dogs treated with BIM-28131 display significantly increased food intake, BW gain and circulating IGF-1 levels. BIM-28131 has been demonstrated to have dual anti-inflammatory properties; it decreases the levels of circulating inflammatory cytokines as well as inhibits their action. The evaluation of BIM-28131 in a rat model of chronic heart failure, demonstrated that BIM-28131 is more efficacious than native ghrelin in increasing BW gain. In addition to the metabolic effects, BIM-28131-treated rats also showed trends of improved cardiac performance. In addition, in rat models of both prostate cancer and renal failure, we have demonstrated that BIM-28131 can reverse the associated cachexia. In summary, the unique efficacy of BIM-28131 in stimulating BW gain in both normal and catabolic states, its dual anti-inflammatory actions, as well as its enhanced plasma stability and smaller molecular size, make BIM-28131 an excellent candidate for therapeutic intervention in cachectic conditions.

Notes:

I38

Immune modulation**Anthony Cerami**

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The old adage "the cure is worse than the disease" regrettably is true with respect to the body's own response to injury or invasion by invaders, e.g. bacteria, viruses, parasites, tumors. In an attempt to stop the spread of tissue damage from a localized, primary site of insult following invasion or injury, the body needlessly targets a large area of tissue surrounding the injury for destruction by the release of protein pro-inflammatory cytokines like TNF. This leads to greater impairment and to a slow, limited recovery and metabolic derangement e.g. anorexia, catabolic state, anemia, malaise (cachexia). This natural defense mechanism evolved long ago to protect the individual from succumbing to invasion by killing the surrounding tissue and the invader. This secondary damage of tissue and the ensuing cachexia serves no survival function and is in fact responsible for the further deterioration of the individual.

Recently Erythropoietin (EPO), the hormone stimulating the production and differentiation of red blood cells, which is widely used for treating anemia of renal disease or anemia of chronic disease has been found to counteract the actions of TNF and reduce the secondary damage to tissues.

In order to avoid the hematopoietic effects of EPO, derivatives of EPO have been identified which separate the blood cell stimulating and tissue protective activities of EPO. This is possible because there are two biologically distinct functions of EPO through its interaction with two different types of receptors. These compounds are being evaluated in several clinical settings.

Notes:

K39

Epidemiology of sarcopenia**Richard N Baumgartner**

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Skeletal muscle mass loss appears to begin around 40 y; rates of loss are higher in men than women and accelerate after age 70 y. Thus, the prevalence of sarcopenia, or low relative skeletal muscle mass, increases from about 8 to 10% at 60 years of age to > 30% in the ninth decade. "Sarcopenic-obesity" is a rarer condition in which an individual has an excess of body fat, or obesity, combined with lower than expected muscle mass, or sarcopenia. The prevalence of this condition also increases with age from about 3% at 60 y to nearly 15% in the ninth decade.

There are few data for risk factors associated with sarcopenia or sarcopenic-obesity. Most studies have been cross-sectional and have not been able to distinguish causes from consequences. Methods of defining sarcopenia have varied, complicating direct comparisons of results. We first reported associations with self-reported disability, problems with balance and gait, falls in the past year, smoking, and physical inactivity in our studies in New Mexico (Baumgartner et al., 1998). Melton et al., (Melton et al., 2000) subsequently reported significant associations with difficulty walking in men and osteoporotic fractures in women. The Rancho Bernardo Study reported that sarcopenic men were twice as likely to have fallen in the past year (Castillo et al., 2003). Sarcopenia was significantly associated with functional limitation in the HEALTH ABC study (Newman et al., 2003). We found significant associations with physical impairment and self-reported disability in NHANES III (Janssen et al., 2004). Szulc et al. reported that low physical activity, tobacco smoking, thinness, low testosterone (AFTC and FTI), and decreased 25(OH)D concentrations were risk factors for sarcopenia (Szulc et al., 2004). Sarcopenia was most recently reported to be associated with cigarette smoking, chronic illnesses, underweight, physical inactivity, poorer well-being and upper limb physical performance in older Chinese (Lee et al., 2007). We found that sarcopenic-obesity was more strongly associated than sarcopenia with incident disability in old age in the New Mexico Aging Process Study (Baumgartner et al., 2004). New analyses of these prospective data suggest that the relative risk of death is 1.8 ($p < 0.05$) in elderly men and women with sarcopenia over 5 years of follow-up.

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Notes:

K40

Sarcopenia: general pathophysiology**Luigi Ferrucci**

National Institute on Aging, Baltimore, MD, USA

Aging is associated with a progressive decline of muscle mass, composition and strength, leading to a severe condition described as sarcopenia of aging. The number of fibers in a given muscle is determined at birth and changes little throughout the life span except in cases of injury or disease. Sarcopenia, in fact, consists in a reduction of the number of myofibrils, and, consequently muscle fiber cross-sectional area and strength. This process is almost universal, and involves most animal species, as well as among healthy, physically active adults. The rate of decline in mass is about 1-2% per year, and can be substantially accelerated by pathological conditions and lack of exercise. Given the lack of a standard, widely accepted definition, the true prevalence of sarcopenia is unknown, but can be estimated as 25-30% in the general population 65 and older.

Studies have suggested that multiple contributing factors are at the root of sarcopenia, including neuronal and hormonal changes, excessive and unopposed oxidative stress, inadequate nutrition, a pro-inflammatory state of aging, and physical inactivity. Over the last few years, a number of studies have provided evidence for the importance of each one of these mechanisms, but there has been little attempted to connect them as an interacting network. The muscle tissue is constantly in a dynamic equilibrium between removal of old, damaged or dysfunctional cell material and development of new cell material from highly specialized stem cells (satellite cells and possibly bone marrow cells). Thus, it is generally hypothesized that the risk factors for accelerated sarcopenia and, to same extent, also the aging process, disrupt this perfect equilibrium toward the catabolic side. It is also postulated that the muscle tissue has a natural tendency to become atrophic, and maintain its mass or function because of a background continuous stimulation and the anabolic effect of muscle contraction (use it or lose it principle). In accordance with this hypothesis, bed rest and muscle denervation are powerful causes of sarcopenia. However, evidence is accumulating that sarcopenia is neither obligatory nor irreversible. In animal models, the knockout of the IKK protein, a protein that is required for the activation of the NF- κ B signaling and consequent inflammatory response, blocks the development of muscle atrophy after prolonged immobilization and even after denervation. Observation of hibernated bears in the wild, show very little muscle atrophy and almost no loss of function over a 6-month of relative immobility. At least 25 participants of the Baltimore Longitudinal study of Aging show no detectable decline of muscle strength over a 30 years follow-up period. Finally, recent experiments of parabiosis between genetically identical old and young mice suggest that the quality and quantity of muscle repair is more related to the circulating environment, possibly because of specific and still unknown growth factors, than to the age of the tissue and of the stem cells. This emerging evidence suggests that age-related sarcopenia can be prevented, and perhaps even cured.

Notes:

K41**Cell death regulation and ageing****Volker Adams**

Heart Center Leipzig, Leipzig, Germany

The loss of muscle mass and strength with ageing, also referred to as sarcopenia, is a highly prevalent condition among older adults and predicts several adverse outcomes.

Although the exact mechanisms underlying sarcopenia are far to be unveiled, accumulating preclinical evidence suggests that an age-related acceleration of myocytes loss via apoptosis might represent a key mechanism driving the onset and progression of muscle loss. Notably, preliminary evidence seems to confirm a causative role for apoptosis in age-related muscle loss in human subjects. Several signaling pathways of skeletal muscle apoptosis are currently under investigation, with a particular focus on the role played by mitochondria and tumor necrosis factor alpha (TNF- α). TNF- α is elevated in the serum as a result of aging and it promotes pro-apoptotic signaling upon binding to the type I TNF receptor. Several evidences will be provided that factors regulating either TNF- α induced or mitochondrial triggered apoptosis are significantly altered in the skeletal muscle of older people.

Besides the activation of apoptosis, the induction of muscle specific E3 ubiquitin ligases like Murf-1 and MAFbx (atrogin-1) has to be considered as a step in muscle fiber loss, finally leading to sarcopenia. Cell culture as well as in vivo animal experiments demonstrate that Murf-1 and MAFbx are activated by TNF- α , thereby leading to an increase in ubiquitinated proteins, which are possibly scheduled for protein degradation via the ubiquitin proteasome system. Evidence will be provided that this system is activated in older individuals, and that alterations of Murf-1 and MAFbx expression has an impact on muscle mass.

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Notes:

K42

Clinical insights into sarcopenia**Cornel Christian Sieber**

University of Erlangen-Nürnberg, Department for Geriatric Medicine, Germany

Malnutrition in Western societies is an age-associated phenomenon, where sarcopenia as its clinical face is a cornerstone of frailty in the old and especially oldest old. Muscle loss seen in the elderly is partly preventable and by that is probably one of the distinction when compared with cachexia, the main topic of this meeting.

Frailty as defined by Fried (1) shares many pathophysiological similarities with sarcopenia. They both have in common that they interfere with the functionality of the elderly, thereby diminishing independence and as a consequence quality of life. Whereas the fall syndrome is closely linked to sarcopenia, other consequences of sarcopenia are less explored such as heart failure (the myocardium is also a muscle!), as well as sarcopenia of the chest wall with regard to the propensity of old people for chest infections. These few examples should demonstrate that sarcopenia as the most important clinical picture of malnutrition in the elderly has eminent consequences for both the people affected as well as for health care expenditures as a whole in the old and especially oldest old.

In addition, both frailty and sarcopenia show signs of chronic (sub)clinical inflammation. Factors perpetuating these inflammatory processes seem to be alteration in hormonal homeostasis and synthesis-action (2), but also an increase in inflammatory cytokines such as interleukin-6 interfering with functional loss (3). This triade of chronic disease (or in most instances diseases as part of the multimorbidity of the elderly), weight loss and signs of inflammation brings it near to pathophysiological processes also seen in cachexia. It is therefore of importance to discuss common backgrounds and differences between these clinical entities in order to define who can profit of nutritional intervention.

Nevertheless, the main aim of the presentation is to show how these alterations influence the clinical presentation of frail elderly persons and how preventive and therapeutic strategies may prove beneficial in the group of persons. What can be said for sure by now is that successful therapeutic strategies in these patients always combine physical exercise with nutritional supplementary support.

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Notes:

K43

Short plenary poster presentation

The effect of weight loss on mobility and frailty in the community-dwelling elderly

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To evaluate the correlation of BMI (Body Mass Index) with parameters of frailty and mobility in community-dwelling older people according to age category.

Data from the Belgian Health Interview Surveys of 1997, 2001 and 2004 (n=37.387) are used. Frailty is measured with the VIP (Variable Indicative of Placement)-tool, which gauges “living alone”, need for assistance with washing and dressing, and mobility outside the own neighborhood. People are then assigned to a high or low-risk group for frailty. Mobility is assessed by limitation in transfers and in walking distance. The relation between BMI, frailty and mobility is examined. To this end, Chi-Square analysis and logistic regression is applied using SPSS for Windows 14.0.

The cohort for evaluation contains 6515 people over 65 years of age out of 37.387. There is a continuous shift of BMI to lower values with increasing age. In the 85+ cohort 9.6% of the participants have a BMI lower than 18.5 compared to 1.8% in the 65-69 yr group (p ≤ 0.001). Mobility problems and risk for frailty score significantly higher in the lower BMI classes (cfr Table: data for all 65+).

BMI	< 17	17 – 18.5	18.5 - 25	25 - 30	30 – 40	> 40
N (total=6515)	75	139	2813	2512	940	36
Severe limitation in mobility: “a few steps” (%)	21.2	23.1	8.6	7.6	14.0	38.0
Normal walking distance: > 200 m (%)	35.9	51.8	75.9	77.5	66.0	42.9
Frailty (%)	30.3	41.8	14 .4	11.6	18.5	35.9

There is a progressive loss of weight with aging. This weight loss is significantly correlated with frailty and loss of mobility in community dwelling elderly.

Notes:

L44

Feeding and wasting disease: the role of nutraceuticals**Connie W Bales**

Duke University, Center for the Study of Aging and Human Development, Duke Medical Center, Durham, NC, USA

While recognizing that factors other than dietary intake figure most prominently in the etiology of wasting disease, nutritional interventions may nonetheless have the potential to ameliorate some of the consequences. However, traditional interventions (e.g., dietary support, protein/energy supplements, tube feedings) have been largely unsuccessful in improving ultimate outcomes related to mortality. Thus, interest in the use of specific nutrients and other bioactive food components (i.e., nutraceuticals) as innovative, targeted approaches to therapy has led to several avenues of clinical study. Major classes of nutrients under study include fish oils (specifically, the predominant omega 3- fatty acid, eicosapentaenoic acid or EPA) and amino acids (especially the branched chain amino acids or BCAA, glutamine, and the related compound, carnitine). While some promising findings have been reported, the field is not at the point of providing clinical recommendations. Because of the complexity and severity of the wasting process and the difficulty of dealing with nutritional deficits along with other metabolic effects, nutraceuticals are increasingly being studied in combinations with traditional interventions and/or with each other. This approach may lead more quickly to the discovery of a beneficial intervention regimen but, obviously, will be slower in yielding a mechanistic understanding of nutraceutical effects. In addition, there are some concerns about the potential for certain nutraceuticals (e.g., BCAA could promote tumor growth) to do harm as well as good. The field of nutraceutical study thus remains at the experimental stage, but could hold promise for health benefits for cachexia in the near future.

Notes:

L45

Eicosapentaenoic acid, BCCA and carnitine**Filippo Rossi Fanelli**

La Sapienza University Rome, Department of Clinical Medicine, Italy

The clinical course of many chronic and acute diseases is frequently characterized by the development of anorexia, i.e., decreased appetite leading almost invariably to reduced food intake. Although anorexia is frequently considered as a symptom contributing to the diagnosis of cachexia, this approach is misleading. Actually, anorexia should be considered as a syndrome per se. Indeed, it is found in different chronic and acute diseases, ranging from cancer to chronic renal failure, and from liver cirrhosis to heart failure. Also, it is characterized by a number of symptoms (i.e., early satiety, nausea, vomiting, changes in smell/taste), and specific diagnostic tools exist. Finally, it represents an independent prognostic factor for survival. Considering anorexia and cachexia as separate entities reflects the basic science approach to the issue of wasting, based on the study of suitable experimental models which are mainly either anorexic or cachectic. On the clinical grounds, it is difficult to find patients who are just anorexic or just cachectic, but the two syndromes almost invariably coexist. In this light, the use in the clinical setting of the term "anorexia-cachexia syndrome", as already used for decades, appears more reflecting the clinical reality. Interestingly, disease-associated anorexia and cachexia are closely linked by their pathogenic mechanisms, which involve the onset of the inflammatory response. As far as anorexia is concerned, cytokine-driven neuroinflammation appears to reduce the sensitivity of the hypothalamic food intake regulating areas to the inputs arising from the periphery and to stimulate the hypothalamic anorexigenic pathway, which include the serotonergic system. Consequently, the use of anti-inflammatory agents may prove beneficial in anorexic patients. Eicosapentaenoic acid (EPA) is an n-3 polyunsaturated fatty acids with anti-inflammatory properties. In experimental models, EPA-supplemented rat chow has been demonstrated to significantly improve food intake of tumour bearing rats. In humans, and particularly in cancer patients, the results obtained are more controversial since in the clinical setting the issue of the dose, timing and duration of supplementation are critical factors in influencing the effects. Considering the involvement of the serotonergic system in the pathogenesis of disease-associated anorexia, brain depletion of serotonin or its precursor, 5-OH-tryptophan, may improve food intake. Branched-chain amino acids (BCAA) have been shown to reduce the entry into the brain of the precursor of serotonin. Experimental and clinical studies consistently demonstrated that BCAA supplementation ameliorates anorexia in different diseases. Recent data show that food intake is also controlled in the brain by energy metabolism and particularly by mitochondrial function. During disease, hypothalamic mitochondrial function might be altered, leading to increased leakage of protons. This would increase the production of reactive oxygen species (ROS) and lead to increased oxidative stress. Oxidative stress may contribute to anorexia per se and by triggering/sustaining the inflammatory response. Carnitine is a nutrient with vitamin-like properties, including an antioxidant effect. Preliminary experimental and clinical studies show that carnitine supplementation improves food intake and ameliorates body composition during cancer, by reducing the inflammatory response.

Anorexia is a critical component of the anorexia-cachexia syndrome associated to different clinical conditions. The better understanding of its pathogenic mechanisms is leading to the development of more targeted and, hopefully, more effective treatments.

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Notes:

L46

Orexigenics**David R Thomas**

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Undernutrition is defined as a state induced by nutrient deficiency that may be improved solely by administration of nutrients. By this definition, provision of adequate protein and energy sources should reverse the clinical presentation and correct the problem. However, a large number of patients who appear to be undernourished fail to respond to refeeding. A developing understanding of the acute phase inflammatory response to illness and the role of cytokines in the pathophysiology of chronic illness has challenged the current diagnostic paradigm of undernutrition.

In the presence of adequate food, weight loss is most often due to cytokine-associated cachexia and anorexia. Failure of appetite, or anorexia, may play a role in involuntary weight loss. Assessment of changes in appetite are essential to evaluating older persons with weight loss. When anorexic changes are identified, a search for reversible causes should be instituted.

Intervention for involuntary weight loss should aim first at the provision of adequate calories and protein, often in the form of high-density nutritional supplements. However, the chief difference between starvation and cachexia is that refeeding reverses starvation, but is less effective for cachexia. Cytokine-mediated cachexia is remarkably resistant to hypercaloric feeding.

With continued weight loss, the use of an orexigenic drug should be considered. Orexigenic drugs have been demonstrated to improve appetite and produce weight gain. The mechanism is unknown, but may relate to suppression of proinflammatory cytokines. General guidelines for the use of orexigenic agents are presented. Although much work remains to be done, anti-cytokine drugs appear to be a promising avenue for the treatment of involuntary weight loss.

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Notes:

L47

Regulation of feeding**Daniel L Marks**

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Under normal circumstances, body weight is regulated with remarkable precision. Indeed, body mass is regulated to within 0.5-1% per year under basal conditions, even in the face of disturbances in energy balance brought about by changes in food intake or exercise. The mechanism whereby this fidelity is maintained has been the subject of an intense research effort. Indeed, a great deal of progress has been made in understanding the central and peripheral mechanisms of body weight control, particularly in the context of obesity. In contrast, cachexia is a state of energy imbalance that accompanies many chronic diseases, producing wasting of lean body mass, increased energy expenditure and a paradoxical loss of appetite. Conditions as diverse as cancer, renal failure and heart failure show a remarkable similarity regarding the symptoms associated with cachexia, the increase in cytokines that accompanies these diseases and the difficulty in successful treatment of the resultant metabolic derangement. This talk will focus on the basic mechanisms of central appetite regulation, and how these systems are affected by chronic illness. Particular attention will be paid to a discussion of the central melanocortin system, an anorexigenic pathway in brain. Humans who have genetic mutations involving Pro-opiomelanocortin or the melanocortin-4 receptor in this pathway exhibit increased appetite and increased lean body mass. Recent research has shown that in models of cancer and renal failure, administration of melanocortin-4 receptor antagonists results in an attenuation of symptoms of cachexia, including a maintenance of appetite, lean body mass and basal energy expenditure. Though this research needs to be substantiated in humans, it provides a promising direction for treating the wasting that is associated with a variety of disease states. We will also discuss how peripheral anorexigenic and orexigenic hormones interact with the central melanocortin system to regulate appetite. It has been shown that long-term treatment with the orexigenic gut peptide ghrelin improves food intake and lean body mass retention in rats with cachexia due to cancer or chronic renal failure. Early clinical trials indicate that this may be of therapeutic benefit in humans with cachexia as well.

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Notes:

M48

Selective androgen receptor modulators as function promoting anabolic therapies**Shalender Bhasin**

Boston University School of Medicine, Boston, MA, USA

Testosterone supplementation increases whole body and appendicular skeletal muscle mass, maximal voluntary muscle strength, and leg power. However, concerns about the long term risks of prostate and cardiovascular disorders in older men treated with testosterone have encouraged efforts to develop selective androgen receptor modulators (SARM) that have the desired anabolic effects on the muscle, but that do not have adverse effects on prostate and cardiovascular outcomes. These nonsteroidal SARMs do not serve as substrates for CYP19 aromatase or 5 α -reductase, act as full agonists in muscle and bone and as partial agonists in prostate and seminal vesicles. The differing interactions of steroidal and nonsteroidal compounds with the AR may at least partially contribute to their unique pharmacologic actions. Bicalutamide adopts a greatly bent conformation in the AR. Although A-ring and amide bond of the bicalutamide molecule overlaps the steroidal plane, the B-ring of the molecule folds away from the plane, pointing to the top of the ligand binding pocket (LBP), which forms a unique structural feature of this class of ligands. These H bonding interactions are believed to be critical for high binding affinity. Structural modifications of aryl propionamide analogs bicalutamide and hydroxyflutamide led to the discovery of the first generation of SARMs. SARM pharmacophores can be classified into four categories: aryl-propionamide, bicyclic hydantoin, quinoline, and tetrahydroquinoline analogs.

The mechanistic basis of the tissue selective actions of SARMs is poorly understood, although several mechanisms have been proposed. Ligand binding induces specific conformational changes in the ligand binding domain, which could modulate surface topology and subsequent protein-protein interactions between AR and other coregulators involved in genomic transcriptional activation or cytosolic proteins involved in nongenomic signaling. Differences in ligand-specific receptor conformation and protein-protein interactions could result in tissue-specific gene regulation, due to potential changes in interactions with ARE, coregulators or transcription factors.

It is generally believed that the downstream signaling mechanisms that mediate the anabolic effects of SARMs on the skeletal muscle are similar to those of testosterone. Testosterone induces hypertrophy of both type I and type II fibers and an increase in the number of satellite cells. Testosterone promotes the differentiation of mesenchymal, multipotent cells into myogenic lineage and inhibits their differentiation into adipogenic lineage. Testosterone and DHT regulate mesenchymal multipotent cell differentiation by promoting the association of AR with β -catenin and translocation of the AR- β -catenin complex into the nucleus, resulting in activation of TCF-4. The activation of TCF-4 modulates a number of Wnt-regulated genes that promote myogenic differentiation and inhibit adipogenic differentiation. The effects of testosterone on myogenic differentiation are mediated through an AR pathway. Testosterone increases fractional muscle protein synthesis and improves the reutilization of amino acids by the muscle. We do not know whether conversion of testosterone to DHT is required for mediating androgen effects on the muscle.

Preclinical studies have demonstrated the ability of SARMs to increase levator ani muscle mass in the castrated rat and to increase bone mass and strength. Efficacy trials of several SARMs in humans are in early stages. The first generation SARMs do not undergo aromatization or 5- α reduction; it is unknown whether this may pose long term risks. The efficacy and the safety of SARMs as function promoting therapy is just beginning to be evaluated.

Notes:

M49

Mechano growth factor / IGF-1 and cachexia**Geoffrey Goldspink**

Departments of Surgery and Anatomy, Royal Free and University College Medical School, London University, UK

Age-related muscle wasting and increased frailty is a major socioeconomic as well as a major medical problem. In our quest to extend the quality of life it is important to increase the strength of elderly people sufficiently so they can carry out every day tasks and to prevent them falling and breaking bones that are brittle due to osteoporosis and the maintenance of other musculoskeletal tissues. At the present time there are proposed strategies in addition to exercise for preventing age-related muscle wasting and these will be briefly mentioned. Here, more attention is paid to the role of the GH/IGF-1 axis and the discovery of mechano growth factor (MGF). The latter is derived from the IGF-I gene by alternative splicing and in the young is associated with increasing contractile strength in response to exercise. This involves activating the muscle satellite (progenitor) cells that kick start local muscle repair and induce hypertrophy. Following a bout of resistance exercise the IGF-I gene is initially spliced to MGF which due to a reading frame shift has a unique C-terminal peptide for which the natural version has a short half life. Recent studies using human primary cultures have shown that this MGF C-peptide activates muscle satellite (progenitor) cells in normal human muscle. Interestingly although the initial yield of these cells was less from dystrophy and ALS patients the numbers were increased by MGF C-peptide within 48 hr. During the second phase following the exercise the IGF-1 gene is spliced to IGF-1Ea, which is the main source of anabolic agent although the other function of IGF-I is to induce the progenitor cells to enter the myogenic pathway and to fuse with the muscle fibres. With the increased number of nuclei and gene copies, IGF-I which is a major metabolic agent increases protein synthesis for the second stage of muscle repair and hypertrophy. During ageing growth hormone levels decline markedly and administration of hGH appears to upregulate the number of primary transcripts of the IGF-I gene so more MGF and IGF-1Ea can be produced in older people by exercise. The unique MGF peptides offer the prospect of treating muscle wasting during the ageing process as muscle cachexia that is associated with many diseases.

Notes:

M50

Ghrelin**Akio Inui**

Department of Behavioral Medicine, Kagoshima University Graduate School of Medical and Dental Sciences

Anorexia/cachexia is a prevalent syndrome in cancer patients in which multiple mechanisms have been implicated. Given the prevalence and the severity of the state leading to poor prognosis and early death, effective treatment is a critical component of patient management. Ghrelin is an orexigenic hormone secreted from the stomach and plays an important role in regulating energy balance. Anorexia/cachexia in cancer patients is associated with insufficient secretion and/or action of ghrelin. Several studies demonstrate that ghrelin increases food intake and body weight in animal models of cachexia and in some cancer patients. Ghrelin, ghrelin agonists or ghrelin-releasing compounds may offer a new therapy for the treatment of cancer anorexia/cachexia.

Serotonin selective reuptake inhibitor (SSRI) is widely used for treatment of depression and anxiety, including those associated with cancer. However, the side effects of the drug including anorexia limit the use of the drug in cancer patients. We examined the effects of SSRI on gastrointestinal (GI) motility and food intake and whether ghrelin has a beneficial effect on these in rats.

We intraperitoneally administered fenfluramine and other SSRIs to rats and measured food intake, gastroduodenal motility and gastric emptying. A strain gauge force transducer was implanted on the wall of gastric antrum and duodenum to measure gastroduodenal motility in a conscious state. Phenol red retention in the stomach at 15 min was calculated to assess the gastric emptying. Plasma acyl ghrelin levels were determined by EIA (Mitsubishi, Japan).

Fenfluramine diminished fasting-induced food intake up to one third of the control at 1 hour and changed motor activity from fasted to fed-like pattern, with decreased phase III-like contractions, motility index and gastric emptying rate. Fenfluramine and other SSRIs decreased plasma acyl ghrelin levels while ghrelin improved the decreased food intake and GI motility. These results indicate that SSRI may affect appetite and GI motility through a decrease in endogenous ghrelin. Insufficient secretion and/or action of ghrelin should be considered to be associated in various ways with anorexia/cachexia in cancer patients. Some drugs such as Rikkunshito, a traditional Japanese medicine, may improve SSRI-induced anorexia and decrease in GI motility through an increase of endogenous ghrelin.

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Notes:

M51

Measuring the response to anabolic therapies by use of kinetic biomarkers**Marc Hellerstein**

University of California at Berkeley, University of California at San Francisco, CA, USA

Cachexia syndromes are characterized by loss of lean body mass and typically an altered capacity to deposit muscle as compared to fat in response to increased nutrient intake. I will discuss the use of kinetic biomarkers for development and testing of anabolic therapies in cachexia. Kinetic biomarkers are defined as measures of flux through metabolic pathways, including biosynthetic pathways, in living systems. Muscle protein synthesis, adipose tissue dynamics, inflammation/anabolic block and mitochondrial biogenesis will be reviewed.

The recently developed $^2\text{H}_2\text{O}$ (heavy water) labeling technique for measuring protein synthesis rates has a number of advantages over previous methods. The effects of denervation (nerve crush, ALS), glucocorticoid therapy, inflammation and the response to anabolic therapies have been sensitively detected. Mass isotopomer distribution analysis (MIDA) of peptides from hydrolyzed proteins represents another accurate approach. A combined $^2\text{H}_2\text{O}$ /MIDA approach is an analytically advanced but powerful strategy.

The dynamics of adipose tissue components (triglycerides, fatty acid synthesis, and cell proliferation) can be measured concurrently by $^2\text{H}_2\text{O}$ labeling. Examples of adipose tissue dynamics in response to dietary and drug interventions (e.g., leptin) will be shown. Changes in metabolic fluxes precede and predict subsequent changes in adipose mass. Interestingly, gene expression data consistently fail to reflect actual metabolic fluxes in adipose tissue.

Biomarkers of inflammation/anabolic block have proven useful in certain wasting conditions. Hepatic *de novo* lipogenesis is a sensitive index of systemic cytokine presence and predicts change in LBM in response to nutritional therapy in HIV/AIDS. Finally, mitochondrial (mt) biogenesis can be measured by $^2\text{H}_2\text{O}$ labeling of mtDNA or phospholipids. The response of mtDNA synthesis is different for treadmill exercise vs. voluntary wheel running in rats. Changes in mtDNA replication precede and are more sensitive than changes in mt enzyme content.

In summary, measurements of biosynthetic rates are more sensitive and detect therapeutic response earlier than static metrics. Several new kinetic biomarker assays are ready for translation into human studies. Potential roles of biomarkers for drug evaluation include generating rapid proof-of-concept data in Phase IB studies; optimization of dose and regimen; and selection of best lead compound in a class. The actual value of kinetic biomarkers in evaluation of anabolic therapies remains to be established, however.

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N52

“Hippocrates” clinical research in cachexia lecture: the metabolic basis of muscle loss**Robert R Wolfe**

University of Arkansas for Medical Sciences, College of Medicine, Center for Translational Research in Aging & Longevity, Little Rock, Arkansas, USA

Over the past 25 years my laboratory has, with the collaboration of a number of clinical investigators, quantified the responses of protein synthesis and breakdown in a variety of patients suffering the effects of a rapid loss of muscle protein not readily reversible by nutrition (ie, cachexia). In a variety of forms of cancer, net protein loss in the post-absorptive state is evident even in the early stages. Net protein loss is greater in advanced cancer due to a significant acceleration of protein breakdown that is not countered by a corresponding increase in synthesis. In trauma, sepsis, and severe burns, with or without sepsis, the same general response is evident. Protein breakdown is accelerated, and synthesis may or may not be elevated as well. The elevated rates of synthesis in these clinical situations can be attributed to an increased availability of amino acids in the intracellular pool as a consequence of the stimulated rate of protein breakdown.

A variety of factors can potentially cause the response of protein metabolism in cachexia. These include, but are not limited to, anorexia, inflammation, reduced tissue blood flow, inactivity, age, and hormonal response. In a series of experiments we have assessed the response to these potential components of cachexia. In contrast to the clinical situation, in most cases including reduced availability of amino acids (simulated anorexia), infusion of tumor necrosis factor, and inactivity, net loss of muscle protein results from a suppression of synthesis rather than accelerated breakdown. Similarly, the catabolic hormone glucagon increases the net loss of protein when infused into normal volunteers as a result of suppressed protein synthesis. The interactive nature of the various components of cachexia is evident when the situation of glucagonoma (glucagon-secreting tumor) is considered. Whereas increased glucagon in normal volunteers suppresses synthesis, when glucagon is increased as a result of secretion by a cancerous tumor, then synthesis is elevated, but not to the same extent as the accelerated rate of breakdown. In an analogous situation, a relatively low rate of cortisol infusion alone induced no significant change in either synthesis or breakdown, and bed rest alone induced net loss of protein as a consequence of a reduction in the rate of muscle protein synthesis (with no change in breakdown), but an increase in cortisol in subjects restricted to bed rest significantly stimulated breakdown and to a lesser extent, synthesis was increased as well. Similarly, while the individual hormone infusions of glucagon, cortisol and epinephrine did not significantly affect the rate of breakdown, when infused together muscle protein breakdown was significantly stimulated, and synthesis responded similarly only to a lesser extent.

The conclusions of this talk are that cachexia results from a stimulation of muscle protein breakdown, and that synthesis may or may not be increased, but is not decreased. The causes of cachexia are multifactorial and, importantly, interactive, meaning that their combined effects are not merely the sum of their individual effects.

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Notes:

N53

Muscle wasting in cancer and ageing: cachexia versus sarcopenia**Josep M Argilés**

Departament de Bioquímica i Biologia Molecular, Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain

Cancer cachexia is a syndrome characterized by a marked weight loss, anorexia, asthenia and anemia. In fact, many patients who die with advanced cancer suffer from cancer cachexia. The degree of cachexia is inversely correlated with the survival time of the patient and it always implies a poor prognosis. Unfortunately, at the clinical level, cachexia is not treated until the patient suffers from a considerable weight loss and wasting. At this point, the cachectic syndrome is almost irreversible. The cachectic state is often associated with the presence and growth of the tumour and leads to a malnutrition status due to the induction of anorexia or decreased food intake. In addition, the competition for nutrients between the tumour and the host leads to an accelerated starvation state which promotes severe metabolic disturbances in the host, including hypermetabolism which leads to an increased energetic inefficiency. Although the search for the cachectic factor(s) started a long time ago, and although many scientific and economic efforts have been devoted to its discovery, we are still a long way from knowing the whole truth. A lot of progress has been made, however, and the suggested mediators (associated with both depletion of fat stores and muscular tissue) can be divided into two categories: of tumour origin (produced and released by the neoplasm) and humoral factors (mainly cytokines). In recent years, age-related diseases and disabilities have become of major health interest and importance. This holds particularly for muscle wasting, also known as sarcopenia, that decreases the quality of life of the geriatric population, increasing morbidity and decreasing life expectancy. Weight loss is a major problem that increases mortality in the geriatric population. Feelings of well-being and the pleasure derived from eating affect the quality of older individuals' lives positively. The connection between eating and good health has been understood for hundreds of years and transcends all cultures. Furthermore, it is understood that when elderly people stop eating their death is imminent. The aim of the presentation is to summarize and evaluate the different mechanisms and catabolic mediators (both humoral and tumoural) involved in cancer cachexia and ageing sarcopenia since they may represent targets for future promising clinical investigations.

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Notes:

N54

Mitochondria and muscle**Christiaan Leeuwenburgh¹**, Jinze Xu¹, Christy Carter¹, Esther E. Dupont-Versteegden², Mitchell D. Knutson³¹Department of Aging and Geriatrics, Division of Biology of Aging, Genomics and Biomarkers Core of The Institute on Aging, University of Florida, Gainesville, FL, USA;²Department of Geriatrics and Physiology and Biophysics, University of Arkansas for Medical Sciences, Little Rock, AR, USA;³Nutritional Biochemistry Food Science and Human Nutrition Department University of Florida PO Box 110370 Gainesville, FL, USA

Sarcopenia is strongly associated with reduced physical activities and the loss of muscle mass and strength. Muscle atrophy is also associated with a loss of muscle fiber nuclei most likely through apoptosis. We investigated age-related differences in the extent of apoptosis in the soleus muscle of young (6 months) and old (32 months) male Fischer 344 X Brown Norway rats subjected to acute disuse atrophy, induced by 14 days of hind limb suspension (HS). Atrophy (reduction in muscle weight and cross-sectional area) induced by HS was associated with a loss of myofiber nuclei in the soleus muscle of young, but not old rats. This resulted in a significant decrease in the myonuclear domain (cross-sectional area/nucleus) in young and old rats, with changes being more pronounced in the old animals. Moreover, levels of apoptosis (TUNEL and DNA fragmentation) were higher in the soleus muscles of old control rats than in young animals. Immunohistochemistry showed that the pro-apoptotic endonuclease G (EndoG; a mitochondrion-specific nuclease) was localized in the subsarcolemmal mitochondria in control muscles and translocation to the nucleus occurred in old, but not in young control animals. These results show that deregulation of myonuclear number occurs in skeletal muscle at old age and that the pathways involved in apoptosis are distinct in young and old muscles. Moreover, apoptosis in skeletal muscle is partly mediated by the subsarcolemmal mitochondria through EndoG translocation to the nucleus in response to hind limb suspension. In addition we studied, male Fischer 344 X Brown Norway rats (8, 29 and 37 months) fed *ad libitum* or a calorie restricted (CR) diet (60% of *ad libitum* food intake starting at 4 months of age) were used to investigate the effects of age and calorie restriction on skeletal muscle non-heme iron levels, iron transport mechanisms, oxidative stress and indexes of sarcopenia (muscle mass and grip strength). The non-heme iron content in the gastrocnemius muscle increased significantly with age in rats fed AL, reaching approximately 2-fold higher levels at 37 months of age than young rats. In striking contrast, the age-related iron accumulation was found to be markedly attenuated by short (4 months) and long (25 and 32 months) calorie restriction. RNA oxidation was increased with age in both the 29 and 37 month old animals compared to the 8-month old and CR attenuated the age-associated rise significantly. Iron levels correlated strongly with the levels of oxidized RNA ($r=0.72$, $p < 0.0001$), but only moderately with oxidized DNA ($r=0.38$, $p = 0.02$). Moreover, there was a highly significant age associated decreases in muscle mass and grip strength, which were both attenuated in CR rats. Striking was that the overall higher grip strength and muscle mass strongly correlated ($r = 0.74$, $p < 0.0001$ and $r = 0.85$, $p < 0.0001$ respectively) with lower non-heme iron content. This study strongly suggests an important role of iron toxicity in skeletal muscle and caloric restriction modulated the levels of iron, oxidative stress and key characteristics of sarcopenia. Supported by NIA R01-AG17994, AG21042 and the University of Florida Institute on Aging and Claude D. Pepper Older Americans Independence Center (1 P30 AG028740).

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N55

Muscle and fat tissue interaction**Liat Mintz**¹, Diego Perez-Tilve², Natanja Slager¹, Matthias H. Tschöp²¹DiaLean, Ltd., East Brunswick, NJ, USA²Department of Psychiatry, Genome Research Institute - University of Cincinnati, Cincinnati, OH, USA

Maintaining body weight is one of the basic needs for maintaining health. In many cases it is the first to be disrupted in cases of disease or stress. Organisms have developed complex mechanisms to maintain body weight. At the base level of the complex system of checks and balances are the numerous genes that were shown to be involved in maintaining an adequate ratio of lean to fat mass and total body weight. Some genes currently identified in this process are Leptin, Ghrelin, PYY, PP, Adiponectin, Insulin, OXM, CCK and GLP-1. The second level of complexity is the checks and balances within each specific gene family. When the human genome draft was completed and only 30,000 human genes were identified people were surprised as many more proteins were thought to exist. It was soon clear that one of the ways organisms use to increase the variety of proteins is derived from alternative splicing. It is now known that in most cases each gene encodes for more than one mRNA and that each mRNA may have a specific function within the organism. For example, the VEGF gene encodes for at least 8 protein splice variants with each one of them responsible for different aspects of vascularisation including fine tuning via specific angiogenic and anti angiogenic splice variants. We would like to suggest that the same phenomenon may occur in the ghrelin gene family. Till now, 13 different splice variants of ghrelin were identified. Their specific mode of control is not clear yet. The acylated form of the known ghrelin variant is involved in induction of food consumption and weight gain, mostly fat mass. We would like to present a novel ghrelin variant which seems to induce food consumption and weight gain in lean mass suggesting an additional factor to be considered in the control of body composition and body weight gain.

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O56

Are cytokines always involved in the pathogenesis of cachexia? PRO

Maurizio Muscaritoli, Paola Costelli, Zaira Aversa, Valentina Tommasi and Filippo Rossi Fanelli
Department of Clinical Medicine, La Sapienza University, Rome, Italy

Cachexia is still seeking for an unanimously accepted definition, but general consensus exists that cachexia, sarcopenia and malnutrition have to be considered clinically distinct entities. However, the boundaries between them are seldom unclear. Loss of body weight and muscle depletion are considered the hallmarks of cachexia, which are determined by a complex interplay between several humoral mediators.

Cytokines such as tumor necrosis factor (TNF)- α , interleukin (IL)-1, IL-6, and interferons, have been proposed as mediators of tissue depletion. Experimental animals treated with TNF α or IL-1 has been shown to develop cachexia, while muscle wasting and ubiquitin hyperexpression in tumor-bearing animals is prevented by the administration of anti-TNF α antibodies, pentoxifylline and suramine. In addition, cachexia induced by the Lewis lung carcinoma is antagonized by treatment with anti-IFN γ antibodies. The murine C-26 adenocarcinoma seems to produce muscle wasting mainly by releasing IL-6, and IL-6 hyperproduction achieved in transgenic mice increases the expression of components of the ATP-ubiquitin-dependent proteolytic systems. Such observations are not confined to experimental studies. Indeed, TNF α plasma levels in prostate cancer patients positively correlate with the mortality rate, and the levels of soluble TNF α receptor I increase with disease progression in gastric or colorectal cancer patients, being higher in severely cachectic ones. Proinflammatory cytokines also play a role in muscle depletion associated with chronic non-malignant diseases such as chronic heart failure, chronic kidney disease and chronic obstructive pulmonary disease. TNF α , IL-1 and IL-6 are frequently elevated in sepsis, and cytokine levels positively correlate with its severity and lethality. AIDS patients show an imbalance of cytokines with an excess of proinflammatory mediators, which probably contributes to muscle ubiquitin overexpression and protein hypercatabolism that affected such patients before the use of the combined therapy. Finally, muscle wasting associated with increased levels of several mediators among which TNF α , IL-1, IL-6 is a common clinical problem in elderly patients, which frequently renders the distinction between sarcopenia and cachexia almost impossible.

In conclusion, it is apparent that the inflammatory response driven by cytokines plays a crucial role in the pathogenesis of cachexia of chronic diseases, and that it differentiates cachexia from simple malnutrition secondary to starvation or underfeeding.

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Notes:

O57

Are cytokines always involved in the pathogenesis of cachexia? CONTRA**Michael W Schuster**

New York Presbyterian Hospital, USA

Determining the etiology of cancer cachexia always reminds me of the Indian fable of the three blind men touching different parts of the elephant to describe what an elephant looks like. Although much experimental evidence points to the role played by proinflammatory cytokines, the etiology of cachexia remains unclear and is probably multifactorial. To lay the blame solely on these cytokines is probably an oversimplification. Metabolic disturbances also play a key role in cachexia. Increased energy expenditure, for example, is seen in cancer cachexia. Many tumors consume glucose anaerobically at a rate, paralleling the present-day world consumption of oil, and release lactate that is used primarily in the Cori cycle. This, in turn, leads to increases in hepatic conversion of lactate to glucose, creating a futile energy-consuming cycle. Experimental evidence in an animal cancer cachexia model demonstrates that tumor-induced decrease in tissue lipoprotein lipase (LPL) activity is caused by insulin resistance. Chronic heart failure (CHF) can also be associated with cachexia and is associated with metabolic abnormalities, including insulin resistance and lack of anabolic hormone activity. Likewise, the cachexia seen in chronic kidney disease is also mediated, in part, by insulin resistance and a metabolic acidosis. Thus, hypermetabolism, insulin resistance and other endocrine disturbances play a central role in causing the cachexia seen in such diverse settings as cancer, heart failure and kidney failure. That cytokines play an important role is certainly true. That cytokines are the only explanation reminds us of the three blind men touching only one aspect of the cachexia elephant and professing to understand the whole beast.

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O58

What needs to be preserved in acute cachexia – fat or muscle tissue?**FAT****Wolfram Doehner**

Division of Applied Cachexia Research, Charite - University Medical School, Berlin, Germany

Obesity is doubtless a plague of epidemic proportions to modern society. Proclaimed like a Hindu mantra, losing weight and especially minimizing body fat tissue stores has been thoroughly established in the public consciousness as a desired aim that guarantees health as well as physical and mental wellbeing. There is, however, a more differentiated view required as fat tissue is an important and vital proportion of our body. Carrying significant functions in healthy conditions such as control of thermoregulation, isolation, mechanical protection and energetic storehouse of the body, conserved fat stores are vitally important in the setting of chronic diseases. Energy requirements of the body are to be met by whatever means, and fat stores prevent functional tissues such as muscle tissue to be degraded for mere energy yield. At this point, a spiral of self-destruction of the body's integrity starts and vital consequences are imminent. Accordingly, while muscle tissue is the key to physical activity, fat tissue should be viewed as the key to survival. Better understanding of the mechanisms of anabolic failure and catabolic dominance in cancer and other chronic diseases will advance new therapeutic approaches to target tissue wasting and prevent cachexia.

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Notes:

O59

What needs to be preserved in acute cachexia – fat or muscle tissue?

MUSCLE

William J Evans

University of Arkansas for Medical Sciences, Little Rock, AR, USA

Skeletal muscle has a number of metabolic properties that are critical for survival during cachexia. Cachexia involves the selective loss of skeletal muscle in individual with an acute illness or chronic disease. During periods of stress and/or starvation, muscle provides amino acids for protein synthesis in tissues that are essential for survival. Even when fat mass is maintained or increased in a cachectic state such as sepsis through total parenteral nutrition, accelerated loss of skeletal muscle occurs and results in death. In chronic illnesses and in very old people low muscle mass and weakness are powerful predictors of early mortality. While nutrition in the form of lipid can be provided to maintain body weight in individual with cachexia, no nutritional supplement can maintain muscle mass.

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O60

Is nutritional support essential for anabolics to work in cachexia? PRO**Arny A Ferrando**

Center for Translational Research in Aging and Longevity, DWR Institute on Aging, Little Rock, AR, USA

Trauma results in altered hormonal profiles of the key anabolic and catabolic hormones which control muscle protein metabolism. With severe injury, production of testosterone dramatically decreases, while skeletal muscle becomes resistant to circulating levels of growth hormone and insulin. Concomitantly, the principal catabolic signal to skeletal muscle, cortisol, is prominently and persistently elevated. For this reason, the anabolic/catabolic hormone ratio favors muscle catabolism during severe stress. Feeding alone is necessary to support patient nutrition and survival, but can not ameliorate the dramatic loss of muscle nitrogen. We have endeavored to restore anabolic influence and ameliorate muscle loss in these patient populations by exogenous administration of several anabolic agents. We have utilized testosterone or an analog, oxandrolone, insulin, growth hormone, and insulin-like growth factor-1 complexed with its principal binding protein-3, in adult and pediatric burn patients. The results indicate a common ability of these agents to restore the effects of feeding on muscle protein metabolism in these severely injured patients such that the substantial loss of muscle nitrogen is ameliorated. To compare with a non-injured population, the effect of prolonged administration of testosterone on muscle protein metabolism in elderly men is presented. Unlike the stressed/injured population, testosterone can reduce the catabolism of fasting in older men. Thus, it appears that a greater physiological stress requires the restoration of anabolic influence and concomitant feeding to ameliorate the loss of skeletal muscle.

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O61

Is nutritional support essential for anabolics to work in cachexia? CONTRA**Khursheed N Jeejeebhoy**

Toronto, Canada

Malnutrition due to starvation or inadequate intake results in weight loss due to loss of fat mass with relative preservation of lean body mass and plasma protein levels. This process is reversed by feeding which occurs with nutritional support. In contrast cachexia is weight loss due to an inflammatory process causing anorexia and hypercatabolism. Weight loss in cachexia is due to loss of both lean and fat mass as well as hypoproteinemia. The treatment of cachexia is reversal or counteraction of the inflammatory reaction. In cachexia patients the gastrointestinal tract is functional and thus once the anorexia is corrected the patient starts to eat without nutritional support. Controlled trials have shown that Megesterol Acetate and anabolic progestin as well as anabolic steroids and growth hormone will result in weight gain and improved quality of life without nutritional support in cachectic patients proving the fact that we do not need nutritional support to treat cachexia.

Notes:

12:15 – 13:45

Cachexia mechanisms 1

- 1.01 Prostaglandin excess is sufficient to induce adipose tissue wasting through increased substrate utilization**
Vegiopoulos A¹, Chichelnitskiy E¹, Ostertag A¹, Müller-Decker K², Herzig S¹
¹Molecular Metabolic Control, ²Tumor Models, German Cancer Research Center, Heidelberg, Germany
- 1.02 Wnt3a promotes beta-catenin signaling and myotube formation during myogenic differentiation**
Langen RC¹, van der Velden JL¹, Kelders MC¹, Laeremans H², Schols AM¹
¹Department of Respiratory Medicine, Nutrition and Toxicology Research Institute, Maastricht University, Maastricht, Netherlands; ²Department of Pharmacology and Toxicology, Maastricht University, Maastricht, Netherlands
- 1.03 Protein breakdown, mitochondria homeostasis, energy balance and muscle wasting**
Mammucari C¹, Masiero E¹, Romanello V², Milan G², Sandri M²
¹Venetian Institute of Molecular Medicine, Padova, Italy; ²Dulbecco Telethon Institute at Venetian Institute of Molecular Medicine, Padova, Italy
- 1.04 Proteasome inhibitor MG 132 has different effect on protein metabolism under in vivo and in vitro conditions**
Holecek M, Muthny T, Kovarik M, Sispera L
 Department of Physiology, Charles University Medical Faculty, Hradec Kralove, Czech Republic
- 1.05 The ubiquitin-proteasome and the apoptotic pathways are sequentially up- and down- regulated during atrophy and recovery following immobilization in rat gastrocnemius muscles**
Vazelle E, Claustre A, Taillandier D, Bechet D, Attaix D, Combaret L
 Human Nutrition Unit, INRA, Ceyrat, France
- 1.06 Uncoupling skeletal muscle damage and regeneration during cachexia: a cellular mechanism underlying muscle wasting?**
Coletti D¹, Berardi E¹, Aulino P¹, Molinaro M¹, Sassoon D², Adamo S¹
¹Sapienza University of Rome, Rome, Italy; ²UMR S 787 - Groupe Myologie INSERM - UPMC Paris VI, Paris, France
- 1.07 Hsp70 overexpression inhibits NF-kappaB activation and skeletal muscle atrophy**
Senf SM, Dodd SL, Judge AR
 Department of Applied Physiology and Kinesiology, University of Florida, Gainesville, FL, USA
- 1.08 Identification of novel signaling molecules involved in muscle atrophy**
Hardeland U, Vegiopoulos A, Gail AM, Lemke U, Chichelnitskiy E, Herzig S
 German Cancer Research Center, Heidelberg, Germany
- 1.09 STAT3 mediates skeletal muscle wasting in cancer cachexia**
Koniaris LG^{1,2}, Aydogdu T², Guo S¹, Jin X¹, Zhang Z¹, Zimmers TA^{1,2}
¹Surgery, University of Miami Miller School of Medicine, Miami, FL, USA; ²Cell Biology & Anatomy, University of Miami Miller School of Medicine, Miami, FL, USA
- 1.10 Investigating TNF inhibition of IGF-1 signaling via JNK in cell culture models of skeletal muscle atrophy**
GebSKI BL¹, Shavlakadze T¹, Ng DCH², Bogoyevitch MA², Grounds MD¹
¹School of Anatomy and Human Biology, the University of Western Australia, Perth, WA, Australia; ²Department of Biochemistry and Molecular Biology, Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Parkville, Vic, Australia
- 1.11 Mass and strength of skeletal muscles over-expressing Class 2 IGF-1 Ea isoform**
Shavlakadze T¹, Chai R¹, Pinniger G², Winn N³, Rosenthal N³, Grounds MD¹
¹School of Anatomy and Human Biology, School of Biomedical, ²Biomolecular and Chemical Sciences, the University of Western Australia, Perth, WA, Australia; ³Developmental Biology, Pasteur Institute, Paris, France; ³Mouse Biology Unit, EMBL Monterotondo Outstation, Monterotondo, Rome, Italy
- 1.12 Insulin-like growth factor (IGF)-I inhibits dexamethasone-induced muscle atrophy through Akt1/GSK-3beta/beta-catenin pathway**
Schakman O, Kalista S, Lause P, Verniers J, Ketelslegers JM, Thissen JP
 Unité de Diabétologie et Nutrition, Université catholique de Louvain, Brussels, Belgium
- 1.13 Myostatin blockade by deacetylase inhibitors fails to counteract muscle wasting in tumor-bearing mice**
Bonetto A, Penna F, Reffo P, Minero VG, Costelli P, Baccino FM
 Department of Experimental Medicine and Oncology, University of Torino, Italy
- 1.14 Muscle overexpression of Follistatin, an antagonist of Myostatin, causes muscle hypertrophy**
Gilson H, Schakman O, Lause P, Verniers J, Thissen JP
 Unité de Diabétologie et Nutrition, Université catholique de Louvain, Brussels, Belgium
- 1.15 Glycogen Synthase Kinase-3 beta suppresses myogenic differentiation through negative regulation of nuclear factor of activated T-cells 3**
Langen RC, van der Velden JL, Kelders MC, Willems J, Schols AM
 Department of Respiratory Medicine, Maastricht University, the Netherlands

- 1.16 **Attenuation of muscle loss and proteolysis by curcumin C3 complex in MAC-16 tumor-bearing mice**
Siddiqui RA¹, Hassan S¹, Harvey K¹, Das T², Mukerji P², DeMichele S²
¹Methodist Research Institute, Indianapolis, IN, USA; ²Abbott Nutrition, Abbott Laboratories, Columbus, OH, USA
- 1.17 **Investigation of hormonal-inflammatory interference in cachexia through in vivo promoter mapping**
Chichelnitskiy E, Vegiopoulos A, Ziegler A, Herzig S
 Molecular metabolic control, German Cancer Research Center, Heidelberg, Germany
- 1.18 **The leucine metabolite HMB: mechanics considerations influencing clinical outcomes**
Baxter JH¹, Kraemer WJ², Tisdale MJ³
¹Abbott Laboratories, Columbus, Ohio, USA; ²Department of Kinesiology, University of Connecticut, Storrs, CT, USA; ³Pharmaceutical Sciences, Aston University, Birmingham, UK
- 1.19 **Identification of pro-cachectic transcription factors by cell-based high throughput screening**
Gail AM¹, Hardeland U¹, Conkright MD², Herzig S¹
¹Molecular Metabolic Control, DKFZ, Heidelberg, Germany; ²Scripps Research Institute, Jupiter, Florida, USA
- 1.20 **Chemotherapy related fatigue: an ex vivo mouse model for skeletal muscle impairment**
van Norren K¹, van Tuijl S¹, Arts K¹, Haagsman H², van der Beek E¹, van Helvoort A¹
¹Numico Research, Wageningen, The Netherlands; ²Faculty of Veterinary Medicine, Utrecht University, The Netherlands

Cachexia in cancer

- 2.21 **Body composition and functional performance characteristics of newly-diagnosed cancer patients with and without weight loss**
Kilgour R¹, Vigano AA², Trutschnigg B², Hornby L², Bacon S¹, Morais JA³
¹Department of Exercise Science, Concordia University, Montreal, Quebec, Canada; ²McGill University, Palliative Care Division, Montreal, Quebec, Canada; ³Division of Geriatrics, McGill University Health Centre, Montreal, Quebec, Canada
- 2.22 **Bioelectrical impedance analysis (BIA) and survival in advanced cancer**
Seyidova-Khoshknabi D, Kirkova J, Lasheen W, Davis MP, Walsh D, Lagman R
 The Harry R. Horvitz Center for Palliative Medicine, Taussig Cancer Center, Cleveland Clinic Health System, Cleveland, OH, USA
- 2.23 **Daily physical-rest activity in cancer cachexia in relationship to nutritional state, metabolism and quality of life**
Fouladiun M¹, Körner U¹, Gunnebo L¹, Sixt-Ammilon P², Bosaeus I², Lundholm K¹
¹Department of Surgery, Sahlgrenska University Hospital, Göteborg Sweden; ²Department of Clinical Nutrition, Sahlgrenska University Hospital, Göteborg Sweden
- 2.24 **Secondary causes of weight loss in patients with cancer cachexia**
Del Fabbro E, Dalal S, Li Z, Freer G, Bruera E
 M.D. Anderson Cancer Center, Houston, USA
- 2.25 **Functional muscle testing may detect early cancer cachectic muscle degeneration**
Krakowski-Roosen H¹, Kinscherf R², Renk H¹, Hildebrandt W³, Martignoni ME⁴, Weber MA⁵
¹Div. G100 (Translational Oncology), German Cancer Research Center, Heidelberg, Germany; ²Anatomy and Developmental Biology, University of Heidelberg, Germany; ³Div. D020 (Immunochemistry), German Cancer Research Center, Heidelberg, Germany; ⁴Dept. of General Surgery, University of Heidelberg, Germany; ⁵Div. E010 (Radiology), German Cancer Research Center, Heidelberg, Germany
- 2.26 **C-reactive protein: a potential predictor of early cachexia in lung cancer?**
Mehrfar P¹, Altit G², Walker E¹, Chevalier S
¹School of Dietetics and Human Nutrition, McGill University, Montreal, Quebec, Canada; ²Medicine, McGill University, Montreal, Quebec, Canada
- 2.27 **Skeletal muscle mass regulation in mildly weight-losing cancer patients**
Op den Kamp CMH¹, Minnaard R², Kelders MC¹, Hesselink MK², Langen RC¹, Schols AM¹
¹Department of Respiratory Medicine, Nutrim, Maastricht University, Maastricht, The Netherlands; ²Department of Movement Sciences, Nutrim, Maastricht University, Maastricht, The Netherlands
- 2.28 **Body composition and exercise intolerance in non small cell lung cancer.**
Paland M¹, Mori Y¹, Karhausen T³, Misgeld C¹, Kuhnke A², Felger D¹, Koch A¹, Suttorp N², Anker SD¹, Rauchhaus M^{1,3}
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- 2.29 Fat loss and lipid metabolism in advanced cancer patients**
Perrine M², Pawlowicz M¹, Mourtzakis M², Lieffers JR¹, Prado C¹, Clandinin MT¹, Baracos VE^{1,2}, Mazurak VC¹
¹Alberta Institute for Human Nutrition, Department of Agricultural, Food and Nutritional Science, Faculty of Agriculture, Forestry and Home Economics, University of Alberta, Edmonton, Alberta, Canada; ²Department of Oncology, Division of Palliative Care Medicine, Faculty of Medicine, University of Alberta, Edmonton, Alberta, Canada
- 2.30 Cachectic colorectal cancer patients show anabolic resistance of muscle signaling and protein turnover to amino acid availability without elevated whole body or muscle proteolysis**
Rennie MJ, Atherton P, Patel R, Larvin M, Liptrot S, Lund JN, Rankin D, Selby A, Smith K, Wilkes E
 University of Nottingham School of Graduate Entry Medicine and Health, Derby City Hospital, Derby, UK
- 2.31 Systemic inflammation and muscle quality in cancer cachexia**
Stephens NA¹, Skipworth RJE¹, Greig CA¹, Gray CD², Ross JA¹, Fearon KCH¹
¹School of Clinical and Surgical Sciences (Surgery), University of Edinburgh, Edinburgh, UK; ²Dept of Medical Physics, University of Edinburgh, Edinburgh, UK
- 2.32 A mass spectrometric approach to the discovery of protein biomarkers in the urine of patients with gastroesophageal cancer and cachexia**
Skipworth RJE¹, Stewart GD¹, Bhana M¹, Christie J², Cronshaw AD², Fearon KCH¹, Ross JA¹
 Repair Group, University of Edinburgh, Edinburgh, UK; ²Institute of Structural and Molecular Biology, University of Edinburgh, Edinburgh, UK
- 2.33 Plasma levels of macrophage inhibitory cytokine-1 in patients with gastro-oesophageal cancer: association with systemic inflammation**
Skipworth RJE¹, Brown DA², Hunter M², Breit SN², Ross JA¹, Fearon KCH¹
¹Clinical and Surgical Sciences (Surgery), School of Clinical Sciences and Community Health, The University of Edinburgh, UK; ²Centre for Immunology, St. Vincent's Hospital and University of New South Wales, Sydney, Australia
- 2.34 Real-imaging cDNA-AFLP gene expression profiling reveals new insights into muscle cachexia development of pancreatic cancer patients**
Skorokhod A¹, Krakowski-Roosen H², Martignoni ME³, von Kalle C²
¹Institute of Molecular Biology and Genetics, Ukrainian Academy of Sciences, Kiev, Ukraine; ²German Cancer Research Center, Heidelberg, Germany; ³Department of Surgery, European Pancreas Center, University of Heidelberg, Germany
- 2.35 Lower intramuscular free amino acids, carnosine and glutathione with reduced myofibrillar protein in cachectic compared to non-cachectic patients with pancreatic cancer**
Schmitt TL¹, Martignoni ME², Kinscherf R³, Friess H², Hildebrandt W⁴
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- 2.36 Fatigue in pancreatic cancer: the potential link between exertional dyspnea, exercise limitation, skeletal musculature and neurohormonal activation.**
Heinz C¹, Mori Y¹, Springer J¹, Karhausen T¹, Stieler J³, Doehner W², Oettle H³, Dietz R², Anker SD¹, Rauchhaus M²
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- 2.37 Interaction of gonadal status with systemic inflammation and opioid use in determining nutritional status and prognosis in advanced pancreatic cancer**
Skipworth RJE¹, Moses AGW¹, Voss AC², Anderson RA³, Ross JA¹, Fearon KCH¹
¹Clinical and Surgical Sciences (Surgery), The University of Edinburgh, Edinburgh, UK; ²Ross Products Division, Abbott Nutrition Research & Development Abbott Laboratories, Columbus, Ohio, USA; ³MRC Human Reproductive Sciences Unit, The University of Edinburgh, Edinburgh, UK
- 2.38 Tertiary cachexia: psycho-social factors that contribute to the problems caused by cancer cachexia syndrome**
Hopkinson JB, Foster C, Wright DNM, Okamoto I
 Macmillan Research Unit, School of Nursing and Midwifery, University of Southampton, Southampton, Hampshire, UK
- 2.39 Living with cancer cachexia: exploring the perspectives of patients and their significant others**
Reid J
 Cancer Centre, Belfast City Hospital / Queens University Belfast, Belfast, Ireland

Methodology studies

- 3.40 The role of measuring REE in the diagnosis and treatment of cachectic states**
Vaisman N¹, Niv E², Lusthaus M², Comaneshter D³
¹Tel Aviv Sourasky Medical Center and Tel Aviv Sackler Medical School, Tel Aviv, Israel; ²Clinical Nutrition Unit, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel; ³Statistic Service, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel
- 3.41 Validation study of inflammatory-based prognostic score in terminal cancer**
Chiang D¹, Glare PA², Clarke SJ³, Clark K², Adelstein S⁴, Sharpe L⁵
²Palliative Care, ³Oncology, ⁵Psychology, Sydney Cancer Centre, ¹University of Sydney, NSW, Australia; ⁴Immunology, RPAH, Camperdown, NSW, Australia
- 3.42 How precise is BIA in cachexia**
Davis M, Seyidova-Khoshtknabi D, Kirkova J, Walsh DT, Lasheen W, Lagman R, LeGrand S
 The Harry R. Horvitz Center for Palliative Medicine, Taussig Cancer Center, Cleveland Clinic Health System, Cleveland, Ohio, USA
- 3.43 Bioelectrical impedance analysis is a valid and accurate method to assess body composition in hemodialysis patients, correlating well to dual-energy X-ray absorptiometry**
Rustom R¹, Wiedemann J², Djurhuus CB³, El Nahas M⁴
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- 3.44 Precision of dual energy X-ray absorptiometry and bioelectrical impedance in patients with advanced cancer**
Vigano AAL¹, Trutschnigg B¹, Morais JA², Lucar E¹, Kilgour R³, Rosenthal L⁴
¹McGill University, Palliative Care Division, Montreal, Quebec, Canada; ²McGill University Health Centre, Division of Geriatrics, Montreal, Quebec, Canada; ³Concordia University, Exercise Science, Montreal, Quebec, Canada; ⁴McGill University Health Centre, Radiology Department, Montreal, Quebec, Canada
- 3.45 Does a nutritional assessment tool correlate to laboratory values indicating cachexia in lung cancer patients?**
Gioulbasanis I¹, Giannousi Z¹, Tsatsanis C², Margioris A², Mavroudis D¹, Georgoulas V¹,
¹Department of Clinical Oncology, University Hospital of Crete, Herakleion, Greece; ²Department of Clinical Chemistry-Biochemistry, University Hospital of Crete, Herakleion, Greece
- 3.46 Validation of a simplified anorexia questionnaire**
Kirkova J, Davis M, Yavuzsen T, Walsh D, LeGrand S, Lagman R
 The Harry R. Horvitz Center for Palliative Medicine, Cleveland Clinic, Cleveland, OH, USA
- 3.47 Assessing quadriceps muscle strength in newly-diagnosed, advanced cancer patients: test-retest reliability and correlational analyses**
Reinglas J¹, Kilgour R¹, Hornby L², Trutschnigg B², Rosenthal L³, Vigano AA²
¹Department of Exercise Science, Concordia University, Montreal, Quebec, Canada; ²McGill University, Palliative Care Division, Montreal, Quebec, Canada; ³Department of Radiology, McGill University Health Centre, Montreal, Quebec, Canada
- 3.48 Handgrip dynamometry measurements in elderly cancer patients: a comparison of two instruments (JAMAR versus BIODEX)**
Trutschnigg B¹, Kilgour R², Reinglas J², Rosenthal L³, Hornby L¹, Vigano AA¹,
¹McGill University, Palliative Care Division, Montreal, Quebec, Canada; ²Department of Exercise Science, Concordia University, Montreal, Quebec, Canada; ³Department of Radiology, McGill University Health Centre, Montreal, Quebec, Canada

Intervention studies

- 4.49 A soluble activin receptor type IIB increases muscle mass in a mouse model of androgen deprivation therapy**
Lachey J, Pullen AE, Wong V, Haigis B, Ucran J, Pearsall RS
 Acceleron Pharma, Inc, Cambridge, MA, USA
- 4.50 Effects of CRF2R agonist on tumour growth and cachexia in mice bearing the Lewis lung carcinoma**
Argilés JM¹, Figueras M¹, Ametller E¹, Fuster G¹, Olivan M¹, Fontes de Oliveira CC¹, F. J. López-Soriano FJ¹, Isfort RJ², Busquets S¹
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- 4.51 Selective androgen receptor modular (SARM) prevents body weight and muscle loss in tumor-bearing mice**
Okolicany J¹, Veverka K², Steiner MS³, Dalton JT⁴
¹Drug Discovery, GTx Inc., Memphis, TN, USA; ²Preclinical Development, GTx Inc., Memphis, TN, USA; ³CEO, GTx Inc., Memphis, TN, USA; ⁴Preclinical R&D, GTx Inc., Memphis, TN, USA
- 4.52 Attenuation of mouse colon adenocarcinoma CT-26-induced cachexia by Chinese herbal medicine extract in BALB/c mice**
Chen BS, Kao WY, Lai TH, Chung YS, Sui PN, Wu RY
 Chinese Herbal Medicine Project, Development Center for Biotechnology, Taipei 221, Taiwan
- 4.53 Fenofibrate (trikor) exacerbates muscle wasting in a mouse model of cancer cachexia**
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¹Department of Cell Biology and Anatomy, University of Miami School of Medicine, Miami, FL, USA; ²Department of Surgery, University of Miami School of Medicine, Miami, FL, USA
- 4.54 Ghrelin improves body weight loss associated with angiotensin II-induced cachexia in mice**
Sugiyama M, Yamaki A, Furuya M
 Biomedical Research Laboratories, Asubio Pharma Co., LTD., Osaka, Japan
- 4.55 AKT-dependent insulin signaling is enhanced in skeletal muscle and reduced in liver following in vivo ghrelin administration**
Barazzoni R, Zanetti M, Cattin MR, Vinci P, Cattin L, Guarnieri G
 Clinica Medica - Dept of Clinical, Morphological and Technological Sciences - University of Trieste, Italy
- 4.56 Effects of beta-hydroxy-beta-methylbutyrate treatment on protein metabolism in rat skeletal muscle**
Kovarik M, Muthny T, Sispera L, Holecek M
 Department of Physiology, Medical Faculty, Charles University, Hradec Kralove, Czech Republic
- 4.57 Lithium administration modulates muscle GSK3-beta but does not prevent muscle loss in experimental cancer cachexia**
Sonni PV¹, Iannuzzi S¹, Aversa Z¹, Frascaria T¹, Costelli P², Rossi Fanelli F¹, Muscaritoli M¹
¹Clinical Medicine, University "La Sapienza", Rome, Italy; ²Experimental Medicine and Oncology, University of Turin, Turin, Italy
- 4.58 Effect of beta-hydroxy-beta-methylbutyrate on weight and muscle loss in cancer cachexia**
Sonni PV¹, Iannuzzi S¹, Aversa Z¹, Ramaccini C¹, Pinto G¹, Minero VG², Costelli P², Rossi Fanelli F¹, Muscaritoli M¹
¹Department of Clinical Medicine, University "La Sapienza", Rome, Italy; ²Department of Experimental Medicine and Oncology, University of Turin, Italy
- 4.59 Endurance training restores hepatic lipid metabolism of cachectic Walker 256 tumour bearing rats**
Lira FS¹, Tavares FL¹, Yamashita AS¹, Koyama CH¹, Alves MJ¹, Caperuto EC¹, Batista ML^{1,2}, Seelaender Jr.& MCL¹
¹Molecular Biology of the Cell Group, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil.
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- 4.60 Effects of proteasome inhibition in an experimental model of cancer cachexia**
Reffo P¹, Minero VG¹, Muscaritoli M², Costelli P¹, Baccino FM¹, Rossi Fanelli F²
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- 4.61 Formoterol and roxithromycin administered individually and in combination prevent tissue wasting in a rat model of cancer cachexia**
Kenley R¹, Ekblom J¹, Denissenko M²
¹Anaborex, Inc., La Jolla, CA, USA; ²Invitrogen, Corp., Carlsbad, CA, USA
- 4.62 Bisoprolol in experimental cancer cachexia**
Springer J¹, Palus S¹, Braun T¹, Flach VC¹, Hartmann K¹, Schmidt K¹, Rauchhaus M¹, Argiles J², Anker SD¹
¹Division of Applied Cachexia Research, Department of Cardiology, Charité Medical School, Berlin, Germany; ²Dept of Biochemistry and Molecular Biology of Cancer, University of Barcelona, Spain
- 4.63 The effects of xanthine oxidase inhibitors oxypurinol and allopurinol in experimental cancer cachexia**
Springer J, Möller N, Palus S, Braun T, Flach VC, Hartmann K, Schmidt K, Doehner W, Anker SD
 Division of Applied Cachexia Research, Department of Cardiology, Charité Medical School, Berlin, Germany
- 4.64 The effects of the aldosterone antagonist spironolactone in experimental cancer cachexia**
Palus S, Springer J, Braun T, Flach VC, Hartmann K, Schmidt K, von Haehling S, Anker SD
 Division of Applied Cachexia Research, Department of Cardiology, Charité Medical School, Berlin, Germany
- 4.65 Proteasome inhibition in CHF rats improves diaphragm function by restoring myosin content**
van Hees HWH¹, Li YP², Dekhuijzen PNR¹, Heunks LMA¹
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- 4.66 Pharmacological intervention against TNF-alpha attenuates the increases in MuRF-1 and MaFbx mRNA in a model of cardiac cachexia**
Steffen BT, Lees SJ
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- 4.67 **The impact of the appetite stimulant megestrol acetate on survival, body weight and cardiac function in a rat model of chronic heart failure**
Springer J¹, Palus S¹, Strassburg S¹, Bockmeyer B¹, Bauersachs J², Waller C², Anker SD¹
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- 4.68 **Proteasome inhibition improves contractility of the emphysematous hamster iaphragm**
Heunks LMA¹, Van Hees H¹, Li YP², Ennen LE¹, Dekhuijzen PNR¹
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- 4.69 **Double-blinded, placebo-controlled plasmid GHRH trial for cancer-associated anemia in dogs**
Draghia-Akli R, Khan AS, Bodles-Brakhop AM, Pope MA, Brown PA
 VGX Pharmaceuticals, Immune Therapeutics Division, The Woodlands, Texas, USA
- 4.70 **Mirtazapine and appetite in advanced cancer**
Kirkova J, Davis MP, Walsh D, Lagman R, Bennani-Baiti N, Seyidova-Khoshknabi D
 The Harry R. Horvitz Center for Palliative Medicine, Cleveland Clinic, Cleveland, Ohio, USA
- 4.71 **Randomised phase III clinical trial to evaluate the efficacy and safety of an integrated treatment (diet, pharmaco-nutritional and pharmacological) in cancer patients with cancer-related anorexia/cachexia and oxidative stress: interim results**
Mantovani G, Madeddu C, Gramignano G, Serpe R, Massa E, Dessì M, Macciò A
 Department of Medical Oncology, University of Cagliari, Italy

Human cachexia - various

- 5.72 **Disuse atrophy reduces muscle protein synthesis in the fasted and fed state in humans**
Glover EI¹, Phillips SM¹, Tang JE¹, Oates BR¹, Tarnopolsky MA², Smith K³, Selby A³, Rennie MJ³
¹Kinesiology and ²Pediatrics, McMaster University, Hamilton, Ontario, Canada; ³University of Nottingham, School of Biomedical Sciences, Derby City General Hospital, Derby, UK
- 5.73 **The effect of weight loss on mobility and frailty in the community-dwelling elderly**
Vandewoude MFJ¹, Hoeck S¹, Geerts J¹, Van Hal G¹, Van der Heyden J², Breda J¹
¹University of Antwerp, Belgium; ²Scientific Institute of Public Health, Belgium
- 5.74 **Muscle quality is maintained in healthy old women: influence of muscle volume optimisation**
Greig CA, Gray CD, Fearon KCH, Beggs, Lewis SJ, Young A
 School of Clinical Sciences and Community Health, University of Edinburgh, UK
- 5.75 **Cytokines and their association with functional parameters in older persons – A pilot study using a biochip with high sensitivity for cytokine measurements**
Bauer JM¹, Wagner JT¹, Cupic D¹, Bertsch T², Braun S³, Sieber CC¹
¹Department for Geriatric Medicine, University of Erlangen-Nuremberg, Germany; ²Institute of Clinical Chemistry, Nuremberg Hospital, Germany; ³Institute of Laboratory Medicine, German Heart Centre, Munich, Germany
- 5.76 **Cachexias: a 2007 state of the art review of the metabolic and biochemical abnormalities in different clinical models of cachexia**
Bennani-Baiti N, Walsh D, Davis MP
 The Harry R. Horvitz Center for Palliative Medicine, Cancer Center, Cleveland Clinic, Cleveland, Ohio, USA
- 5.77 **Natriuretic peptide-and catecholamine-induced lipolysis in cardiac cachectic patients**
Tedeschi S, Cremaschi E, Cabassi A
 Department Internal Medicine, Nephrology and Health Science, University of Parma, Parma, Italy
- 5.78 **Differences in body mass changes after the onset of heart failure symptoms. Impact on body composition, biochemical characteristics and prognosis.**
Rozenryt PJ¹, Nowak JU¹, von Heahling S², Sikora J¹, John A¹, Wilczek U¹, Poloński L¹, Anker SD²
¹III Department of Cardiology, Silesian Centre for Heart Diseases, Zabrze, Poland; ²Applied Cachexia Research, Charite Campus Virchow-Klinikum, Berlin, Germany.
- 5.79 **Upregulation of STAT-3, SOCS-1, pro-inflammatory and anti-inflammatory circulating cytokine mRNA gene expression in CHF patients with and without cachexia compared to healthy control subjects**
Sandek A¹, Wüst J¹, von Haehling S¹, Szabó T¹, Bauditz J², Swidsinski A², Doehner W¹, Rauchhaus M¹, Lochs H², Anker SD¹, Volk HD³
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- 5.80 **Direct medical costs of HIV wasting: a retrospective analysis of a national managed care database**
Siddiqui J¹, Meletiche D², Phillips AL², Freedland E²
¹University of California, Davis, Sacramento, USA; ²EMD Serono, Inc., Rockland, MA, USA

- 5.81 Hypermuscularity does not abrogate burn injury induced muscle wasting**
Cheung MC, Spalding PB, Gutierrez JC, Namias N, Koniaris LG, Zimmers TA
 Department of Surgery, University of Miami, Miami, FL, USA
- 5.82 Nutrition management of patients with major burns**
Cernea D, Vladoianu NAM, Novac MB, Stoica M
 Intensive Care, Clinical Emergency Hospital Craiova, Romania
- 5.83 Metabolic and nutritional aspects of traumatic injury in critically traumatic patients**
Vladoianu NAM¹, Comanescu A², Cernea D¹
¹Intensive Care, Clinical Emergency Hospital Craiova, Romania; ²Department of Obstetrics and Gynecology, Clinical Emergency Hospital Craiova, Romania
- 5.84 Reversibility of cachexia and course of exercise capacity over 1 year after heart-, lung- or kidney-transplantation**
Habedank D¹, Kung T¹, Karhausen T¹, Ewert R³, Hetzer R³, Reinke P², Anker SD¹
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- 5.85 Exercise capacity and body composition after renal transplantation of living donors: changes over time**
Kung T¹, Habedank D¹, Karhausen T¹, Reinke P², Anker SD¹
¹Division of Applied Cachexia Research, Department of Cardiology, Charité Campus Virchow-Klinikum, Berlin, Germany; ²Department of Nephrology, Charité Campus Virchow-Klinikum, Berlin, Germany
- 5.86 Plasma high- but not low-molecular weight adiponectin is positively associated with resting energy expenditure in male non-diabetic hemodialysis patients**
Barazzoni R¹, Stulle M¹, Panzetta G², Biolo G¹, Zanetti M¹, Guarnirei G¹
¹Clinica Medica - Dept of Clinical, Morphological and Technological Sciences, University of Trieste, Italy; ²Division of Nephrology, Azienda Ospedaliera "Ospedali Riuniti", Trieste, Italy
- 5.87 Pathogenic mechanisms of muscle loss in patients undergoing liver transplantation**
Iannuzzi S¹, Costelli P², Merli M³, Bonetto A², Gentili F³, Rossi M³, Aversa S¹, Sonni PV¹, Giusto M³, Tommasi V¹, Rossi Fanelli F¹, Muscaritoli M¹
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Cachexia mechanisms 2

- 6.88 Animal models for cancer cachexia: What are the options?**
Bennani-Baiti N, Walsh D
 The Harry R. Horvitz Center for Palliative Medicine, Cancer Center, Cleveland Clinic, Cleveland, Ohio, USA
- 6.89 Effect of cancer cachexia upon the balance pro and anti-inflammatory cytokines in the adipose tissue of trained rats**
Lira FS¹, Yamashita AS¹, Rosa JC², Koyama CH¹, Batista Jr. ML^{1,3}, Seelaender MCL¹
¹Molecular Biology of the Cell Group, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil; ²Department of Physiology, Federal University of São Paulo, São Paulo, Brazil; ³School of Physical Education, University of Mogi das Cruzes, São Paulo, Brazil
- 6.90 New insights into adipose atrophy in cancer cachexia**
Bing C¹, Russell S², Tisdale MJ², Jenkins JR¹
¹School of Clinical Sciences, University of Liverpool, Liverpool, UK; ²School of Life and Health Sciences, Aston University, Birmingham, UK
- 6.91 Serine-protease activity is increased in experimental cancer cachexia**
Aversa Z¹, Costelli P², Sonni P¹, Iannuzzi S¹, Ramaccini C¹, Pinto G¹, Frascaria T¹, Rossi Fanelli F¹, Citro G³, Muscaritoli M¹
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- 6.92 The IGF-1 signaling pathway is not down-regulated in cancer cachexia**
Penna F¹, Bonetto A¹, Costelli P¹, Muscaritoli M², Rossi Fanelli F², Baccino FM¹
¹Dipartimento di Medicina e Oncologia Sperimentale, Università di Torino, Torino, Italy; ²Dipartimento di Medicina Clinica, Università La Sapienza, Roma, Italy
- 6.93 Genetic and pharmacologic inhibition of myostatin for muscle preservation in cancer cachexia**
Link M¹, Aydogdu T², Guo S¹, Koniaris LG³, Zimmers TA³
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- 6.94 Impaired immune function in an animal model for cancer cachexia prior to weight loss**
van Helvoort A¹, Faber J¹, Kegler D¹, Vos P¹, Garssen J^{1,2}
¹Numico Research, Wageningen, The Netherlands; ²Department of Pharmacology & Pathophysiology, Utrecht University, The Netherlands
- 6.95 Impaired daily activity and muscle function in cachectic C26 adenocarcinoma bearing mice**
van Norren K, Kegler D, Luiking YC, Gorselink M, van der Beek EM, van Helvoort A
Numico Research, Wageningen, The Netherlands
- 6.96 Metabolomics of cancer cachexia**
Ardeshirpour F¹, O'Connell TM², Asher SA¹, George JR¹, Guttridge DC³, Couch ME¹
¹Otolaryngology, University of North Carolina School of Medicine, Chapel Hill, NC, USA; ²Molecular Pharmaceutics, University of North Carolina School of Medicine, Chapel Hill, NC, USA; ³Human Cancer Genetics, Ohio State University, Columbus, Ohio, USA
- 6.97 The obesity paradox in dogs with spontaneously-occurring heart failure**
Slupe JL, Freeman LM, Rush JE
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1.01

Prostaglandin excess is sufficient to induce adipose tissue wasting through increased substrate utilization

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Background and aims: Prostaglandins are products of the cyclooxygenase (Cox) pathway and are known to be involved in the regulation of inflammatory processes. Increased Cox-2 expression has been detected in various tumor types and serum prostaglandin levels were found to be elevated in several animal models of cancer cachexia. Moreover, pharmacological inhibition of prostaglandin synthesis ameliorated certain symptoms of cachexia in cancer patients as well as tumor-bearing mice. Since the mechanism by which prostaglandins influence metabolism is not understood we examined the effect of prostaglandin excess as a single factor on metabolism.

Methods: The metabolic phenotype of transgenic mice overexpressing Cox-2 through the keratin-5 promoter in defined epithelia (K5COX2) was characterized.

Results: Mimicking cancer cachexia prostaglandin E2 serum levels were increased in K5COX2 mice, whereas no systemic inflammation could be detected. This was associated with severe wasting of abdominal adipose tissue, a process which developed with age and could be reversed by treatment with a Cox-2 inhibitor. To our surprise, K5COX2 mice displayed improved glucose tolerance and hyperphagia, suggesting that prostaglandins could be excluded as mediators of insulin resistance and anorexia. Finally, molecular and histological characterization of adipose tissue in K5COX2 mice provided evidence for uncontrolled substrate utilization linked to energy dissipation.

Conclusions: Prostaglandin excess is sufficient to induce adipose tissue wasting through the promotion of intense substrate utilization and increased energy expenditure. Since this mechanism could substantially contribute to the pathogenesis of cancer cachexia there is good indication that research on treatment strategies for cachexia should include Cox-2 inhibitors.

1.02

Wnt3a promotes beta-catenin signaling and myotube formation during myogenic differentiation

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Background and aims: Satellite cells are muscle precursor cells, required for postnatal skeletal muscle growth and regeneration. Glycogen synthase kinase 3beta (GSK-3beta) has been implicated in the negative regulation of skeletal muscle growth and muscle differentiation. Two cellular pools of GSK-3beta exist, which are selectively inhibited by IGF-I/Akt- or canonical Wnt signaling, respectively. Since canonical Wnt signaling and subsequent stabilization of beta-catenin is involved in embryonic muscle formation, the effects of Wnt3a on differentiating myoblasts were investigated, and compared to those resulting from GSK-3beta inactivation by LiCl or IGF-I.

Methods: Myogenic differentiation was evaluated by muscle specific gene expression and myotube formation in differentiating C2C12 myoblasts following inhibition of the two GSK-3beta pools separately, by IGF-I or Wnt3a, or simultaneously using LiCl. Wnt signaling was measured as beta-catenin-dependent induction of TCF-LEF transcriptional activity.

Results: Wnt3a promoted myoblast fusion, and induced beta-catenin signaling in differentiating myoblasts in a time and concentration dependent fashion. In addition, beta-catenin-dependent transcriptional activation was also induced after GSK-3beta inhibition using LiCl, but not by Insulin-like Growth Factor I (IGF-I). Furthermore, stimulation of Muscle Creatine Kinase or Troponin-I expression was only observed in response to pharmacological inhibition of GSK-3beta, which uncouples the promoting effects of Wnt3a on myoblast fusion from muscle specific gene expression.

Conclusions: These data suggest that myoblast fusion and muscle specific gene expression are controlled by distinct cellular pools of GSK-3beta, and provide further evidence for a role of GSK-3beta as a central regulator of muscle differentiation.

1.03

Protein breakdown, mitochondria homeostasis, energy balance and muscle wasting

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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 systems. Autophagy is an evolutionarily conserved mechanism that allows cell survival during starvation through the bulk degradation of proteins and organelles by lysosomal enzymes. However, the mechanisms responsible for the induction and regulation of the autophagy program are poorly understood. Here we show that FoxO transcription factors are required for the induction of autophagy in skeletal muscle. Suppression of FoxO3 activity prevents autophagosome formation induced by starvation. Akt/PKB activation blocks FoxO activation and autophagy, and this effect is not prevented by TORC1 inhibition. FoxO3 is able to induce autophagosome formation and up-regulation of the autophagy genes and Bnip3. Bnip3 appears to be a major mediator of FoxO3, as FoxO3-dependent autophagy is markedly reduced by knockdown of Bnip3. FoxO3 activates the proteasomal system by inducing the ubiquitin ligases atrogin-1 and MuRF1, however FoxO3-dependent autophagy is not affected by loss of atrogin-1 and MuRF1 or by blockade of proteasome. We further identified the substrates of autophagy which proved to be mitochondria. FoxO3 in adult muscle fibers causes a reduction in mitochondrial content, and mitochondrial

fragmentation induced by Bnip3 pathway is sufficient to induce time-dependent muscle loss. Conversely inhibition of the fission machinery suppressed mitochondrial fragmentation and FoxO3-mediated muscle atrophy. Thus FoxO3 controls independently the two major proteolytic systems in skeletal muscle, the ubiquitin-proteasome and autophagy-lysosome systems.

1.04

Proteasome inhibitor MG 132 has different effect on protein metabolism under in vivo and in vitro conditions

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Ubiquitin-proteasome system plays an important role in degradation of myofibrillar proteins in skeletal muscle. Two separate studies were performed using Wistar rats to evaluate the effect of proteasome inhibitor MG 132 on protein metabolism.

In the first study, m. soleus or m. extensor digitorum longus were incubated in medium with 30 μmol/L MG 132 or without inhibitor (control). Changes in proteolysis and protein synthesis were determined according to the rate of the tyrosine release, chymotrypsin-like activity and L-[1-14C]leucine incorporation into the muscle protein. In the second study, rats were injected with MG 132 (10 mg/kg b.w.) or with solvent. Changes in whole-body protein metabolism were estimated using infusion of L-[1-14C]leucine. The results were analyzed using unpaired Students' test.

In *in vitro* study, MG 132 significantly decreased both proteolysis and protein synthesis. In *in vivo* study MG 132 induced the increase in whole-body proteolysis and protein synthesis. Proteasome-dependent proteolysis was inhibited in skeletal muscle and activated in the liver and kidney. Protein synthesis increased in skeletal muscle, liver and kidney.

We conclude that MG 132 affects both protein anabolic and protein catabolic pathways via the direct effect on proteasome-dependent proteolysis and indirect effect on proteolysis and protein synthesis via unidentified mediators. The results also demonstrate that the observations *in vitro* may not necessarily reflect the *in vivo* situation.

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1.05

The ubiquitin-proteasome and the apoptotic pathways are sequentially up- and down- regulated during atrophy and recovery following immobilization in rat gastrocnemius muscles

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Background and aim: Muscle wasting during disuse is associated with an activation of the ubiquitin(Ub)-dependent pathway, of some proapoptotic signals, and an impairment in the regenerative potential of myogenic cells. However, the precise links between apoptosis and proteolysis during disuse and recovery remain to be elucidated. Thus, we have studied the regulation of these two processes and the impact on muscle myogenic markers during cast-induced atrophy and recovery in the rat.

Methods: Animals were subjected to unilateral hindlimb immobilization for 4 (I4), 6 (I6) or 8 (I8) days, the contralateral non-casted leg being the control. For regeneration studies, cast were removed at I8 and rats were allowed to recover for 10 (R10), 15 (R15), or 20 (R20) days.

Results: The gastrocnemius muscle atrophied progressively from I4 to I8. Ub-proteasome pathway activation was detectable from I4 and largest at I6. The regulation of apoptosis was assessed by measuring the activation of the mitochondria-associated apoptotic pathway, which increased maximally at I8. A reduction of protein levels for myogenic markers (MyoD and Myf 5) prevailed from I4 to I8. After cast removal, muscles recovered progressively. Ub-proteasome pathway and myogenic markers protein levels were rapidly normalized early as R10, whereas apoptotic processes were first down-regulated below basal levels (R15) before being completely normalized (R20).

Conclusion: Altogether our data suggest a two-stage process in which Ub-proteasome pathway is rapidly up- and down- regulated when a muscle atrophies and recovers, respectively, whereas apoptotic processes may be involved in the late stages of atrophy and recovery.

1.06

Uncoupling skeletal muscle damage and regeneration during cachexia: a cellular mechanism underlying muscle wasting?

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Elevated levels of pro-inflammatory cytokines and degradation of structural protein such as dystrophin lead to muscle damage. Several lines of evidence demonstrate that cytokines inhibit muscle repair. Our hypothesis is that a perturbation of the balance between damage and repair play a role in the onset of muscle wasting in cachexia. C26-tumor bearing (C26) mice were compared to controls, in the absence or presence of free exercise in wheel-equipped cages. While C26-mice run less than control mice, their number of damaged fibers was increased about 4-fold over running control mice. Stem cells were increased in the musculature of C26-mice but we did not observe different levels of regeneration as compared to controls. If muscle regeneration was boosted by freeze injury, reduced and delayed repair was observed in C26-mice. End stage C26-mice displayed a decreased muscle tissue DNA content associated to protein loss, as well as a lower fiber number per section. To unveil mechanisms involved in muscle response to cytokines, we delivered TNF-alpha to non tumor-bearing mice. TNF-alpha inhibited

muscle regeneration and activated caspases in interstitial stem cells, an event mediating TNF- α effects. We also found that perturbing the function of PW1, a key intracellular mediator of TNF- α signaling, inhibited caspase activation and rescued the block of muscle regeneration in the presence of TNF- α . Taken together, these observations suggest that muscle wasting results from a combination of protein degradation and muscle fiber loss. In the presence of cytokines, fibers are not efficiently repaired/replenished due to compromised muscle precursor cell function.

1.07
Hsp70 overexpression inhibits NF- κ B activation and skeletal muscle atrophy

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Heat Shock Protein 70 (Hsp70) protein expression is significantly decreased in skeletal muscle during disuse; and over-expression of Hsp70 by whole body hyperthermia is associated with the attenuation of muscle atrophy during disuse. However, hyperthermia may differentially regulate the expression of multiple proteins, therefore a direct role of Hsp70 in the attenuation of muscle atrophy is lacking. We injected, and electroporated, EGFP or EGFP-Hsp70 into the soleus muscle of control or 7-day immobilized rats. Overexpression of Hsp70 attenuated muscle fiber atrophy by 73%. In addition, immobilization caused a 50% increase in total ubiquitin-conjugated protein in control injected muscle, which was abolished in muscles injected with Hsp70. Furthermore, mRNA expression of the E3 ubiquitin ligases, MAF-bx/atrogen-1 and MuRF1 was increased 4-fold and 6-fold, respectively, in immobilized muscles injected with the control plasmid, which was attenuated by 65% and 56%, respectively, in Hsp70 injected muscle. Since NF- κ B may regulate the expression of atrogen-1 and MuRF1, is required for disuse muscle atrophy, and has been shown in various cell types to be inhibited by Hsp70, we co-injected Hsp70 (or a control plasmid) and an NF- κ B reporter plasmid into skeletal muscle prior to 3 or 7 days of immobilization. NF- κ B activity increased 2-fold after 3 days and 5-fold after 7 days of immobilization, which was completely abolished at both time points by Hsp70. These findings show a direct role of Hsp70 in the maintenance of muscle mass during disuse which is mediated, at least in part, through the ubiquitin-proteasome pathway and NF- κ B signaling.

1.08
Identification of novel signaling molecules involved in muscle atrophy

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The increased degree of protein degradation in skeletal muscle is considered to be the major cause of lean body mass wasting in cancer cachexia. Though several pro-cachectic factors have been identified, their signaling mechanisms have been elucidated only partly. Therefore, we aimed to identify new signaling molecules mediating the cachectic phenotype in skeletal muscle, particularly focusing on post-translational protein modifications as critical determinants of intracellular signal transduction. In this respect, we treated C2C12 myotubes with or without the pro-cachectic cytokine TNF α . Extracted proteins were separated by 2-D SDS-PAGE, stained by Coomassie or analyzed by immunoblotting to detect serine, threonine and tyrosine phosphorylated proteins. Based on subsequent mass spectrometry, a small G-protein, the receptor of activated kinase C (Rack1), was found among the differentially phosphorylated proteins, showing decreased phosphorylation upon TNF α treatment. Interestingly, protein levels of Rack1 were decreased in muscle tissue of several mouse models of muscle atrophy (Lewis lung carcinoma, colon 26 carcinoma, glucocorticoid treatment and starvation), while mRNA levels remained unchanged. One common feature of these models is represented by elevated glucocorticoid levels, and indeed, treatment of C2C12 myotubes with the glucocorticoid analogue dexamethasone led to the downregulation of Rack1 protein levels. Preliminary experiments indicated that Rack1 might be degraded by ubiquitination and proteasomal degradation in this context. Based on these results, we hypothesize that Rack1 might represent a novel component of the TNF α signaling pathway, the dysregulation of which contributes to the cachectic phenotype under various pathophysiological conditions.

1.09
STAT3 mediates skeletal muscle wasting in cancer cachexia

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Background and aims: Serum IL-6 levels and acute phase response (APR) generally correlate with wasting in cancer cachexia. IL-6 induces the hepatic APR through STAT3, however the mechanism by which IL-6 might cause muscle wasting in cachexia is unknown.

Methods: The contribution of IL-6, STAT3 and target genes was evaluated in C2C12 myotube cultures, by electroporation of plasmid DNA into mouse muscle, and in mouse models of genetic and transplantable cancer and sepsis.

Results: IL-6 activated STAT3 (pSTAT3) and wasting in C2C12 myotubes. In mice, IL-6 induced cachexia along with pSTAT3 and a STAT3 program of gene expression in skeletal muscle. Skeletal muscle activation of STAT3 was also observed in transplantable (LL2, B16.F10, colon-26) and genetic (ApcMin) models of cancer cachexia, as well as in models of sepsis-induced wasting (LPS, cecal ligation and puncture). Gene transfer of constitutively activated STAT3 (cSTAT3) was sufficient to induce muscle fiber wasting in C2C12 myotubes and in intact mouse gastrocnemius. Skeletal muscle from mice with

cachexia showed an 80 to 1000-fold increase in expression of APR genes, including fibrinogen, haptoglobin, LBP, and serum amyloid A. Quantitation of muscle vs. liver fibrinogen expression suggested that up to 40% of serum fibrinogen in cachexia could be muscle-derived.

Conclusions: STAT3 activation is associated with muscle wasting in cancer cachexia and sepsis. STAT3 activation is sufficient to induce wasting in normal skeletal muscle. The activation of a skeletal muscle-specific APR in cachexia may contribute to muscle wasting independently of known cachexia-inducing pathways by redirecting protein synthesis towards secreted proteins.

1.10
Investigating TNF inhibition of IGF-1 signaling via JNK in cell culture models of skeletal muscle atrophy

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The pro-inflammatory cytokine tumour necrosis factor (TNF) plays a critical role in muscle atrophy and necrosis. It is proposed that TNF is increased in Duchenne muscular dystrophy as a consequence of the sarcolemmal damage and myofibre necrosis and that increased TNF then exacerbates inflammation and muscle necrosis; however the precise signaling events are unknown. We investigate interactions between TNF and the insulin-like growth factor-1 (IGF-1) signaling pathways in dystrophy and other situations of muscle wasting: specifically, the involvement of TNF activated c-Jun NH2-terminal kinase (JNK) in abrogating IGF-1 signaling via phosphorylation of insulin receptor substrate-1 (IRS-1) on serine 307. Cross-talk between TNF and IGF-1 signaling is demonstrated in differentiated C2C12 muscle cells in culture using morphological and phospho-protein western analysis. Morphological studies show that IGF-1 induces myotube hypertrophy, whereas TNF decreases myotube size. In these treated C2C12 myotubes, western blots reveal TNF induces phosphorylation of JNK and IRS-1 (Ser 307), which indicates involvement of JNK in the cross-talk between IGF-1 and TNF. The specific role of JNK in inhibition of IGF-1 signaling was further investigated using JNK inhibitors. Three inhibitors were assessed for biological toxicity in C2C12 myotubes and two, SP600125 and TAT-TIJIP, were used for studies in C2C12 myotubes. Both were similarly applied to primary cultures of skeletal muscle cells isolated from transgenic mice which over-express Class 2 IGF-1 Ea in skeletal muscles. Both JNK inhibitors appear to protect against TNF induced atrophy in C2C12 myotubes. In vivo studies are also in progress.

1.11
Mass and strength of skeletal muscles over-expressing Class 2 IGF-1 Ea isoform

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Insulin like growth factor-1 (IGF-1) plays a central role in muscle hypertrophy and wasting. IGF-1 exists as different isoforms due to different exon splicing. IGF-1 isoforms that initiate from exon 1 are termed Class 1 (C1) isoforms, while isoforms that initiate from exon 2 are termed Class 2 (C2) isoforms. Differential splicing at the terminal end yields Ea or Eb isoforms. Different IGF-1 isoforms have different biological effects and may act through different signaling pathways. Previous studies show that over-expression of Class1 IGF-1Ea (IGF:C1) causes skeletal muscle hypertrophy and delays onset of necrosis in dystrophic (mdx) skeletal muscles. Novel strains of non-dystrophic and mdx transgenic mice that over-express Class2 IGF-1Ea (IGF:C2) isoform show more pronounced muscle hypertrophy compared to IGF:C1 mice. In sedentary mdx male and female mice aged 3 and 12 months, IGF:C2 significantly increases the weight of quadriceps muscles. Myofibre cross sectional area (CSA) examined in male mice was significantly increased at 3 months; however, CSA decreased at 12 months of age (despite the muscle weight increase) possibly due to confounding effects of myofibre splitting. This marked hypertrophy of the dystrophic (mdx/IGF:C2) muscles at 3 months of age was not dependent on the classical Akt/mTOR/p70S6K signaling pathway downstream of the IGF-1 receptor. Specific muscle force was not increased and myofibre necrosis was not reduced in the sedentary mdx/IGF:C2 mice at either age. The potential benefits of IGF:C2 induced muscle hypertrophy have yet to be tested in other animal models of muscle wasting due to denervation, inflammation and ageing.

1.12
Insulin-like growth factor (IGF)-I inhibits dexamethasone-induced muscle atrophy through Akt1/GSK-3beta/beta-catenin pathway

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Decrease of Insulin-like Growth Factor (IGF)-I plays a critical role in muscle atrophy caused by glucocorticoids. Indeed, we have demonstrated that muscle IGF-I gene electrotransfer prevents muscle atrophy caused by glucocorticoids. The goal of this study was therefore to identify the intracellular mediators responsible for the IGF-I anti-atrophic action in glucocorticoid-induced muscle atrophy. First, we assessed by western blot the IGF-I transduction pathway alterations caused by glucocorticoids administration and their reversibility by local IGF-I overexpression performed by electrotransfer. Muscle atrophy induced by dexamethasone (dexa) administration was paralleled by a decrease in Akt1 (-42%, P<0.05) and GSK-3beta (-26%, P<0.05) phosphorylation and a decrease in beta-catenin levels (-40%, P<0.001). Prevention of atrophy by IGF-I was associated with partial

or total restoration of Akt1 and GSK-3beta phosphorylation and beta-catenin levels. We then investigated whether muscle overexpression of these intracellular mediators could mimic the IGF-I anti-atrophic effects. Cross sectional area (CSA) of transfected muscle fibers identified by immunohistochemistry were analysed. Overexpression of a constitutively active form of Akt1 induced a marked increase in fiber CSA in dexamethasone-treated animals (+175%; P<0.001), which prevented dexamethasone-induced atrophy. This hypertrophy was associated with an increase in phosphorylated GSK-3beta (+40%, P<0.01) and in beta-catenin levels (+35%, P<0.05). Furthermore, overexpression of a dominant negative GSK-3beta or a stable deltaN-beta-catenin increased fiber CSA by about 23% (P<0.001) and 29% (P<0.001) in dexamethasone-treated rats, preventing completely the atrophic effect of glucocorticoids. In conclusion, this work indicates that Akt1, GSK-3beta and beta-catenin probably contribute to the IGF-I anti-atrophic effect in glucocorticoid-induced muscle atrophy.

1.13
Myostatin blockade by deacetylase inhibitors fails to counteract muscle wasting in tumor-bearing mice

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Background and aims: Muscle wasting, as occurring in cancer cachexia, is primarily characterized by protein hypercatabolism and increased expression of ubiquitin ligases, such as atrogen-1 (Costelli et al., 2006). Recent data suggest that myostatin, a member of the TGFbeta superfamily, negatively regulates skeletal muscle mass (McPherron et al., 1997). Several evidences reported deacetylase inhibitors (DIs), such as valproic acid (VPA) or trichostatin-A (TSA), may increase myofiber size in mdx mice by inducing the expression of follistatin, an antagonist of myostatin (Minetti et al., 2006). This work aimed to evaluate whether DIs could restore muscle mass in tumor-bearing mice.

Methods: Balb-c mice were divided into controls and tumor bearers. The latter, inoculated s.c. with C-26 colon adenocarcinoma cells, received either saline, or VPA, or TSA. After 13 days mice were sacrificed and the gastrocnemius muscle excised and stored at -80 °C. Myostatin expression was determined at both mRNA (semi-quantitative RT-PCR) and protein levels (western blotting). Follistatin and atrogen-1 were evaluated at mRNA level only.

Results: C26 growth was responsible for a progressive loss of body and gastrocnemius weight, and for the increase in myostatin and atrogen-1 levels. Only VPA administration proved effective in reducing myostatin by increasing follistatin levels, even though both of DIs were unable to reduce atrogen-1 expression and to counteract muscle atrophy in tumor bearers.

Conclusions: Reducing myostatin signaling by DIs treatment was unable to prevent muscle mass loss and protein degradation in tumor-bearing animals. Furthermore, apparently there is no direct link between myostatin activation and ubiquitin ligases expression.

1.14
Muscle overexpression of Follistatin, an antagonist of Myostatin, causes muscle hypertrophy

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Follistatin is known to antagonize the function of several members of the TGF-beta family, including Myostatin, a powerful inhibitor of muscle growth. The goal of this study was to investigate whether local overexpression of Follistatin by gene transfer could induce muscle hypertrophy. Localized Follistatin overexpression in tibialis anterior muscle was performed by electroporation. Our results showed that Follistatin overexpression led to a marked increase in muscle mass. Indeed, seventeen days after transfection, the weight of the transfected muscle was increased by 24% compared with the contralateral muscle electroporated with a control plasmid (p<0.001). The increase of muscle mass reflected a true muscle hypertrophy since the muscle protein content of the Follistatin-transfected muscle was also significantly increased (+24%, p<0.001). In addition, the immunohistomorphological analysis of the transfected cells showed that this hypertrophy was caused by increased cross-sectional area of the muscle fibers (+42%, p<0.001). The increase of muscle DNA content (+28%, p<0.001) together with the MHC neonatal mRNA (6-fold, p<0.001) in Follistatin-transfected muscle indicated that the muscle hypertrophy resulted from cell proliferation and differentiation. In order to investigate whether muscle hypertrophy observed in our model was mediated through IGFs, we measured the muscle IGFs expression. In contrast to IGF-I mRNA which remained unchanged, muscle IGF-II mRNA was increased by Follistatin overexpression (+54%, p<0.05).

In conclusion, Follistatin overexpression in adult animals stimulates muscle growth, by enhancing muscle cell proliferation and differentiation. This hypertrophic effect may involve IGF-II gene. Further studies will investigate the role of IGF-II in the hypertrophic effect of Follistatin.

1.15
Glycogen synthase kinase-3 beta suppresses myogenic differentiation through negative regulation of nuclear factor of activated T-cells 3

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Background: Skeletal muscle atrophy is a prominent and disabling feature in many chronic diseases. Prevention or reversal of muscle atrophy by stimulation of skeletal muscle growth could be an important therapeutic strategy. Glycogen synthase kinase 3beta (GSK-3beta) has been implicated in the negative regulation of skeletal muscle growth.

Aims: To investigate if inhibition of GSK-3beta is sufficient to stimulate myogenesis, since myogenic differentiation is an essential part of muscle growth. To assess whether this is regulated by controlling the transcription factor Nuclear Factor of Activated T-cells (NFAT). **Methods:** Myogenesis was studied in C2C12 myoblasts or myogenically converted Mouse Embryonic Fibroblasts (MEFs), in which GSK-3beta was depleted by RNAi or knock-out approaches, respectively.

Results: Deficiency of GSK-3beta protein (activity) resulted in enhanced myotube formation and muscle specific gene expression during differentiation, which was reversed by reintroduction of wt, but not kinase-inactive (K85R) GSK-3beta. GSK-3beta deficient MEFs or myoblasts displayed enhanced nuclear translocation of NFATc3, and elevated NFAT-sensitive promoter transactivation, which was reduced by re-introducing wt-, but not K85R-GSK-3beta. Over-expression of NFATc3 increased muscle gene promoter transactivation, which was abolished by co-expression of wt-GSK-3beta. Finally, stimulation of muscle gene expression observed following GSK-3beta inhibition was strongly attenuated in NFATc3-deficient myoblasts, indicating that this response requires NFATc3.

Conclusions: Negative regulation of myogenic differentiation by GSK-3beta occurs through a transcriptional mechanism which depends on NFATc3. Inhibition of GSK-3beta may be a potential strategy in prevention or treatment of muscle atrophy.

1.16
Attenuation of muscle loss and proteolysis by curcumin C3 complex in MAC-16 tumor-bearing mice

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Background and aims: Muscle wasting or cachexia is caused by accelerated muscle protein breakdown via the ubiquitin-proteasome complex. Recent attempts have been made to inhibit or reverse cachexia by nutrients that specifically inhibit muscle proteolysis. Curcumin has been shown to inhibit proteasome complex activity and muscle specific ubiquitin ligases (atrogen-1/MAFbx and MURF1) expression in both in vivo and in vitro systems. However, curcumin has failed to reverse cachexia in experimental animal models. The aim of the present study was to compare the effects of curcumin and an improved curcumin preparation known as Curcumin C3 complex (CurC3) on inhibiting protein degradation.

Methods: In vitro studies were performed on human skeletal muscle cells whereas in vivo studies were conducted using MAC-16 tumor-bearing cachectic mice.

Results: CurC3 was 30-50 times more potent than that of curcumin for inhibiting protein degradation and 20S proteasome activity. The in vivo data indicates that low doses of CurC3 (100 mg/kg BW), were able to prevent weight loss in mice exhibiting cachexia where as higher doses of CurC3 (250 mg/kg BW) resulted in 25% weight gain. The effects of CurC3 treatment were also evident by an increase in the gastrocnemius muscle weight (30-50%), hind quarter weight (15-40%) and muscle fibers size (30-65%). The effect of CurC3 appeared to be due to inhibition of ubiquitin-proteasome complex activity via its effect on atrogen-1/MAFbx and MURF1 expression.

Conclusion: Our data imply that CurC3 may have an effective therapeutic or an adjuvant therapeutic potential against cachexia.

1.17
Investigation of hormonal-inflammatory interference in cachexia through in vivo promoter mapping

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Interference between hormone signaling and inflammatory pathways is a potential trigger of the aberrant metabolism in cachexia. Sepsis and acute inflammation are tightly associated with cachectic phenotype and characterized by the suppression of stress compensatory pathways, e.g. de novo glucose production in the liver (gluconeogenesis). The inhibition of key gluconeogenic enzymes, phosphoenolpyruvate carboxykinase (PEPCK), during cachectic inflammation represents a critical determinant for septic patient outcome, however, its molecular mechanisms remain unknown.

Therefore, the present study aimed to define dysfunctional PEPCK gene regulatory elements and associated transcriptional complexes involved in impaired gene regulation during cachexia-associated inflammatory states in vivo.

To this end, we developed adenoviral PEPCK promoter-reporter constructs containing various 5'-regulatory flanking regions of the PEPCK gene. The viral promoter-reporter system was successfully tested in cultured liver cells in response to endogenous cellular signals and glucocorticoid treatment. Subsequent trials in wt-mice verified that PEPCK reporters respond to physiological stimuli, e.g. fasting, in vivo.

By using these PEPCK deletion promoter viruses in a mouse model of sepsis, associated with cachexia, we were able to map the inflammation-responsive site within the PEPCK gene to the -490/-355 promoter region, corresponding to the GRU. Current experiments are designed to specify the role of individual response elements and transcriptional complexes on the PEPCK gene as targets for pro-inflammatory/pro-cachectic pathway action in vivo.

These studies might ultimately help to unravel molecular mechanisms of cross-talk between inflammatory and hormonal pathways in cachexia and pave the way to improved therapeutic options.

1.18

The leucine metabolite HMB: mechanics considerations influencing clinical outcomes

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Beta-Hydroxy-beta-methylbutyrate (HMB) is a leucine metabolite, has a history of sports nutrition use, and is found in a medical nutritional product that affects disease-induced lean mass loss and enhances wound healing. HMB has been shown to enhance protein homeostasis in skeletal muscle of cachectic mice. HMB has significant impact on both protein degradation (NFkB, PKC, ubiquitin-proteasome system) as well as protein synthesis (mTOR, the 70kDa ribosomal S6 kinase and initiation factor 4E binding protein). In various models of tumor-induced weight loss, HMB treatment mitigates the signaling events that result in rapid lean mass loss. Supplemental arginine and glutamine additionally impacted protein synthesis but not degradation.

Intense physical training also places a significant stress on muscle tissues, and overtraining syndrome is a catabolic state with muscle impact similar to cancer cachexia. A recent clinical assessed the impact of HMB, glutamine, arginine and taurine (versus an isocaloric and isonitrogenous control) on training gains (strength and lean mass) as well as muscle stress markers when subjects engaged in rigorous resistance training. Supplementation resulted in significantly greater performance, lean body mass gains and fat mass losses. Additionally, cortisol and plasma CK levels were reduced, and testosterone levels increased in the supplemented group, indicating a greater anabolic response to exercise and reduced exercise-induced muscle damage.

Coupled with results of similar supplements evaluated in cancer cachexia and AIDS clinical studies, these data suggest that HMB can serve a pivotal role as a regulatory molecule, and clinically substantial results can result with suitable protein nutrition.

1.19

Identification of pro-cachectic transcription factors by cell-based high throughput screening

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The significant loss of skeletal muscle mass represents a prominent feature of the cachexia wasting syndrome, occurring in patients with advanced gastrointestinal, pancreas and lung cancer. Pro-cachectic factors, such as glucocorticoids and tumor necrosis factor- α (TNF- α), are critical mediators of this complex disorder. Over the past decade, numerous studies have aimed to decipher the intracellular pathways triggered upon binding of these mediators to the target tissue, in particular, skeletal muscle. However, on the molecular level the pro-cachectic transcriptional factors and target genes still remain elusive.

Our aim was to identify transcription factors dysregulated upon cachectic stimulation. For this purpose we performed a cell-based High-Throughput Screen consisting of a cDNA library of ~1500 transcription factors, cloned in-frame with the GAL4 DNA-binding domain driving expression of a co-transfected GAL4 luciferase reporter. Following transfection, cells were treated for 24h with either TNF- α , glucocorticoids or both stimuli. Thereby, we found 16% of transcription factors with altered transcriptional activity upon cachectic treatment. Of these selected target factors, 71% showed a diminished activity and 29% a significantly enhanced activity. Based on these results, we have validated the functional inhibition of a distinct Ets transcription factor by TNF- α treatment in cultured myotubes. In addition, promoter analysis in these cells confirmed the inhibition of a representative Ets target gene by TNF- α . These data support the hypothesis that the Ets transcription factor controls gene activity downstream of cachectic signaling pathways, and will now allow for the identification of distinct regulatory mechanisms in the development of cancer cachexia.

1.20

Chemotherapy related fatigue: an ex vivo mouse model for skeletal muscle impairment

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Fatigue is one of the most common side effects of chemotherapy. We hypothesize that the direct effect of chemotherapy on skeletal muscle functionality plays a significant role. In this study doxorubicin (DOX) was used to induce fatigue in mouse skeletal muscle as a models for chemo-induced fatigue. Contractile characteristics of the extensor digitorum longus (EDL) were assessed ex vivo upon incubation with DOX. The effect of DOX on calcium fluxes was measured in vitro with FURA-fluxes in C2C12-myotubes. After 30 min. of incubation, maximal relaxation velocity of the EDL was increased for all concentrations of DOX used (50-175 μ M). One hour incubation with 100-175 μ M DOX resulted in > 30% reduction in maximal force compared to control (P>0.05). The maximal contraction velocity started to decrease after 1.5h of incubation. Moreover fatigability increased significantly. These results indicate that EDL muscle function impairment started off with a reduced relaxation velocity. Relaxation velocity is directly related to the decrease rate of cytoplasmic calcium levels. Subsequent in vitro experiments in C2C12 myotubes showed that spontaneous Ca²⁺ responses could be provoked by stimulation with ATP or caffeine. On incubations with DOX augmented this calcium response.

We conclude that the chemotherapeutic treatment induces impaired muscle function and may explain part of the asthenia. The in vitro results indicate that this could be a consequence of a Ca²⁺ overload the result of impaired transporter function. Future

interventions with these models may lead to a better understanding of the mechanism behind chemotherapy-induced fatigue and identification of beneficial interventions

2.21

Body composition and functional performance characteristics of newly-diagnosed cancer patients with and without weight loss

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Background: Cachexia-induced weight loss (WL) is associated with fatigue and weakness. Little is known about the impact of WL on muscle mass, strength and subjective performance indices, i.e., Edmonton Symptom Assessment Scale (ESAS), Brief Fatigue Inventory (BFI), Patient-Generated Subjective Global Assessment (PG-SGA), McGill Quality of Life (MQoL), in advanced cancer patients (ACP).

Methods: Handgrip and quadriceps muscle strength were measured using BIODEX dynamometry in 59 newly diagnosed (median age 61.5 yrs) cancer patients with NSCLC (n = 12) and GI (n = 47) tumours. Other variables included body composition (dual-energy X-ray absorptiometry; DEXA), and 24-hour dietary recall.

Results: Patients were grouped according to WL (<5% n = 29 vs. >5% n = 30) as determined by the PG-SGA. Both groups were similar according to age, cancer staging, and distribution of tumour types. Handgrip, quadriceps strength, and muscle mass were not different between groups (all p's > .05). Despite similar strength output, ESAS (p<.001), PG-SGA (p<.001), BFI (p<.005), and MQoL (p=.026) were different between groups. DEXA fat mass was different between groups (23±11 vs. 14±9 kg; p<.001), whereas caloric intake remained unchanged (1992±720 vs. 1881±591 kcals; p=.499). White blood cells were different (p=.012) while C-reactive protein showed a statistical trend (15.2 ± 16.3 vs. 31.9 ± 47.9 g/L; p=.085) between groups.

Conclusions: Patients with WL had lower levels of fat mass and subjective performance indices. However, measures of strength and caloric intake were not different. Further research is needed to explore the relationships and mechanisms to explain WL in cancer patients.

2.22

Bioelectrical impedance analysis (BIA) and survival in advanced cancer

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Background and aims: Bioelectrical impedance analysis (BIA), a noninvasive method to measure resistance (mainly fat mass), capacitance (stored charge within membranes) and phase angle (PA) (vector relationship between capacitance and resistance). Extracellular (ECW), intracellular (ICW) and total body water (TBW) can also be directly measured and body composition determined through conversion equations. We correlated BIA parameter changes during hydration with survival in cancer.

Methods: Patients hydrated parenterally were eligible after written informed consent. BIA was performed at baseline and for 2 consecutive days during hydration. Solution, rate, clinical evaluation, and the six parameters of BIA (capacitance, resistance, PA, TBW, ICW, and ECW) were recorded at baseline and for 2 days. Survival was correlated using hazard ration (HR) compared from study entrance.

Results: Fifty cancer inpatients participated: 29 were females; 40 have died. Mean age was 63 years. 40 received NaCl 0.9 at various rates, 2 received D5W 0.45 NaCl, and 8, D5W. Changes in resistance and TBW from day 1-3 did not correlate with survival. Shorter survival correlated with: increased PA (HR: 1.2 (CI=1.04-1.3) (P=0.008) and capacitance (HR: 1.02 (CI=1.00-1.04) (P=0.018)). Longer survival correlated with increased ECW/ICW (HR: 0.14 (CI=0.03-0.79) (P=0.025)).

Conclusion: Increased capacitance and PA during hydration predicts shorter survival while increased ECW/ICW predicts improved survival. The results are counterintuitive but may indicate a pre-existing "sick cell" syndrome which influences fluid migration during hydration and membrane capacitance/predhydration. Correlations with serum sodium and potassium are being sought at the present.

2.23

Daily physical-rest activity in cancer cachexia in relationship to nutritional state, metabolism and quality of life

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Aims: To evaluate daily physical-rest activities in weight losing cancer patients in relationship to disease progression.

Patients and Methods: Physical activity-rest rhythms were measured (Actigraph, Armband Sensor of Body Media) in relationship to body composition (DEXA) energy metabolism, exercise capacity (walking test) and self-scored quality of life (SF-36, HAD scale) in weight losing out-patients with systemic cancer (mean age 71 years, n=53). Well-nourished age-matched and previously hospitalized non-cancer patients served as controls (mean age 74 years, n=8). Middle-aged healthy individuals were used as reference subjects (mean age 49 years, n=23).

Results: Quality of life was globally reduced in cancer patients (p<0.01), accompanied by significantly reduced spontaneous physical activity (SPA) during both weekdays and weekends compared to reference subjects (p<0.01). SPA declined over time during follow up in cancer patients (p<0.05). However, overall physical activity and the extent of sleep and bed-rest activities did not differ between cancer patients and age matched non-cancer

patients. SPA correlated weekly to maximum exercise capacity in univariate analysis ($r=0.41$, $p<0.01$). Multivariate analysis showed that SPA was related to weight loss, Hb, CRP and to subjectively scored items of physical functioning and bodily pain (SF-36) ($p<0.05-0.004$). Anxiety and depression were not related to SPA. Patient survival was only predicted by weight loss and s-albumin ($p<0.01$), while SPA had no such prediction.

Conclusions: Daily physical-rest activities represent variables which probably reflects complex mental physiological and metabolic interactions. Thus, activity-rest monitoring provides a new dimension in evaluation of medical and drug interventions during palliative treatment of cancer patients.

2.24

Secondary causes of weight loss in patients with cancer cachexia

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The clinical effects of cachexia may be exacerbated by conditions capable of decreasing energy intake such as dysgeusia, anxiety, depression, pain, early satiety, nausea and constipation. The objective of this study was to determine the causes and the frequency of these conditions in a cohort of patients with cancer cachexia.

Methods: We reviewed the charts of 84 consecutive advanced cancer patients who underwent a structured assessment in a specialized cachexia clinic at a comprehensive cancer center.

Results: All patients gathered criteria for cachexia, including a weight loss of >5% within the preceding 6 months. The conditions identified most frequently were early satiety in 61 patients (73%), uncontrolled pain in 45 (54%), constipation in 43 (51%), depression or anxiety in 37(44%), chronic nausea in 33 (39%), dysgeusia in 19(23%), dental problems in 5(6%), dysphagia in 5 (6%) chronic aspiration in 2 (2%) and oral candidiasis in 1 (1%). 80 patients (95%) presented with at least one condition contributing to decreased energy intake, and 55 (65%) presented with ≥ 3 . The median number of causes was 3 (range 0-5). Metoclopramide was initiated or increased in 56 (67%) and laxatives in 46 (55%). 21 patients (25%) were subsequently enrolled in clinical trials of interventions for "primary" cachexia.

Conclusions: Secondary causes of weight loss should be ruled out in all cancer patients who have cachexia, since the majority referred to a cachexia clinic have 3 or more conditions contributing to involuntary weight loss. Inexpensive effective treatments are available for these conditions but are probably underused.

2.25

Functional muscle testing may detect early cancer cachectic muscle degeneration

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Purpose: Muscle weakness and cancer related fatigue (CRF) are major problems reducing quality of life in cachectic cancer patients. Knowledge about muscle performance in these patients is scarce and the mechanisms for muscle atrophy are unsolved yet. Thus, it is of clinical interest to identify parameters before the onset of cachexia, especially before patients reveal the usually used criterion of 10% weight loss.

Methods: 10 cachectic patients and 29 healthy subjects performed a combined concentric isokinetic (IK) and isometric (IM) strength training protocol for 8 weeks (twice per week). Maximal IK peak torque (PT; range of motion 80°) and maximal IM PT with knee position fixed at 40° were measured for right and left leg extensors and flexors. Ratios from IK PT and IM PT were calculated. Maximal cross sectional area (CSA) was assessed by magnetic-resonance tomography.

Results: Before training both IK and IM maximal PT were significantly lower in patients in comparison with healthy subjects (mean reduction IK 40.63±1.53% IM 36.52±5.81%). Most of the IK PT-reduction may be explained by the significantly reduced CSA in the patients (e.g. right extensors $r=0.92$ $p=0.00$). Interestingly, analyzing IM data shows a smaller deficit in patients and concordantly IK/IM-ratios are significantly lower in patients (Extensors -10.71%, Flexors -3.91%).

Conclusion: We conclude that muscle weakness in cancer cachexia is explained to a great portion by reduction in CSA but additionally involves presently unidentified factors possibly also central neuromuscular mechanisms. Thus, dynamically performed muscle functional tests may be reasonable as non-invasive procedures for detecting cachexia related muscle atrophy.

2.26

C-reactive protein: a potential predictor of early cachexia in lung cancer?

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Background: Muscle loss leading to cachexia is rarely identified early in lung cancer. Objective: To identify biological markers related to early weight loss to predict cachexia onset in newly diagnosed non-small cell lung cancer (NSCLC) patients.

Methods: NSCLC and non-cancer (controls) patients were recruited and assessed using Patient Generated-Subjective Global Assessment (PG-SGA) before their planned thoracotomy. Fasting blood samples and muscle biopsy (latissimus dorsi) specimens were collected at the beginning of surgery to measure circulating anabolic/catabolic /

inflammatory factors and key regulators of muscle protein synthesis and proteolysis pathways.

Results: From 59 NSCLC and 15 control patients, average %weight loss (\pm SEM) was higher (-1.9 ± 0.7 vs $0.1\pm0.4\%$), serum C-reactive protein (CRP) levels were higher (15 ± 3 vs 4 ± 2 mg/L, $p=0.00$ 1) and albumin was lower (38 ± 1 vs 36 ± 1 g/L, $p=0.03$) in NSCLC. Among NSCLC, CRP levels correlated negatively with %weight loss ($r=-0.56$, $p<0.001$), albumin ($r=-0.53$, $p<0.001$), hemoglobin ($r=-0.47$, $p=0.001$) and positively with platelet count ($r=0.73$, $p<0.001$) and physical activity score ($r=0.43$, $p=0.001$). Preliminary muscle data ($n=24$) showed higher MuRF1 mRNA expression (a regulating ligase of the ubiquitin-proteasome proteolysis system) in NSCLC with weight loss vs. none ($p=0.028$), suggestive of higher muscle proteolysis. MuRF1 expression also correlated with CRP levels ($r=0.42$, $p=0.02$). Serum CRP was the most important predictor of %weight loss ($r=0.56$, $p<0.001$) whereas albumin best predicted total PG-SGA ($r=0.55$, $p<0.001$).

Conclusion: Inflammation and compromised nutritional status were prevalent in early NSCLC. Serum CRP may serve as an early indicator of increased muscle proteolysis underlying weight loss and functional limitations in the early stages of NSCLC.

2.27

Skeletal muscle mass regulation in mildly weight-losing cancer patients

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Background: Cancer cachexia, characterized by loss of muscle mass and systemic inflammation, occurs commonly in non-small-cell lung cancer (NSCLC). Experimental models of cachexia have revealed increased ubiquitin-proteasome pathway (UPP) activity resulting in increased muscle protein degradation and decreased muscle anabolism.

Aims: To investigate whether UPP activity is increased and/or muscle anabolism is decreased in skeletal muscle of NSCLC patients.

Methods: Vastus lateralis muscle biopsies were obtained from 11 NSCLC patients (5% weight loss) and 8 healthy, age-matched controls. Plasma sTNF-R55 and CRP were determined. NF-kappaB f activity was determined indirectly by IkappaB f mRNA assessment using Q-PCR. UPP activity was evaluated by chymotrypsin- and caspase-like proteasome activities, as well as Q-PCR analysis of MuRF1 and Atrogin. Muscle anabolism was assessed as MyoD, Myogenin and IGF-1eA mRNA transcript levels.

Results: Muscle IkappaB f and TNFalpha mRNA levels were unchanged. Strong correlations between IkappaB f and both plasma sTNF-R55 ($R=0.74$, $p=0.01$) and CRP ($R=0.86$, $p=0.001$) were observed in patients but not in controls. Both proteasome activities, as well as MuRF1 and Atrogin levels were unchanged, although a trend for an increase in MuRF1 ($p=0.076$) was observed. IGF-1eA and MyoD levels were not different. Myogenin mRNA levels normalized to actin were significantly increased ($p=0.025$) in patients compared to controls.

Conclusions: Our results show evidence for an association between systemic inflammation and muscle NF-kappaB activity in NSCLC patients. In this patient group with very mild weight loss, no gross changes in UPP activity were observed, although the increase in myogenin expression may reflect a compensatory muscle regenerative response.

2.28

Body composition and exercise intolerance in non small cell lung cancer.

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Background: Lung cancer is a major killer in both men and women. An estimated 173,700 new cases of lung cancer and 160,440 deaths from lung cancer will occur in the United States in 2007. In non-small cell lung cancer (NSCLC) cachexia, impaired exercise capacity, fatigue, and shortness of breath is frequently seen. We aimed to test underlying causes leading to impaired exercise capacity and the impact of cachexia.

Methods: 29 NSCLC patients (18 male, age 61 ± 6.2 years) were examined prospectively and compared with 22 healthy controls matching for age and gender (age 61 ± 7.8 years, 8 female). Patients and controls underwent maximal exercise testing with measurement of peak oxygen uptake (pVO2) and body composition analysis using dual energy X-ray absorptiometry (DEXA) in addition to conventional echocardiography, 24h-holter-ECG, and measurements of peripheral blood flow.

Results: LV ejection fraction (LVEF) was not different between controls (64 \pm 5%) and NSCLC patients (61 \pm 10%, $p=0.43$). BMI (24 ± 4 vs 26 ± 4 , $p=0.4$) was not significantly reduced in NSCLC patients. In 7 patients (24 %) cachexia was diagnosed (weight loss >5% within the last year or BMI <20 kg/m²). In comparison to controls, NSCLC patients had different total lean tissue (48 ± 9 vs 53 ± 10 kg, $p=0.02$) and bone mineral content (2.6 ± 0.5 vs 3 ± 0.6 kg, $p=0.017$) whereas there was no difference in total fat tissue (21.6 ± 11 vs 22.4 ± 10 kg, $p=0.8$). Exercise capacity (pVO2 18 ± 3.6 vs 29 ± 6.9 mL/min/kg, $p<0.001$) and anaerobic threshold (12 ± 3 vs 15 ± 4 mL/min/kg, $p=0.024$) were reduced in NSCLC patients. The VE/VCO2-slope was increased (33 ± 5 vs 27 ± 3 , $p=0.0002$). Exercise capacity was reduced in cachectic vs non-cachectic patients (pVO2 14 ± 6.2 vs 20 ± 5.5 mL/min/kg, $p=0.0771$). FEV1 (2.2 ± 0.6 vs 3.3 ± 0.7 L, $p<0.0001$), FVC (3 ± 0.8 vs 4 ± 1 L, $p=0.0004$), and FEV1/FVC (74.3 ± 8.5 vs $81.4\pm7.6\%$, $p=0.0052$) were dramatically reduced in NSCLC-patients. FEV1 related to pVO2 ($r=0.6$, $p=0.002$). Haemoglobin concentration (12.5 ± 1.7 vs 14.4 ± 1.1 mg/dl, $p<0.0001$) was reduced in NSCLC patients and related to total pVO2 ($r=0.56$, $p<0.05$) whereas in controls this relation was not present. Multivariate analysis showed a significant correlation for

FEV1 to predict pVO2 ($r=0.68$; $r^2=0.47$; $p=0.0019$) while only a trend was seen for haemoglobin ($p=0.08$).

Conclusions: Exercise capacity is reduced in patients with NSCLC and cachexia. Only 46% of the reduction of exercise capacity, described by the multivariate analysis, is explained by limited pulmonary function and low haemoglobin. Therefore we hypothesize that factors released directly from the tumor diminish exercise capacity according to the muscle hypothesis of heart failure, i.e. by reducing the intrinsic quality of lean skeletal muscle.

2.29

Fat loss and lipid metabolism in advanced cancer patients

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Background and aims: Extensive loss of adipose tissue is a key feature of cancer cachexia. Low levels of plasma phospholipids (PL) have also been reported in advanced cancer patients. The nature of altered lipid metabolism and the relationship between depletion of phospholipids and extensive lipolysis are unknown. We aimed to characterize loss of adipose tissue and changes in plasma PL fatty acids over the disease trajectory.

Methods: A retrospective cohort of patients who died of colorectal and lung cancers ($n=71$) who underwent > 2 computed tomography (CT) scans in the last 500 days of life, had body composition assessed using Slice-O-Matic software. Amounts and types of fatty acids in plasma PL were assessed in a similar cohort of patients with metastatic cancer of the lung / colorectum ($n=76$) using gas liquid chromatography.

Results: Patients began to lose fat tissue mass at 264 days before death. Adipose loss increased exponentially ($p<0.05$) as patients approached death. Fat loss was maximal at a mean time to death of 92 ± 26 (SD) days, at which time it was 3.60 ± 5.45 kg /100 days (1.85 ± 2.97 kg/m² of height /100 days). Plasma PL fatty acids declined and were lower in the 264 days prior to death than beforehand ($p<0.01$).

Conclusions: Depletion of plasma PL and particularly essential fatty acids indicates a fatty acid deficit in the periphery which may contribute to loss of both adipose and lean tissue mass. These observations are unlikely to be wholly attributable to changes in dietary intake.

2.30

Cachectic colorectal cancer patients show anabolic resistance of muscle signaling and protein turnover to amino acid availability without elevated whole body or muscle proteolysis

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Background and aims: We previously showed (Emery et al BMJ, 289, 1984) that postabsorptive lung cancer patients had depressed muscle protein synthesis (MPS); leg efflux of 3-methylhistidine indicated no elevation of protein breakdown (MPB) (Lundholm et al Cancer Res 42, 1982). We now hypothesize that cancer patients show blunted anabolic responses to amino acids (AA).

Methods: We studied 11 patients (Duke's A-C2, $69 \pm 12y$), losing 16 g/day of lean mass, scheduled for hemicolectomy, both postabsorptively and during infusion of AA. Muscle was biopsied to measure MPS (incorporation of $[1,2^{13}C]_{2}$ leucine), phosphorylation of mTOR-pathway components, mRNA for atrogens, ubiquitin, and myostatin, and Western analysis for 14kDa actin fragment and other proteolytic "markers". Whole body proteolysis (WBP) and MPB were measured as arterialized and femoral vein dilutions of D₅-phenylalanine and $[1,2^{13}C]_{2}$ leucine.

Results: Compared to healthy weight and age-matched controls, postabsorptive patients showed $40 \pm 15\%$ lower MPS ($P<0.01$), the predominant cause of muscle loss since MPB was not elevated, and neither was WBP. Muscle "markers" of proteolytic activity and myostatin mRNA were normal. Furthermore, on AA infusion, the patients failed to show any increased phosphorylation of anabolic signaling elements, nor any increase in MPS or depression in MPB, whereas controls showed marked anabolic responses (e.g. $40 \pm 10\%$ increase [$P<0.001$] in MPS). After curative resection, despite no improvements in stress markers (cortisol, C-reactive protein, TNF- α), the patients showed a restoration of anabolic responses.

Conclusions: Not all cancer cachexia is due to unrestrained protein breakdown in muscle or whole body. Supported by Derby NHS Trust, RCSEng and CRUK.

2.31

Systemic inflammation and muscle quality in cancer cachexia

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Background and aims: Muscle wasting and loss of function are key features of both inflammation and cancer cachexia. We report preliminary results of lower limb muscle strength and power in cancer patients, along with a method of quantifying muscle quality with a view to assessing the role of inflammation in muscle dysfunction and developing novel end-points for intervention studies.

Methods: Newly diagnosed upper GI cancer patients awaiting surgery were recruited. Ten females and four males (average weight-loss 8.6%) participated. Voluntary isometric knee extensor strength and lower limb extensor power were measured in each leg. Magnetic resonance imaging of the thigh was performed ($n=8$ analysed) and the quadriceps muscle cross-sectional area (CSA) at the midpoint of the femur calculated. This estimate of mass was combined with the functional measurements to give a reading of muscle quality. Systemic inflammation (SI) was assessed with plasma CRP (>10 mg/L).

Results: Cancer patients demonstrated a 34% mean reduction in strength ($p=0.0012$) and a 38% reduction in power ($p<0.0001$) when compared with age and sex-matched predicted values. There was no correlation with weight loss. Patients with SI had a significantly lower strength (0.8 vs 1.1 N/kg, $p=0.0464$) but not power (1.2 vs 1.4 W/kg, $p=0.3171$) compared with those without SI. However when these variables were expressed in relation to quadriceps CSA (muscle quality), both were significantly reduced (2.7 vs 5.0 Ncm⁻², $p=0.0269$; 1.2 vs 1.8 Wcm⁻², $p=0.0172$ respectively).

Conclusions: Systemic inflammation appears to be an important factor in reduced muscle strength, power and quality in the early stages of cancer cachexia.

2.32

A mass spectrometric approach to the discovery of protein biomarkers in the urine of patients with gastroesophageal cancer and cachexia

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Background and aims: Increased understanding of the mechanisms regulating muscle proteolysis is important for the development of therapeutic strategies aimed at preventing/reversing cancer cachexia. Identification of proteins within the systemic compartment of cachectic patients may identify relevant circulating mediators and degradation products.

Methods: Urinary protein from 8 cachectic ($>10\%$ weight loss) gastro-oesophageal cancer (GOC) patients, 8 weight-stable GOC patients, and 8 healthy controls was precipitated using cold acetone containing 20mM dithiothreitol. Proteins were re-suspended and analysed by 1D sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Gel lanes were pixelated and proteins were digested typically. Peptide digests were analysed using liquid chromatography-mass spectrometry (LC-MS) and matrix-assisted laser desorption-ionisation time-of-flight (MALDI-TOF) MS.

Results: Cachectic patients exhibited a median weight loss of 17.9% (range 12.7-25.6). Nutritional parameters were significantly reduced in cachectic GOC patients compared with weight-stable patients and controls (body mass index $p<0.05$; mid-arm circumference $p<0.01$; mid-arm muscle circumference $p<0.05$). More proteins were identified in cachectic samples (median 45; range 28-65; total 206) compared with weight-stable (median 17; range 9-28; total 83) and control samples (median 13; range 5-18; total 54) ($p<0.001$). Many of these proteins have not previously been reported in the urine of cancer patients. Certain proteins (immunoglobulin κ -light chain and zinc α -2 glycoprotein) appear to represent markers of cancer, whereas other muscle (myosin), cytoskeletal (spectrin, talin and nischarin), and microtubule-associated proteins (microtubule-actin crosslinking factor and microtubule-associated protein-1B) represent specific markers of cachexia.

Conclusion: Urinary proteomics can identify candidate molecules for potential targeting as novel biomarkers of cachexia, prognosis and response to therapy in patients with GOC.

2.33

Plasma levels of macrophage inhibitory cytokine-1 in patients with gastro-oesophageal cancer: association with systemic inflammation

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Background and aims: High circulating levels of macrophage inhibitory cytokine-1 (MIC-1) are associated with poor prognosis in colorectal and prostate cancer. Systemic inflammation (defined by plasma C-reactive prote in [CRP] >10 mg/L) and poor nutritional status are adverse prognostic factors in gastro-oesophageal cancer (GOC). We aimed to measure circulating levels of MIC-1 in patients with GOC, and analyse the relationship between MIC-1 and systemic inflammation/nutritional status.

Methods: Plasma MIC-1 and CRP were measured in 293 GOC patients (198 males; 95 females) at diagnosis. Assessment of nutritional status and Karnofsky performance score (KPS) were performed. 37 healthy controls were recruited for comparative MIC-1 analysis.

Results: MIC-1 was elevated in GOC patients (median=1372pg/ml; range=141-39053) compared with controls (median=377pg/ml; range=141-3786) ($p<0.001$). Patients with gastric tumours (median=1592pg/ml) demonstrated higher MIC-1 levels than patients with oesophagogastric junction (median=1337pg/ml) and oesophageal (median=1180pg/ml) tumours ($p<0.015$). Patients with poorly-differentiated tumours (median=1480pg/ml) demonstrated higher MIC-1 levels than patients with moderately-differentiated (median=1103pg/ml) and well-differentiated (median=875pg/ml) tumours ($p=0.010$). GOC patients exhibited a median weight loss of 9.1% (range=-7.5-33.4), and 42% of patients had CRP >10 mg/L (median=9mg/L; range=1-200). MIC-1 correlated positively with CRP ($r^2=0.314$; $p<0.001$), disease stage ($r^2=0.217$, $p<0.001$) and patient age ($r^2=0.332$; $p<0.001$), and correlated inversely with KPS ($r^2=0.269$; $p<0.001$). However, MIC-1 did not correlate with body mass index, mid-arm circumference or triceps skinfold thickness.

Conclusions: Variation in MIC-1 levels between different tumour types, grades and stages implicates a tumour-specific mechanism in the induction of MIC-1. MIC-1 may play a role in the aetiology of systemic inflammation in GOC. Measurement of MIC-1 may be useful in patient prognostication.

2.34

Real-imaging cDNA-AFLP gene expression profiling reveals new insights into muscle cachexia development of pancreatic cancer patients

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Introduction: Most patients with unresectable pancreatic cancer lose body weight and frequently develop cachexia syndrome. The skeletal muscle appears to be significantly affected by cachexia, resulting in altered energy and altered protein metabolism of these patients. However, gene expression profiles of affected muscle tissue have not been studied yet and there is no suitable commercial gene chip available.

Methods: RNA was isolated from rectus abdominis muscle biopsies of 5 patients with pancreatic cancer and 5 controls (median age 62±8y). To perform gene expression profiling, the Real-Imaging cDNA-AFLP was developed to detect and isolate differentially expressed cDNA fragments using near-infrared fluorescence detection of ReadIR 4200 Sequencer and Odyssey Imaging System (LI-COR Biosciences).

Results: About 500 differentially expressed cDNA fragments were isolated resulting in 200 transcript tags. Identified genes were organized into cachexia-associated gene expression local database and clustered according to their annotations. Target genes were validated by quantitative RT-PCR using the same biopsy samples.

Discussion: A panel of cachexia-associated genes is presented. Our results document for the first time the altered expression of several genes that were not previously associated with muscle cachexia of patients with advanced pancreatic cancer. The Real-Imaging cDNA-AFLP is shown to be a powerful and reliable alternative approach to DNA microarray in high-throughput gene expression studies of cachexia development in skeletal muscle of pancreatic cancer patients. High sensitivity and reproducibility of this approach make possible to analyze up to 64 patients simultaneously using as little material as skeletal muscle biopsy.

2.35

Lower intramuscular free amino acids, carnosine and glutathione with reduced myofibrillar protein in cachectic compared to non-cachectic patients with pancreatic cancer

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Background and aims: During increased muscle proteolysis the free pool of amino acids (AA) is expanded. Little data are available on muscle free AA concentrations in patients with cancer cachexia. Accordingly, we examined the size of intramuscular pools of AA, carnosine and glutathione as well as of myofibrillar proteins in biopsies obtained from cachectic and non-cachectic patients with pancreatic cancer.

Methods: We studied patients with histologically-confirmed pancreatic adenocarcinoma (all UICC II-IV) either cachectic (n=7, 12.4% weight-loss) or non-cachectic (n=5, 2.7% weight-loss). Rectus abdominis biopsies were obtained during resection of suspected pancreatic cancer. We analysed total free AA concentrations and carnosine (HPLC), glutathione (spectrophotometry) and myofibrillar proteins (PAGE/Coomassie blue).

Results: Myofibrillar protein concentrations were lower in cachectic patients (myosin heavy chain -30±12%, actin -23±11%, both P<0.05). Reduced concentrations (nmol/mg protein, mean±SD) were found with cachexia for essential AA (23.6±12.7 vs. 43.7±18.6, P=0.05), leucine (1.67±0.86 vs. 2.64±0.84, P=0.08) as well as the non-metabolizable AA phenylalanine (1.30±0.45 vs. 1.96±0.27, P=0.02). 3-Methylhistidine was not significantly higher (1.30±1.4 vs. 0.68±0.41, P=0.31). Moreover, significant reductions were found for carnosine (28.3±21.7 vs. 79.1±49.6, p=0.04) as well as reduced (10.6±5.2 vs. 23.6±14.3, P=0.05) and oxidized glutathione (0.18±0.19 vs. 0.46±0.26, P=0.05).

Conclusions: As intramuscular free AA, carnosine and GSH were in fact lower in cachectic compared to non-cachectic cancer patients, our results suggest that muscle proteolysis was not elevated but that antioxidant defences were compromised in the cachectic patients. The decreased concentration of intramuscular leucine would be consonant with a decreased anabolic drive.

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2.36

Fatigue in pancreatic cancer: the potential link between exertional dyspnea, exercise limitation, skeletal musculature and neuro-hormonal activation.

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Background: In cancer, dyspnea and reduced exercise capacity are frequently seen, their origin is unclear. We suggest, symptoms are due to metabolic changes within the skeletal musculature as has previously been shown for patients with heart failure.

Methods: We examined 50 patients with pancreatic cancer (PaCA, age 60±10 years [mean±SD]). Symptom limited exercise capacity (treadmill), body composition (DEXA), systolic and diastolic function (echocardiography) and limb post-ischemic peak blood flow were assessed. 40 healthy subjects served as controls (age 57±10 years).

Results: 49% of PaCA patients were classified as NYHA class II or III. In PaCA, exercise capacity (peak VO₂) was reduced by 30%, anaerobic threshold by 13% and peak VO₂/kg lean tissue by 33%, while VE/VCO₂-slope was increased by 14%. Compared to controls, patients with PaCA had reduced limb lean mass (9%), lower fat tissue mass (32%). Total peak VO₂ closely related to limb lean mass in controls (r=0.81, p<0.0001), but much less in PaCA (r=0.42, p=0.004). Markers of neurohormonal activation like mid-regional (MR) pro-adrenomedullin (60%) and MR pro-ANP (73%) as well as markers of inflammation (sTNFR's, procalcitonin) were increased in PaCA (all p<0.05).

Resting blood flow was elevated in PaCA in arm (35%) and leg (72%), flow mediated flow was increased in PaCA by 46%.

Conclusion: Exercise capacity is significantly impaired in PaCA. We hypothesise that symptom generation and exercise intolerance develop due to metabolic aberrations leading to intrinsic changes within the skeletal muscle. The pathophysiology of shortness of breath in cancer is similar to that in heart failure.

Table 1:

Parameter	Patients (50)	Controls (40)	p-value
Peak VO ₂ [mL/min/kg]	21 ± 5.69	29.5 ± 7.8	<0.0001
Anaerobic threshold [mL/min/kg]	12.7 ± 3.06	15 ± 3.27	0.003
Peak VO ₂ /kg lean tissue [mL/min/kg]	28.6 ± 7.87	43.1 ± 8.43	<0.0001
VE/VCO ₂ slope	32.5 ± 7.8	27.5 ± 4.94	0.002
BMI [kg/m ²]	22.8 ± 3.06	25.5 ± 3.9	0.0007
Limb lean tissue mass [kg]	20.5 ± 4.42	23.2 ± 6.44	0.025
Lean tissue mass [kg]	49.8 ± 9.26	51.8 ± 12.3	0.4
Fat tissue mass [kg]	15 ± 7.39	21.7 ± 8.85	0.0003
LV ejection fraction [%]	60 ± 8	62 ± 5	0.3
MR pro-adrenomedullin [nmol/L]	0.75 ± 0.69	0.47 ± 0.1	0.0002
MR pro-atrial natriuretic peptide [pmol/L]	123 ± 177	71 ± 35	0.05
TNF-receptor 1 [pg/mL]	1943 ± 1441	1131 ± 265	<0.0001
TNF-receptor 2 [pg/mL]	2684 ± 1413	1472 ± 409	<0.0001
IL-6 [pg/mL]	6.88 ± 8.69	2.03 ± 0.93	<0.0001
Procalcitonin [ng/mL]	1.005 ± 4.3	0.019 ± 0.008	<0.0001
Resting blood flow arm [mL/100g*min]	6.71 ± 3.54	4.96 ± 1.93	0.02
Resting blood flow leg [mL/100g*min]	6.03 ± 4.25	3.51 ± 1.61	0.006
Flow mediated flow [mL/100g*min]	10.2 ± 6.16	7 ± 3.63	0.02

2.37

Interaction of gonadal status with systemic inflammation and opioid use in determining nutritional status and prognosis in advanced pancreatic cancer

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Background and aims: Studies of advanced pancreatic cancer (APC) have demonstrated worsened prognosis in young females and elderly males. Other studies have demonstrated low serum testosterone in male patients and high estradiol in females. Opioids may induce male hypogonadism. We aimed to investigate the interaction of gonadal status with systemic inflammation and opioid use in determining nutritional status and prognosis in APC.

Methods: Serum levels of total testosterone (TT), calculated free testosterone (CFT), estradiol, sex-hormone-binding globulin (SHBG), FSH, LH, CRP, IL-6, and TNF-receptor75 were measured in 175 APC patients (92 males; 83 post-menopausal females) and 19 age-matched, non-cancer controls. Nutritional assessment and opioid use were recorded.

Results: Male patients demonstrated lower TT (p=0.050) and CFT (p=0.006), and elevated SHBG (p=0.034) compared with controls. 75% of male patients were hypogonadal (defined by CFT parameters). CFT correlated positively with BMI (p<0.001) and grip strength (p=0.045), and inversely with weight loss (p=0.004), CRP (p<0.001) and IL-6 (p=0.002). Hypogonadal male patients (defined by either TT or CFT parameters) demonstrated worsened survival compared with eugonadal patients (TT definition - OR of death=2.64; p<0.001; CFT definition - OR=2.17; p=0.008). Male opioid use was associated with decreased TT (p<0.001) and CFT (p<0.001), and was associated independently with worsened survival (OR=1.99; p=0.008).

Although female patients also exhibited higher SHBG (p=0.001), there was a trend towards higher estradiol compared with controls (p=0.072). Estradiol correlated positively with TNF-receptor75 (p=0.012). Hyperestrogenal females demonstrated worsened survival compared with eugonadal patients (OR=2.08; p=0.040).

Conclusions: Low testosterone in males and high estradiol in females are associated with adverse prognosis in APC. Systemic inflammation in both sexes, and opioids in males, may influence adversely gonadal status to affect prognosis.

2.38

Tertiary cachexia: psycho-social factors that contribute to the problems caused by cancer cachexia syndrome

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Background: Cancer cachexia syndrome (CCS) is a constellation of symptoms that typically include weight loss, anorexia and fatigue. It is caused primarily by metabolic change and secondarily by compromised nutritional intake. This paper proposes that CCS

might usefully be examined as a constellation of problems. Psycho-social factors then become visible as a third important contributor to the development of the syndrome.

Methods: This study draws on data from the Macmillan Weight and Eating Studies (MWES) and a review of non-pharmacological interventions for CCS. The MWES include a content and thematic analysis of interviews with 127 patients receiving community palliative care services in the South of England, 2002-2007. Findings are discussed in light of the literature review.

Results: Psycho-social factors can contribute to sub-optimal food intake, thereby exacerbating secondary cachexia, but in addition can lead the problem of symptom distress. An example is conflict over food within a family. Hence, psycho-social interventions may improve health outcomes for patients with CCS and need to be tested empirically.

Conclusions: Understandings of CCS have been informed by the biomedical model of disease leading to interventions that change the balance of biochemicals and/or nutrients within the body with the purpose of managing symptoms. This conceptualisation can be augmented by considering psycho-social factors that contribute to cachexia related distress. To conceptualise CCS as a constellation of problems with primary, secondary and tertiary causative factors (biochemical, nutritional and psycho-social), opens up a new field of study. Intervention for tertiary cachexia could contribute to the management of CCS.

2.39

Living with cancer cachexia: exploring the perspectives of patients and their significant others

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Background and aim: Cancer cachexia has received scant research attention despite being highly prevalent in patients with advanced malignancy. At present there is a dearth of evidence focusing on the impact that cancer cachexia has on patients and their families.

The aim of this study was to explore the lived experience of cancer cachexia in patients and their significant others.

Methods: An interpretative phenomenological approach was used in this study. A purposive sample of 15 patients and 12 significant others was recruited from a large teaching hospital in Northern Ireland. Each participant was interviewed once, using an unstructured technique about their / their significant other's experience of cancer cachexia. Analysis combined a two-stage approach comprising thematic and interpretative phenomenological analysis.

Results: Findings reflected the complex dynamics in the experience of cancer cachexia. These centred on the physical and psychosocial implications of weight loss and the family's focus on food consumption as an indicator of well being. There was a lack of insight and understanding of participants regarding food and its relationship to cachexia and professional interventions to address this was perceived as limited.

Conclusions: This study is distinctive in that it provides an in-depth understanding of the experience of cachexia for patients with cancer and their significant others by illuminating the challenges that they face. Findings from this study have the potential to contribute to the development of practice and delivery of the best possible standards of care that are anticipatory and supportive of the needs of this client group.

3.40

The role of measuring REE in the diagnosis and treatment of cachectic states

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Background and aim: Undernutrition is accompanied by compensatory decrease in cellular metabolism, indicated by reduced REE compared to predicted. Different diseases on the other hand have been shown to increase REE. Both involve changes in body composition. REE should, therefore, be normalized by LBM and energy intake monitored along. Our aim is to explore the relationship between these components in different disease states, in order to understand the possible use of REE and body composition measurements in the process of diagnosis and treatment of cachectic states.

Methods: REE was measured by indirect calorimetry, LBM by DEXA machine and energy intake was calculated based on 3 days food records.

Results: In patients with Anorexia Nervosa, a pure model of starvation, REE/LBM was 26.9±4.0 kcal/kgLBM on admission and 32.4± 0.3 after nutritional intervention (62 vs 87% predicted). In malnourished patients with CF (a model of mixed undernutrition and inflammation) REE/LBM was 42.5±3.2 kcal/kgLBM on admission and 47.6±2.7 after one year treatment (115 vs. 131% predicted) and in patients with ALS (a model of progressive disease) 36.05 ± 5.26 vs. 38.93±5.02 after one year. Comparing well nourished vs malnourished Crohn's patients REE was higher the first group inspite of similar caloric intake but different absorptive capacity.

Conclusions: Both REE and body composition change in response to energy intake and disease course. Multiple measurements of the above and absorption, when needed, are needed for tailoring the exact needs of the patients in order to improve the nutritional status and prevent unnecessary weight loss.

3.41

Validation study of inflammatory-based prognostic score in terminal cancer

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Background: Serum albumin (<35 g/L=1) and C-reactive protein (CRP) levels (> 10 mg/L=1) may be combined to form the Glasgow Prognosis Score (GPS) of 0,1 or 2. GPS has been used to categorize patients with advanced lung or gastrointestinal cancer into iso-prognostic groups, but its discriminatory ability in a heterogeneous sample or the terminally ill has not been evaluated.

Methods: Advanced cancer patients referred to a hospital-based palliative care team were recruited to a study of nutritional status, quality of life and systemic inflammation. Serum albumin and CRP levels were measured for GPS scores. Survival differences were analysed with Mann-Whitney U-test and log-rank test.

Results: 102 patients were recruited, with median observed survival of 2 months. Fifteen tumor types were represented, 53 with lung/GI malignancies. 85% were malnourished. Inflammation was common (elevated CRP in 72, hypoalbuminemia in 50). The GPS scores and survival are shown in the

GPS	N	Median Survival	U test	p-value
0	17	6 months	-	-
1	37	2 months	580.5	0.036
2	42	1 month	1647	>0.1

The difference between groups 0 and 2 was significant (u=700, p=0.002). In patients (n=50) with malignancies associated with inflammation (lung, gastrointestinal, prostate, renal), the difference between Group 1 and 2 (2.5 months vs. 1 month) became significant (U=512, p=0.022) The log-rank test was significant (LR[d.f.2]=7.678, p=0.022).

Conclusion: GPS is a simple, objective prognostic tool that may be useful in palliative care. Tumour type may be relevant. Large scale studies are required to determine appropriate cutpoints in this population.

3.42

How precise is bioelectrical impedance in cachexia

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Background and aims: Bioelectrical impedance (BIA) is based on resistance and membrane capacitance to a fixed low voltage, high frequency current conducted through body fluid compartments. Total body water (TBW) is derived from resistance. This assumes a consistent relationship between intracellular and extracellular water. This relationship is altered in cachexia. BIA using a standard equation [RJL1 (Clinton Twp, MI)] may then become imprecise. Resistance at 50 Hz (R50) may not adequately assess intracellular water (ICW) and TBW. Cachexia is associated with reduced ICW. Simon et al.2 validated a TBW equation in cachexia. (7.55 + 0.528 HT2/R50) using deuterium dilution. We compared TBW by RJL to the Simon equation.

Methods: Advanced cancer patients had BIA measurements 3 consecutive days during hydration. Resistance Day 1 was used to calculate TBW. The RJL TBW uses height (HT), weight, and resistance; the Simon equation uses resistance and height. Pearson correlation was used for comparison.

Results: 50 patients with median weight loss of 16% underwent hydration. Mean age 63 (±12 years), 29 females, mean ECOG score 1.9 (±0.8). Day 1 BIA TBW: RJL = 35.4 ± 7.8 liters; Simon = 36 ± 10 liters. Pearson correlation = 0.65, (0.53 females, 0.63 males).

Conclusion: TBW by RJL has moderate precision in cancer cachexia compared to the Simon equation. TBW calculated in normal populations lose precision in cachexia (particularly in females).

Reference:

1. Chumlea WC, Guo SS et al. Int J Obesity 2002; 26:1596-1609.
2. Simon JP, Schols A et al. Am J Clin Nutr 1995; 61:741-5.

3.43

Bioelectrical impedance analysis is a valid and accurate method to assess body composition in hemodialysis patients, correlating well to dual-energy X-ray absorptiometry

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Introduction: Bioelectrical impedance analysis (BIA) represents a simple and inexpensive way of assessing body composition in chronic hemodialysis (HD) patients. However, other methodologies [(eg. Dual-energy X-ray absorptiometry (DEXA))] have been claimed to be more reliable.

The present study examined the correlation between body composition parameters obtained by both BIA and DEXA in 139 patients on HD over a 6-month period. These assessments were part of an investigation of the efficacy and safety of growth hormone supplementation [Norditropin® (Somatropin)] in improving nutritional status of adult patients on CHD.

Methods: 139 adult HD patients were studied. Multifrequency BIA was measured at 5, 50 and 100 kHz using the central calibrated impedance analysis apparatus Nutriguard-M, and Qc measurements performed daily for each apparatus at 50 kHz. All DEXA scans were standardized and reanalyzed correcting for inter-scanner variability. A whole-body phantom was used for cross-calibration and long-term stability evaluation. Both BIA and DEXA were applied three times during the 6-months period; carried out on the day after regular dialysis. Linear regression analysis was applied to estimate the fat-free mass using analysis of correlations.

Results: The BIA-derived assessments were transformed into fat-free mass using different linear regression models: The Kyle et. al. (2001) approach based on healthy

subjects; and an estimated model based on the HD patients. Assessments were compared to the DEXA findings (Table 1), and comparisons made using analysis of correlation and correlation coefficients (Table 2).
Table 1: Fat-free mass assessments (DEXA and BIA)
 Method Number of Mean Standard Min; max(kg) observation (kg) deviation(SD)
 DEXA 310 46.18 10.79 20.91; 81.13
 BIA: Kyle et al 295 46.76 10.91 25.89; 81.48
 BIA: Estimated 293 46.30 10.05 28.05; 85.59
Model
Table 2: Analysis of correlation
 Method BIA: Kyle. et. al. BIA: Estimated model
 DEXA 0,92 (p<.0001) 0,94 (p<.0001)
Conclusion: Correlation coefficients of 0.92 and 0.94 were obtained, respectively, when comparing fat-free mass derived from BIA and DEXA. Thus, the BIA method is an equally valid and simpler methodology to assess body composition in HD patients.

3.44 Precision of dual energy X-ray absorptiometry and bioelectrical impedance in patients with advanced cancer

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Background: The acquisition of equipment to quantify body tissue composition in patients for diagnosis and to monitor changes over time, and/or response to treatment, necessitates an in-house determination of the precision of these instruments.
Method: 86 patients (50 males, 36 females) were studied with DXA (LUNAR Prodigy densitometer) and bio-impedance analysis (TANITA). Consecutive paired measurements, with repositioning between measurements, were obtained for each patient. The precision was calculated from the expression $CV\% = \frac{[\sum d^2/2N]}{M}$, where CV% denotes the coefficient of variation percent, d is the difference between each paired measurement, N is the number of patients and M is the mean of all the measurements.
Results: Mean age 60.2 years, range 22 to 83 years; mean BMI 24.2, range 16 to 38.8. The DXA results are tabulated as follows:

	Upper extremity	Lower extremity	Lower+Upper	Trunk	Total Body
BMD CV%	3.38	1.18		0.93	0.86
BMC CV%	1.78	0.81		4.17	1.31
Fat CV%	4.22	1.70	1.64	2.46	1.38
Lean CV%	2.03	1.58	1.21	1.49	0.78

For the BIA, CV% for total body fat was 1.19%, and 0.4% for total body lean tissue. By the Bland-Altman analysis the limits of agreement were ± 6.92 kg, and ± 7.60 kg, respectively. **Conclusion:** The limits of agreement between DXA and BIA may be too wide in order to use one as the surrogate of the other in a clinical context. The high precision of both modalities justifies monitoring patients for changes in total body soft tissue composition as a function of time, whether for observation or response to treatment.

3.45 Does a nutritional assessment tool correlate to laboratory values indicating cachexia in lung cancer patients?

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Background and Aim: Early recognition of cachexia in cancer patients is problematic because specific symptoms and reliable objective measurements are lacking. Screening questionnaires like the Mini Nutritional Assessment (MNA)-score (www.mna-elderly.com) may help to identify early the cachectic patients. The aim of our study was to evaluate the relation between the MNA-score and laboratory measurements indicative of cachexia in lung cancer patients before the onset of chemotherapy.

Methods: Patients with metastatic lung cancer referred to our hospital during the last 9 months were enrolled in this study. Patients were classified into 3 groups according to MNA-score (A: adequate nutrition; B: risk of malnutrition; C: malnutrition). Laboratory values indicating malnutrition, inflammation and cachexia were measured and correlated with these 3 groups.

Results: Seventy patients (64 males) entered the study. They were stratified according to the MNA-score into group A (N=18), B (N=32) and C (N=20). Laboratory results in the three groups are depicted in the following table:

MNA Group	A (mean±SD)	B (mean±SD)	C (mean±SD)	A+B vs C	A vs B+C
Lymphocytes	1344 (±745)	1552 (±684)	1542 (±473)	0.876	0.432
Albumin	4.1 (±0.3)	3.4 (±0.6)	3.4 (±0.5)	0.097	0.0002*
CRP	2.5 (±4.1)	9.2 (±11.9)	8.1 (±5.4)	0.027*	0.001*
Ferritine	146.4 (±64.8)	358.7 (±248.4)	248.0 (±178.2)	0.944	0.174
IL-6	7.6 (±8.6)	83.7 (±213.2)	22.7 (±20.0)	0.107	0.001*
IL-8	20.7 (±35.2)	84.6 (±176.0)	257.4 (±694.3)	0.036*	0.003*
TNFa	677.1 (±865.6)	1206.2 (±2619.7)	1687.3 (±3136.1)	0.741	0.160

*p<0.05

Conclusions: The MNA-score is an easy and reliable questionnaire that can be used to identify early cachexia in lung cancer patients.

3.46

Validation of a simplified anorexia questionnaire

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Introduction: A two-item simplified anorexia questionnaire (SAQ) was compared to the anorexia subscale (A/CS-12) of the Functional Assessment of Anorexia and Cachexia Therapy (FAACT).

Methods: The SAQ was 1) Appetite rated by numerical scale (NRS) (0 – normal appetite, 10 – no appetite) 2) Appetite loss by categorical scale (none, mild, moderate, severe). A cross-sectional design involved patients referred to palliative medicine. Patients completed A/CS-12 and SAQ day one (D1) and one week later (D2). Questionnaires were presented in random order. Agreement was defined as: 1) subject ordered in the same way (D1). 2) Scores in the same direction. Validation was determined by SAQ correlations to A/CS-12 and survival.

Results: 117 completed A/CS-12 D1, 111 D2; 111 completed SAQ D1, 89 D2. 69 females (median age 59 [18–87], ECOG-PS 2 [0–4]) participated. Agreement between A/CS-12 and SAQ at one time point was 0.64 (0.63–0.66), NRS -SAQ agreement was 0.56 (0.45–0.66). Agreement over time 0.53 (0.45–0.66). Median survival was 2.8 months (7 days–17.7 months). Adjusted for ECOG-PS, higher A/CS-12 score D1 and D2 predicted longer survival (P<0.004); HR 0.97 (0.95–0.99) D1, HR 0.96 (0.94–0.99) D2. SAQ did not predict survival. For SAQ and A/CS-12, no relationship was found between scores on D1 and D2. The A/CS12 question “Do you feel your family is forcing you to eat?” consistently predicted better survival. **Conclusion:** SAQ modestly correlated with A/CS-12 but loses sensitivity in predicting survival. Patients’ concerns about being forced to eat consistently predicted better survival.

3.47

Assessing quadriceps muscle strength in newly-diagnosed, advanced cancer patients: test-retest reliability and correlational analyses

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Background: Objective strength measurements have typically been limited to handgrip dynamometry testing in cancer patients. Considering the importance of lower limb muscle strength toward maintaining the quality of life, activities of daily living, and functional mobility of the patient, we evaluated the dynamic strength characteristics of the dominant leg.

Methods: We measured quadriceps extensor leg strength expressed as Newton-meters (Nm) of peak torque (pKT) at a contraction speed of 60°/s using isokinetic dynamometry in male (n = 35) and female (n = 22) patients, median age of 62.0 yrs with advanced gastrointestinal and non-small cell lung cancer. All patients were assessed twice on the same day by the same evaluator.

Results: The interclass correlation for pKT (Nm) was calculated to be 0.985 (%CV=9.2). This correlation and %CV are within the ranges published by Symons et al and Goodpaster et al in healthy elderly subjects of similar ages. As expected, men produced significantly greater extensor strength (103.30 ± 50.00 vs 57.58 ± 18.58 Nm at 600/s; p<0.001) when compared to women. These values are similar to those obtained by Katsiaras et al in groups of healthy elderly men and women. The pKT values in our patient cohort were moderately correlated with the lean muscle mass measured by dual energy X-ray absorptiometry of the leg (r2=0.298; p<0.001) segments.

Conclusion: Isokinetic dynamometry is a reliable and acceptable mode of assessment for quadriceps extensor leg strength in newly diagnosed advanced cancer patients.

3.48

Handgrip dynamometry measurements in elderly cancer patients: a comparison of two instruments (JAMAR versus BIODEX)

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Background: Isometric handgrip strength measured by the portable hydraulic dynamometer (JAMAR) is a reliable and commonly used instrument among rehabilitation specialists in different patient populations. The purpose of this study was to compare a novel handgrip dynamometry research instrument (BIODEX) with the JAMAR unit in a group of newly diagnosed, advanced cancer patients (ACP).

Methods: JAMAR (kg) and BIODEX (peak torque- pKT, Newton-meters (Nm)) handgrip strength was recorded in 47 male and 26 female ACP (median age 62 yrs) twice on the same day by the same evaluator.

Results: Using the JAMAR, the handgrip strength in males (35.9 ± 9.6 kg) was significantly greater (p<0.001) than females (22.6 ± 6.0 kg). The interclass correlation coefficient (ICC) of the JAMAR was 0.985 with a percent coefficient of variance (%CV) of 5.3. When the same patients were measured using the BIODEX isometric dynamometer, the pKT was significantly (p<0.001) higher in males (53.3 ± 13.7 Nm) than females (35.7 ± 8.6 Nm). The ICC was robust (r = 0.915) with a %CV of 12.4. The Spearman Rank Correlation between the JAMAR and BIODEX was 0.823 (p<0.001). The handgrip pKT was also strongly correlated (r2 = 0.524; p<0.001) with the pKT measured in the extensors of the quadriceps. Both instruments were correlated with lean muscle mass of the arm measured by dual energy X-ray absorptiometry.

Conclusion: Isometric strength dynamometry using the BIODEX grip apparatus is reliable and the pKT measures are well correlated with the output from the portable JAMAR unit in ACP.

4.49

A soluble activin receptor type IIB increases muscle mass in a mouse model of androgen deprivation therapy

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Background: Androgen deprivation therapy is an effective treatment of prostate cancer. However, men receiving LHRH agonists show significant muscle loss. Increasing muscle mass in these patients can contribute to improvements in morbidity and mortality. Myostatin (GDF-8) and other negative regulators of muscle mass signal via the activin receptor type IIB (ActRIIB) to elicit muscle mass effects. Treatment with a soluble ActRIIB receptor fusion protein (RAP-031) inhibits the negative signals and therefore promotes muscle growth.

Materials and methods: To test the efficacy of RAP-031 in a model of androgen deprivation therapy C57BL/6 mice were castrated (CAST) or sham-operated (SHAM) and began treatment with RAP-031 two weeks later. Body weights and in vivo body composition measurements were determined over 10 weeks.

Results: The body weight of RAP-031 treated mice was significantly higher than vehicle controls (CAST= +16%, SHAM +20%). This was due to changes in body composition as assessed by NMR analysis. CAST-PBS mice decreased lean body mass by 8% and increased fat mass by 280%, while the CAST-RAP-031 mice increased lean body mass by 18% and had significantly less increase in fat mass (200%) The SHAM-PBS mice increased lean mass by 10% and fat mass by 125% while SHAM-RAP-031 mice had a 35% increase in lean tissue and no increase in fat mass.

Conclusions: These data demonstrate that RAP-031 improves lean tissue mass and inhibits fat accumulation after castration. Thus a soluble ActRIIB-Fc fusion protein can be an effective therapy for treatment of muscle loss associated with androgen deprivation therapy.

4.50

Effects of CRF2R agonist on tumour growth and cachexia in mice bearing the Lewis lung carcinoma

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Background and aims: The aim of this study is to evaluate the effects of a corticotropin-releasing factor 2 receptor agonist in preserving skeletal muscle in a mouse cachexia model.

Methods: Implantation of a fast growing tumour to mice (Lewis lung carcinoma) resulted in a clear cachectic state characterized by a profound muscle wasting.

Results: Administration of 100 micrograms/Kg/day CRF2R agonist (PG-873637) by means of osmotic minipumps to tumour-bearing mice resulted in beneficial effects on muscle weight loss. Thus, muscle loss was partially reversed by the CRF2R agonist at different stages of tumour growth (at day 14 after tumour inoculation: 12% tibialis, 9% gastrocnemius and 48% soleus muscles). Moreover, administration of the CRF2R agonist did significantly reduce both the metastases number and its mass (at day 19 after tumour inoculation: 66% and 61% respectively).

Conclusions: Altogether, the data suggested a potential beneficial effect of the CRF2R agonist in preserving skeletal muscle during cancer cachexia and opens an interesting line of research for the development of new therapeutic approaches for the treatment of muscle wasting associated with cancer.

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4.51

Selective androgen receptor modular (SARM) prevents body weight and muscle loss in tumor-bearing mice

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Introduction: Cachexia is a condition frequently associated with cancer and chronic disease. We examined the ability of a novel, nonsteroidal selective androgen receptor modulator (SARM) to prevent cachexia in immunodeficient mice bearing subcutaneous PC3M prostate tumors.

Methods: 24 mice received subcutaneous injections of 2.5*(10)⁷ PC3M cells, while twelve control animals received culture medium only. Animals were weighed and checked for tumor growth three times/week. Ten days later, tumor-bearing mice were divided into two groups and began oral treatment with either 0.5 mg/day of SARM or vehicle. Mice were euthanized when body weight (BW) loss reached 15% or tumor size exceeded 2 g. M. gastrocnemius (MG), levator ani (LA) and seminal vesicles (SV) were excised and weighed. MG was processed for protein and/or RNA extraction.

Results: The BW of tumor-bearing mice treated with vehicle was significantly lower than controls (30.2 g vs. 22.7 g, p<0.05). SARM significantly prevented the tumor-induced decrease in BW (26.3 g, p<0.05 vs. Veh). LA and SV weight significantly decreased in cachectic mice, but was maintained in SARM-treated animals. No differences in tumor weight between groups were observed. Expression of Atrogin-1, a molecular marker of muscular atrophy, was increased 8.8 fold in tumor-bearing animals and decreased 33% by

SARM. Conclusions. SARM prevents BW and muscle loss induced by PC3M xenograft-associated cachexia in mice.

4.52

Attenuation of mouse colon adenocarcinoma CT-26-induced cachexia by Chinese herbal medicine extract in BALB/c mice

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Cachexia is a metabolic disorder characterized by anorexia and loss of lean body mass. It is a relatively common disorder in some diseases such as cancer, HIV infection and renal failure. Cachexia is estimated to be responsible for over 20% of all cancer-related deaths. Currently, available drugs are ineffective, and new therapies are urgently needed. In the present study, we have conducted the anti-cachexia efficacy testing of a Chinese herbal medicine extract, which was coded as 114-ES, in a mouse colorectal tumor CT-26 model. An adequate cell number (2 x 10⁴ cells/ mouse) of CT-26 cells were intra-splenically implanted into the mice followed by the oral administration of test article once a day (0.15 to 0.6 g/kg) for 23days. Food intake and body weight were measured, and net body weight, serum leptin, triglyceride and IL-6 also were assessed. The results showed that the administration of 114-ES significantly attenuated the drop of the net body weight of tumor-bearing mice (p < 0.01). Serum biochemistry and immunology analyses represented that the administration of 114-ES significantly attenuated the drop of the serum albumin, leptin and triglyceride, and the increase of serum IL-6 (p < 0.05). These data support the view that 114-ES will be a suitable approach for the treatment of cancer cachexia and may have potential as drug candidate.

4.53

Fenofibrate (tricolor) exacerbates muscle wasting in a mouse model of cancer cachexia

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Background and aims: Fenofibrate (Tricolor), a peroxisome proliferator-activated receptor-alpha (PPAR alpha) agonist, is known to increase insulin sensitivity and inhibit inflammatory gene expression in mice and humans. Two other isoforms of the same family (PPAR gamma and delta) have been implicated in muscle wasting during cancer cachexia. Our objective was to determine the role of PPAR alpha in muscle wasting in a mouse model of cancer cachexia.

Methods: CD2F1 mice were given either fenofibrate or normal chow diets ad libitum for the duration of the experiment. One week after introduction of the diet, mice were injected with the colon-26 (C26) adenocarcinoma cell line or PBS as a control. Mice were euthanized when weight loss equaled 10% or tumor size reached 20% of starting body weight.

Results: Fenofibrate did not affect C26 tumor growth. Fenofibrate alone induced profound hepatomegaly and a significant reduction in muscle mass, while the combination of fenofibrate and C26 tumor further increased liver mass and reduced muscle mass over either treatment alone. Paradoxically, fenofibrate treatment reduced levels of atrogin-1. Fenofibrate reduced STAT3 phosphorylation ~90% in the liver and ~50% in the skeletal muscle, and coordinately reduced expression of several STAT3-dependent APR genes including fibrinogen, LBP1, and haptoglobin, in both the liver and muscle.

Conclusion: Fenofibrate treatment caused a significant reduction in muscle mass in the C26 model of cancer cachexia. Although the mechanisms mediating fenofibrate-induced muscle wasting are unknown, these results suggest caution in use of Tricolor and similar drugs in patients diagnosed with cancer.

4.54

Ghrelin improves body weight loss associated with angiotensin II-induced cachexia in mice

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Background: Ghrelin is a growth hormone (GH) releasing peptide, identified as an endogenous ligand for GH secretagogue receptor. Ghrelin also stimulates food intake and gastric motility, reduces fat utilization, and suppresses sympathetic nerve activity. These observations suggest that ghrelin may be beneficial to prevent cachexia. The blood angiotensin II (AngII) levels in patients with chronic heart failure (CHF) associated with cachexia were higher than those in CHF patients without cachexia. It has also been reported that continuous administration of AngII to mice results in suppressed body weight gain and decreased muscle mass. Therefore, we investigated the effect of ghrelin on the body weight and body composition in mice with AngII-induced cachexia.

Methods: AngII was administered to mice for 6 days by subcutaneous infusion. Ghrelin (0.1, 1.0 mg/kg) was subcutaneously administered twice daily for 5 days. Body composition and IGF-1 content in the gastrocnemius muscle were measured by DEXA and by ELISA, respectively.

Results: AngII significantly decreased body weight, lean mass and fat mass. The administration of ghrelin (1.0 mg/kg) significantly improved body weight. DEXA analysis revealed that ghrelin increased both lean mass and fat mass. AngII also decreased IGF-1 content of the gastrocnemius muscle, which was significantly recovered by ghrelin. Ghrelin also increased daily food intake.

Conclusion: We demonstrated that ghrelin improved body weight loss associated with AngII-induced cachexia. Ghrelin-induced orexigenic effect and activation of the GH-IGF-1

axis are considered to be involved in the increase in body weight. Ghrelin could have a therapeutic ability to ameliorate cachexia.

4.55
AKT-dependent insulin signaling is enhanced in skeletal muscle and reduced in liver following in vivo ghrelin administration

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Background and Aims: Enhanced appetite and weight gain occur following ghrelin administration, which therefore represents a potential therapeutic strategy for cachexia. Diet-induced weight gain and obesity are associated with reduced insulin action in skeletal muscle and liver, and reduced whole-body insulin sensitivity (mainly reflecting muscle insulin action) can be also associated with cachexia thus contributing to cachexia-associated catabolism. Potential ghrelin effects on muscle and liver insulin action in vivo remain undetermined.

Methods: Effects of sustained ghrelin administration at a non-orexigenic dose on AKT-dependent muscle and liver insulin signaling were determined in young-adult male rats receiving 4-day, twice-daily subcutaneous ghrelin (200 µg/injection) or saline. We measured 1) skeletal muscle (mixed: gastrocnemius; oxidative: soleus) and liver activated (phosphorylated-P) and total (T) AKT and glycogen synthase kinase (GSK, reflecting AKT-dependent GSK inactivation).

Results: Ghrelin increased body weight (+1.4%) and blood glucose (both $P < 0.05$ vs saline) but not ($P = NS$) total food intake, plasma insulin and free fatty acids (FFA). Ghrelin enhanced P/T-AKT and P/T-GSK ratios as well as GLUT-4 mRNA in soleus ($P < 0.05$) while not in gastrocnemius muscles. In contrast, ghrelin reduced hepatic P/T-AKT and P/T-GSK.

Conclusions: Sustained ghrelin administration enhances oxidative muscle AKT signaling. Reduced liver AKT activation could be aimed at preserving glucogenic potential thus contributing to concomitant blood glucose increments. These findings support ghrelin as a novel tissue-specific modulator of lean tissue AKT signaling in vivo, independent of changes in food intake. Insulin-sensitizing effects in muscle could per se favor muscle anabolism in ghrelin-treated models of cachexia.

4.56
Effects of beta-hydroxy-beta-methylbutyrate treatment on protein metabolism in rat skeletal muscle

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Background and aims: Beta-hydroxy-beta-methylbutyrate (HMB) is the leucine metabolite, which could contribute to reversing AIDS- and cancer-related cachexia. The aim of our study was to determine changes after HMB treatment in protein metabolism in skeletal muscle of intact and septic rats.

Methods: Intact and septic rats (i.p. administration of endotoxin, 5 mg/kg) were implanted with osmotic pump with or without HMB content (0.5 g/kg/day) ($n \geq 9$ for each group). After 24 hours extensor digitorum longus (EDL) and soleus (SOL) muscles were isolated and used for determination of total and myofibrillar proteolysis (PL), protein synthesis (PS), leucine oxidation (OL), chymotrypsin like activity (CTLA) and alpha subunits of proteasome.

Results: Sepsis induced a stimulation of PL, CTLA and OL in both types of muscles and an attenuation of PS in EDL only. In HMB-treated septic animals we observed a decrease in OL in both types of muscles, myofibrillar PL and CTLA in SOL and total PL in EDL only. In intact rats treated with HMB we found a decrease in OL and CTLA in SOL, in EDL an increase in PS and total PL and reduction in myofibrillar PL.

Conclusions: The results indicate positive effect of HMB treatment on protein metabolism both in intact and septic animals. This effect is muscle-type dependent and is caused by attenuation of proteasome activity and protein breakdown and by stimulation of protein synthesis.

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4.57
Lithium administration modulates muscle GSK3-beta but does not prevent muscle loss in experimental cancer cachexia

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Rationale: Cancer cachexia (CC)-associated muscle atrophy results in an imbalance between the rates of protein synthesis and breakdown. Insulin-like growth factor-1 (IGF-1) is a positive regulator of skeletal muscle mass. A intracellular target in the IGF-1 pathway is glycogen synthase kinase (GSK-3beta) which negatively regulates the anabolic process. Indeed, GSK-3beta inhibition results in the appearance of a hypertrophic phenotype. Lithium is a natural inhibitor of GSK-3beta. The present study aimed at verifying if a lithium-enriched diet may prevent/attenuate muscle loss in cancer bearing rats.

Methods: Wistar rats ($n=32$) were randomized to receive a regular (R) or a lithium-carbonate-supplemented (Li+) chow (Li_2CO_3 2.5g/kg/day). After 2 weeks, 8 rats/group received an intraperitoneal inoculum of 10^8 cell/2ml of AH-130 ascites hepatoma cells (tumour bearing, TB). All rats were weighed and sacrificed on day 21. The state of

activation of GSK-3beta (pGSK-3beta) was evaluated in the gastrocnemius muscle by western blotting analysis.

Results: Treatment with Li_2CO_3 effectively inhibited GSK-3beta activity. Indeed, the levels of phosphorylated GSK-3beta were significantly higher in Li+ than in unsupplemented rats ($p < 0.05$). However, both body and muscle weight loss were unaffected in TB, where pGSK-3beta levels were significantly higher than in R (1.22 ± 0.24 vs 0.64 ± 0.15 , $p < 0.001$).

Table:

	R(n=8)	Li+(n=8)	TB(n=8)	TB Li+(n=8)
Carcass and gastrocnemius weights				
Carcass wt (g)	400±41	371±35	249±20**	299±44**
Gastrocnemius (mg/% i.b.w.)	591±43	605±36	509±29**	489±41**

Mean ± SD; ** $p < 0.001$ vs R (ANOVA)

Conclusions: In vivo Li_2CO_3 administration can modulate skeletal muscle GSK-3beta activity, but cannot prevent muscle loss, at least in this experimental model of CC.

4.58
Effect of beta-hydroxy-beta-methylbutyrate on weight and muscle loss in cancer cachexia

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Background and aims: Beta-hydroxy-beta-methylbutyrate (HMB), a leucine metabolite, has been reported to exert anabolic effects on muscle. This study aimed at evaluating the effects of HMB administration in an experimental model of cancer cachexia (CC).

Methods: Wistar rats were randomized to receive standard (C) or 4% HMB-enriched (C+HMB) chow. Rats from both groups were randomized to receive an i.p. inoculum of AH-130 cells (TB and TB+HMB). All rats were weighed and sacrificed at day 24. Liver, heart, and muscles were excised and weighed. The protein levels of p-p70, p-elf2α and p-4EB-P1 were evaluated by Western Blotting on gastrocnemius (GSN).

Results: HMB significantly increased GSN and heart weight in C. In TB+HMB body weight was significantly increased, and GSN loss was significantly attenuated with respect to TB.

p-elf2α decreased in C+HMB, while in TB was reduced vs C, but unaffected by HMB. p-p70 was increased by HMB in C and further increased in TB. Phosphorylated 4-EB-P1 was increased in TB but unaffected by HMB.

	Body wt diff (g)	GSN (d24-d16) g%/init. BW	Soleus g%/init. BW	Heart g%/init. BW	Liver g%/init. BW
C(n=13)	48.8±4.9	0.63±0.05	0.04±0.01	0.32±0.03	5.83±0.47
C+HMB(n=16)	50.5±4.5	0.68±0.03*	0.04±0.01	0.36±0.03**	5.82±0.53
TB(n=12)	-2.8±7.3	0.54±0.03	0.04±0.01	0.30±0.02	5.04±0.25
TB+HMB(n=15)	13.8±16.4**	0.57±0.04*	0.037±0.01	0.31±0.04	5.21±0.56

*: $p = 0.002$; **: $p = 0.001$ vs C; *: $p = 0.03$; **: $p = 0.003$ vs TB ('t' test)

Conclusion: HMB attenuates weight and muscle loss in experimental CC, possibly by stimulating anabolic pathways. HMB should be regarded to as a promising nutritional substrate to counteract muscle loss in CC.

4.59
Endurance training restores hepatic lipid metabolism of cachectic Walker 256 tumour bearing rats

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Background and aims: Cancer cachexia is a syndrome that causes profound metabolic disruption including changes of lipid metabolism in the liver. Moderate endurance training modulates hepatic lipid metabolism. We tested the influence of a moderate intensity training running for (6 weeks) upon VLDL assembly and secretion, apoB and MTP gene expression in the liver of cachectic tumour-bearing rats. **Materials and methods:** Animals were randomly assigned to a sedentary control (SC, $n=7$), sedentary tumour-bearing (ST, $n=7$), or trained control (TC, $n=5$) and trained tumour-bearing (TT, $n=5$) groups. Trained rats ran on a treadmill ($60\%V_{O_{2max}}$) for 60min/day, 5 days/week, for 6 weeks. Serum levels of TAG, cholesterol, VLDL-TAG, VLDL-cholesterol, HDL-cholesterol were assessed, as well as the profile of VLDL secretion during 3 hours. In the liver, TAG content was measured; apoB and MTP protein expression were assayed by RT-PCR.

Results: ST showed lower VLDL secretion ($p < 0.05$), and reduced gene expression of apoB ($p < 0.001$) and MTP ($p < 0.001$), when compared with SC. These parameters were restored to values near that found on control group after 6-week of moderate endurance training.

Conclusion: Exercise training promoted the reestablishment of lipid metabolism in cachectic tumour-bearing animals, especially with regards to VLDL secretion and its assembly. These results might be related to the direct effect of moderate training in the modulation gene expression of apoB and MTP, which are essential to these parameters.

4.60
Effects of proteasome inhibition in an experimental model of cancer cachexia

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Muscle wasting in cancer cachexia mainly depends on degradation of skeletal muscle proteins due to activation of the ubiquitin-proteasome pathway. Bortezomib has been described as a potent reversible and selective NF- κ B and proteasome inhibitor with anti-neoplastic and anti-angiogenic properties.

Aim of the present work was to investigate if the pharmacological inhibition of proteasome counteracts skeletal muscle wasting in rats bearing the Yoshida AH-130 hepatoma.

Methods: Male Wistar rats were divided in: controls (C) and tumor-bearers (TB). TB animals were inoculated i.p. with 108 Yoshida hepatoma cells and 2 days after C and TB were randomized to receive bortezomib (BTZ+) i.v. Rats were weighed and sacrificed on days 3, 4 and 7 after tumor inoculation. NF- κ B activation (EMSA) and MuRF1 and MAFbx/atrogen expression (semi-quantitative RT-PCR) were analyzed in the gastrocnemius muscle.

Results: A marked loss of body and tissue weight was observed in both TB and TB-BTZ+. Besides, a reduction in food intake was shown in TB-BTZ+ 24h after BTZ administration, probably due to a transient toxic effect of the drug. DNA-binding activity of NF- κ B increased in TB+BTZ+ 3 days after transplantation, while at day 7 the activity was close to control values. Moreover, mRNA levels for muscle ubiquitin-ligases comparably increased both in TB and in TB-BTZ+ at day 4 and 7 with respect to controls.

Conclusions: These results would suggest that administration of BTZ in vivo is unable to prevent body weight and muscle loss, at least in the AH-130 model of cancer cachexia. In addition, BTZ exerts transient toxicity in TB rats.

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4.61
Formoterol and roxithromycin administered individually and in combination prevent tissue wasting in a rat model of cancer cachexia

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Purpose: We probed the effects of the beta2 adrenergic agonist, formoterol, and the macrolide antibiotic, roxithromycin, on muscle wasting in a well-characterized animal model of cancer cachexia.

Procedure: Female Wistar rats were inoculated with Yoshida AH130 ascites hepatoma (AH) cells to induce rapid and severe cachexia as demonstrated by wet weight determinations of hearts, gastrocnemius muscles, and carcasses. Control animals received saline (vehicle) inoculations. AH-inoculated rats were treated once daily for four days by i.p. injection with: vehicle control, 1 mg/kg formoterol, 5 and 50 mg/kg roxithromycin, or 1 mg/kg formoterol plus 5, 25, 40, and 50 mg/kg roxithromycin. Saline-inoculated animals were treated by i.p. injection with: vehicle control, 1 mg/kg formoterol, 5 and 40 mg/kg roxithromycin.

Results: Formoterol alone reduced the loss of muscle mass in AH-inoculated rats, consistent with literature reports (Busquets et al., 2004). Roxithromycin alone at 5 mg/kg did not affect muscle mass in AH-inoculated rats. Roxithromycin given alone at 50 mg/kg significantly antagonized the loss of muscle mass in AH-inoculated animals. With respect to antagonizing muscle loss, formoterol combined with either 5 or 25 mg/kg roxithromycin did not reach statistical significance as compared with formoterol alone, while formoterol plus either 40 or 50 mg/kg roxithromycin enhanced protection against muscle loss compared with formoterol alone. Gastrocnemius weights in AH-inoculated rats treated with formoterol combined with 40 mg/kg roxithromycin were not significantly different from muscle weights in saline-inoculated controls.

Conclusion: Formoterol and roxithromycin apparently exert anti-cachectic effects in an additive fashion and may offer the potential for combination therapy in cachexia.

4.62
Bisoprolol in experimental cancer cachexia

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Background: Cachexia is a common co-morbidity in cancer patients (up to 80% depending on the tumour type), which drastically reduces quality of life and survival. In chronic heart failure, beta-blockers have been shown to reduce the onset of cachexia and induce weight gain (mainly fat mass).

Methods: Juvenile rats (weight approx. 200 g) were inoculated intra-peritoneally with 10⁸ AH-130 hepatoma cells and treated with bisoprolol (0.5, 2, 5 and 50 mg/kg/d) or placebo. Food intake and locomotor activity were assessed before inoculation and on day 11 (d11) of the 16-day protocol. Weight and body composition (NMR-scan) were assessed on day 0 and day 16 after sacrifice (without tumour).

Results: Placebo-treated animals developed severe cancer cachexia, which was partially prevented by bisoprolol (Biso; see table). The effects of bisoprolol similarly affected muscle and fat tissue mass. Biso showed an improvement of food intake and locomotor activity as indicators of quality of life. Cardiac function was improved by Biso compared to placebo – best effects were seen at 50 mg/kg/d Biso. Survival was significantly improved

by 5 mg/kg/d Biso (HR: 0.32, 95%CI: 0.17-0.62, p=0.0007), and 50 mg/kg/d Biso (HR: 0.45 95%CI: 0.22-0.89, p=0.021).

	Placebo (n=49)	0.5 mg/kg/d Biso (n=13)	2 mg/kg/d Biso (n=14)	5 mg/kg/d Biso (n=23)	50 mg/kg/d Biso (n=20)
Delta BW [g]	-50.2±2.2	-37.8±7.5*	-25.6±13.2**	-21.8±10.6**	-30.6±9.8**
Delta fat [g]	-11.2±0.4	-9.3±1.2	-6.4±3.0**	-5.9±1.9***	-8.4±1.6*
Delta lean [g]	-37.1±2.0	25.3±5.7*	-20.4±9.4**	-16.7±7.7***	-24.4±6.9*
Food intake [g/d]	4.10±0.55	5.80±1.98	16.71±6.86**	10.93±2.01**	10.06±1.95***
Physical activity [counts/d]	29209±2270	33701±6559	38777±6981	43755±3741**	43124±5722**
LVEF, d11 [%]	57.3±2.7	69.9±4.0*	62.8±3.6	57.4±5.3	69.1±2.0**
FS, d11 [%]	31.5±1.9	40.7±3.2*	35.2±2.9	32.3±3.5	40.0±1.7*
SV, d11 [μl]	105.6±8.4	131.4±19.9	108.9±30.0	159.5±21.9**	173.6±22.6**

BW: body weight, LVEF: left ventricular ejection fraction, FS: fractional shortening, SV stroke volume

*: p<0.05, **:p<0.001 improved vs placebo.

Conclusion: In this animal model the beta-blocker bisoprolol partially improves important aspects of cancer cachexia. Compared to placebo, bisoprolol reduced muscle and fat tissue wasting and increased food intake, physical activity and survival.

4.63
The effects of xanthine oxidase inhibitors oxypurinol and allopurinol 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: Juvenile rats (weight approx. 200 g) were inoculated intra-peritoneally with 10⁸ AH-130 hepatoma cells and treated with allopurinol (Allo, 40 mg/kg/d), oxypurinol (Oxy, 4 and 40 mg/kg/d), or placebo. Food intake and locomotor activity were assessed before inoculation and on day 11 (d11) of the 16-day protocol. Weight and body composition (NMR-scan) were assessed on day 0 and day 16 after sacrifice (without tumour).

Results: Wasting of both fat and lean tissue was significantly reduced (see table) by 4 mg/kg/d Oxy and to a somewhat lesser degree by the 10 times higher dose of Allo. The latter resulted in less overall weight loss. The food intake and spontaneous activity were significantly higher in the low dose Oxy and Allo groups, indicating a higher quality of life. Survival was significantly improved by 4 mg/kg/d Oxy (HR: 0.37, 95%CI: 0.17-0.79, p=0.011) and by 40 mg/kg/d Allo (HR: 0.40, 95%CI: 0.19-0.86, p=0.019).

	Placebo (n=49)	Oxy 4 mg/kg/d (n=11)	Oxy 40 mg/kg/d (n=12)	Allo 40 mg/kg/d (n=11)
Delta BW [g]	-50.2±2.2	-15.4±14.2***	-45.3±11.8	-26.4±17.1*
Delta fat [g]	-11.2±0.4	-6.8±2.6**	-14.4±1.9*	-8.1±2.9
Delta lean [g]	-37.1±2.0	-10.6±10.4***	-32.4±9.6	-19.9±12.6*
Food intake [g/d]	4.1±0.6	13.0±2.9***	5.8±2.2	9.8±3.2**
Physical activity [counts/d]	29209±2270	48343±7724**	31777±6738	40046±6854

BW: body weight, *: p<0.05, **:p<0.01, ***p<0.001 improved vs placebo.

Conclusion: Inhibition of XO can reduce tissue wasting and improve survival in an animal model of cancer cachexia. A low dose of the second generation XO-inhibitor oxypurinol had superior properties compared to 10-fold higher dose of allopurinol. Interestingly, the dose-response relationship for oxypurinol was inverse. Studies using even lower doses of oxypurinol are necessary.

4.64
The effects of the aldosterone antagonist spironolactone in experimental cancer cachexia

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Background: Cachexia is a very common co-morbidity in cancer patients. It significantly reduces quality of life and survival. Aldosterone is part of the neurohormonal activation cascade and linked to fibrosis, inflammation and oxygen free radical generation. We hypothesised that the aldosterone antagonist spironolactone can positively impact on cancer cachexia.

Methods: Juvenile rats (weight approx. 200g) were inoculated intra-peritoneally with 10⁸ AH-130 hepatoma cells and treated with 2 (n=6), 5 (n=16), or 50 mg/kg/d (n=16) spironolactone or placebo (n=49). Food intake and locomotor activity were assessed before inoculation and on day 11 (d11) of the 16-day protocol. Weight and body composition (NMR-scan) were assessed on day 0 and day 16 after sacrifice (without tumour).

Results: The severe cachexia seen in placebo-treated animals was partially prevented by 5 and 50 mg/kg/d spironolactone (see table) and similarly affected muscle and fat tissue mass. Spironolactone at 2 mg/kg/d was ineffective. Food intake and activity as measure of quality of life were improved by 5 and 50mg/kg/d spironolactone. Cardiac function was significantly improved only in the 50 mg/kg/d spironolactone group (table). Survival was improved by 5 mg/kg/d (HR: 0.26, 95%CI: 0.12-0.55, p=0.0004) and 50 mg/kg/d spironolactone (HR: 0.31, 95%CI: 0.15-0.64, p=0.0015) compared to placebo.

	Placebo	Spiro 2 mg/kg/d	Spiro 5 mg/kg/d	Spiro 50 mg/kg/d
Delta BW [g]	-50.2±2.2	-64.5±4.1*	-28.9±8.5**	-21.0±11.0***
Delta fat [g]	-11.2±0.4	-13.8±1.5	-7.7±1.9**	-6.7±2.1**
Delta lean [g]	-37.1±2.0	-50.6±3.6*	-21.5±6.4**	-11.9±8.7***
Food intake, d11 [g/d]	4.1±0.6	3.8±1.0	9.0±1.1***	9.8±1.7***
Physical activity, d11 [counts/d]	29209±2270	24128±5878	38948±3524*	44817±5286**
LVEF, d11 [%]	57.3±2.7	ND	63.8±3.8	70.5±2.6**
FS, d11 [%]	31.5±1.9	ND	36.2±3.0	41.2±2.3**
SV, d11 [µl]	105.6±8.4	ND	124.4±18.6	178.9±18.3***

BW: body weight; LVEF: left ventricular ejection fraction, FS: fractional shortening, SV stroke volume, ND: not done; *: p<0.05, **: p<0.01, ***: p<0.001 improved vs placebo, #. p<0.05, ###: p<0.01 reduced vs placebo.

Conclusion: In this animal model, aldosterone antagonism with spironolactone can improve important aspects of cancer cachexia. Compared to placebo, 5 and 50 mg/kg/d of spironolactone reduced muscle and fat tissue wasting and increased food intake, physical activity and survival. At 50 mg/kg/d, spironolactone also improved cardiac function.

4.65 Proteasome inhibition in CHF rats improves diaphragm function by restoring myosin content

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Rationale: Diaphragm weakness is known to occur in congestive heart failure (CHF) and is associated with myosin loss and activation of the ubiquitin-proteasome pathway. The effect of modulating proteasome activity on myosin loss and muscle function is unknown. The present study investigated the effect of in vivo proteasome inhibition on myosin loss and diaphragm function.

Methods: CHF was induced by coronary artery ligation in rats and sham-operations served as controls. Animals were intravenously treated with the clinically used proteasome inhibitor bortezomib or received saline injections. Maximal force generation and myosin content were measured in chemically skinned diaphragm single fibers. Proteasome activity, caspase-3 activity, myosin ubiquitination, MuRF-1 and MAFbx mRNA levels were determined in diaphragm homogenates.

Results: Proteasome activities were increased in CHF diaphragm compared to sham and were reduced by bortezomib treatment (p<0.05). Maximum force was lower in CHF diaphragm fibers than sham and was improved by bortezomib treatment of CHF rats (p<0.05). Myosin content was decreased in CHF diaphragm fibers compared to sham, and was restored by bortezomib treatment (p<0.05). Myosin ubiquitination was significantly higher in CHF diaphragm homogenates than sham. Treatment with bortezomib increased levels of ubiquitinated myosin. CHF significantly increased MuRF-1 and MAFbx mRNA expression in the diaphragm and bortezomib treatment diminished this rise. Caspase-3 activity was higher in CHF diaphragm homogenates than sham and was reduced by bortezomib treatment (p<0.05).

Conclusions: The present study demonstrates that treatment with a clinically used proteasome inhibitor improves diaphragm weakness by restoring myosin content in CHF.

4.66 Pharmacological intervention against TNF-alpha attenuates the increases in MuRF-1 and Mafbx mRNA in a model of cardiac cachexia

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Cardiac cachexia is a serious complication to congestive heart failure (CHF) and is characterized by systemic inflammation, a decrease in muscle mass, and an unintentional loss of body weight in affected individuals. Those diagnosed with the disease face higher rates of morbidity and mortality than those with CHF alone. Despite the severity of cardiac cachexia, an effective treatment has yet to be developed. It was the aim of the current study to determine whether targeting the pro-inflammatory cytokine TNF-alpha would attenuate the deleterious effects of cardiac cachexia on skeletal muscle mass. CHF was induced via monocrotaline (MCT) injection and treatment animals were given either the general inhibitor of TNF-alpha production, pentoxifylline, or the specific inhibitor sTNFR1. We report that MCT-induced cardiac cachexia resulted in a significant decrease in body weight, EDL and TA muscle weights compared to control animals (p<0.05); PTX and sTNFR1 treatment attenuated these losses in EDL and TA muscle mass as well as body weight in cachectic animals (p<0.05). Real-time PCR revealed that losses in EDL mass were accompanied by a concomitant increase in mRNA of ubiquitin proteasome pathway E3 ligases Mafbx and MuRF-1 over control animals (p<0.05). The increase in Mafbx and MuRF-1 mRNA was attenuated by treating cachectic animals with either PTX or sTNFR1 (p<0.05). Overall, the results indicate that blockers of TNF-alpha attenuate cachexia-

induced increases in genes of the ubiquitin proteasome pathway. Based upon their positive effects on body weight and muscle mass, these treatments may have clinical efficacy in combating cardiac cachexia.

4.67 The impact of the appetite stimulant megestrol acetate on survival, body weight and cardiac function in a rat model of chronic heart failure

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Megesterol acetate (MA) is well known for its appetite stimulation effects to treat AIDS and cancer related cachexia. To assess the therapeutic potential of MA in heart failure a rat myocardial infarct model was used.

Methods: From day 14 after surgery (LAD occlusion), rats received frusemide. On day 42, rats were randomized to receive 26 weeks treatment with placebo (PL: n=38), ramipril (n=38, 1 mg/kg/d), the standard MA formulation (MA-I: n=37) or a new nano-cristal MA formulation (MA-II: n=40, both 100mg/kg/d; provided by PAR Pharmaceutical) until sacrifice. Cardiac function was assessed using echocardiography. Additionally, body composition was analyzed by NMR-scanning, and in a subgroup cardiac function was assessed by MRI scanning and invasive hemodynamics.

Results: Mortality was high with MA-I (65%) and PL (51%, p=0.34), but similarly low with MA-II and ramipril (both 13%, MA-II vs PL p<0.001; ramipril vs PL, p<0.001, ramipril vs MA-II, p=0.68). Rats treated with any formulations of MA displayed a retarded growth (tibia length: PL 4.3cm, ramipril 4.3cm, MA-I 4.1cm, MA-II 3.9cm), likely due to testosterone suppression.

	Placebo	Ramipril	MA-I	MA-II
Body weight, end of study	599±11	575±8	576±15	507±11 ^{§€}
Fat/tibia, g/cm	47.3±8.6	45.3±5.9	66.7±3.7	65.2±3.5 [€]
Lean/tibia, g/cm	86.2±3.7	83.2±2.5	60.5±1.6 ^{§€}	48.6±3.8 ^{§€}
EF %	38±3	40±2	45±3	41±3
CO/BW ml/min/kg	238±6	178±8 [§]	218±18 [†]	215±17 [†]

LVEF: left ventricular ejection fraction; CO: cardiac output; BW: body weight; *: p<0.05 vs Plac; #: p<0.01 vs Plac; §: p<0.001; †: p<0.05 vs Rami; €: p<0.001 vs Rami; £: p<0.05 vs MA-I.

Conclusion: In an animal model of chronic heart failure, has no clear anti-cachexia effect. However, megestrol acetate in nano-cristal formulation improves survival in a rat model of heart failure similar to that seen with an ACE inhibitor.

4.68 Proteasome inhibition improves contractility of the emphysematous hamster diaphragm

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Rationale: Diaphragm muscle weakness is of clinical importance in patients with chronic obstructive pulmonary disease (COPD). Recent data from our lab show that in patients with COPD impaired force generation of the diaphragm is associated with activation of the ubiquitin-proteasome pathway. Whether inhibition of the proteasome improves diaphragm muscle function is unknown.

Methods: In adult hamsters emphysema was induced by intratracheal instillation with elastase. After 6 months emphysematous hamsters and healthy controls were treated with the clinically used specific proteasome inhibitor bortezomib (iv, 1.3 mg/m2 at day 1, 4, 8 and 11) or equal volume saline 0.9%. Hamsters were sacrificed within 24 h after last injection. The diaphragm was analyzed for myosin heavy chain content, submaximal and maximal force generation in vitro and components of the caspase-3 – ubiquitin – proteasome pathway.

Results: Bortezomib did not affect the severity of emphysema. As expected, bortezomib almost completely inhibited proteasome activity in the normal and emphysematous hamster diaphragm. Myosin heavy chain content was significantly lower in emphysematous- compared to normal hamster diaphragm. Bortezomib increased myosin heavy chain content in the emphysematous hamster diaphragm. Bortezomib-induced elevation in myosin heavy chain content was associated with increased (sub)-maximal force generation of emphysematous hamster diaphragm muscle bundles. Interestingly, bortezomib reduced caspase-3 activity in the emphysematous hamster diaphragm. In vitro experiments showed that bortezomib did not directly inhibit caspase-3 activity.

Conclusion: The data of the present study show that activation of the ubiquitin-proteasome pathway plays a critical role in diaphragm muscle weakness in COPD. Inhibition of the proteasome with bortezomib improves in vitro force generation by increasing myosin heavy chain content of the diaphragm.

4.69 Double-blinded, placebo-controlled plasmid GHRH trial for cancer-associated anemia in dogs

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Growth hormone-releasing hormone (GHRH) plasmid-based therapy to treat companion dogs with spontaneous malignancies and cachexia-associated anemia receiving a cancer-

specific treatment was examined in a double-blind, placebo-controlled trial. Dogs (age 10.51 ± 2.5 years, weight 24.88 ± 12.9 kg) received a single 0.35 mg dose of plasmid or placebo intramuscularly followed by electroporation, and were analyzed up to 120 days post-treatment. Response rate was defined as a 5% or greater increase above the nadir in red blood cell count, hemoglobin and hematocrit. Placebo-treated controls showed a decrease in response rate during the study. The initial response rate for the plasmid-treated dogs was approximately 35%-40% at day 40 and 60 which increased to 50%-60% at day 90. Although the response rate decreased to 45% by day 120, it was still 10%-20% higher than in the control dogs. On average, treated dogs survived 30% longer, 130±15 days post-treatment, while controls survived 97±31 days post-treatment. Insulin-like growth factor-I levels, a marker for GHRH activity, increased in treated dogs with a significant percentage change from baseline at day 60 (55.76% ± 12.06, p=0.02) and positively correlated with body weight, hematological parameters and improved protein metabolism. Changes in the placebo treated group were not significant. This study shows that plasmid-based therapy could be an effective and efficient method for reversing cachexia-associated anemia in patients, while increasing their chance of survival during specific cancer therapy. Overall, the importance of a growth hormone releasing hormone plasmid-based therapy in cancer-afflicted subjects is substantial and therefore warrants a human trial.

4.70
Mirtazapine and appetite in advanced cancer

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Background: There is limited data about the orexigenic effect of mirtazapine.
Objectives: To determine if mirtazapine improves anorexia and weight loss.
Methods: A 2 week (W) open-label trial of mirtazapine for QOL and symptoms (insomnia, anorexia, nausea, fatigue, worry, depression) in advanced cancer A night time dose of 15 mg increased W2 to 30mg if no improvement and limiting toxicity. Follow-up was twice weekly for 2 weeks. Evaluable patients completed W1. Improved QOL and/or symptom improvement of > 1 point at W1 and W2 was a response. Secondary outcomes to QOL were improved anorexia, ECOG PS, weight (kg) assessed at D1 and D14. We compared anorexia, weight, QOL and symptoms for all subgroups (responders, non-responders and discontinued patients) with Wilcoxon-Signed Rank Test, ANOVA, Chi-square analysis and median tests.
Results: N=45 entered. Completed: W1 (N = 30), W2 (N=18), median age (range) 63 (48-92); male 17/30; mean (SD) weight (kg) 70.05 (13.5); median ECOG (range) 2 (1-3). Drop outs W2 did not differ by weight, overall health, overall QOL, and 7 symptoms. Mean (SD) appetite improved D1-D7, mean (SD) 0.5 (1.0), p<0.01; 37% (11/30) responded with 1.6 (0.7), (p<0.0001). Mean appetite increase D1-14 was 0.2 (1.1), p=NS. 33% (6/18) responded with 1.33 (0.5), p=0.5. No further improvement in appetite occurred D7-14. 9/18 had doses increased without appetite improvement. Weight did not increase (p=0.7).
Conclusions: Mirtazapine increased appetite W1. Dose response was not found. There was no significant influence on weight.

4.71
Randomised phase III clinical trial to evaluate the efficacy and safety of an integrated treatment (diet, pharmaco-nutritional and pharmacological) in cancer patients with cancer-related anorexia/cachexia and oxidative stress: interim results

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Background: Cachexia is a multifactorial syndrome characterized by tissue wasting, loss of body weight, particularly of lean body (muscle) mass and to a lesser extent adipose tissue, metabolic alterations, fatigue, reduced performance status, very often accompanied by anorexia leading to a reduced food intake: it accompanies the end stage of many chronic diseases and especially cancer and therefore it is termed "cancer-related anorexia/cachexia syndrome" (CACS). In April 2005 we started a phase III randomised study to establish the most effective and safest treatment able to improve the identified "key" variables of CACS/OS: lean body mass (LBM), resting energy expenditure (REE), total daily physical activity, IL-6 and TNF-alpha and fatigue. The sample size is 475 patients. All patients enrolled receive as basic treatment: polyphenols + alpha lipoic acid + carbocysteine + Vitamins E, A and C, oral. Patients are then randomised to one of the following 5 arms: 1) Medroxyprogesterone acetate (MPA)/Megestrol acetate (MA); 2) Pharmaco-nutritional support containing EPA; 3) L-carnitine; 4) Thalidomide; 5) MPA/MA + Pharmaco-nutritional support + L-carnitine + Thalidomide. Treatment duration 4 months. At July 2007, 165 patients with advanced stage (96% stage IV) tumors at different sites (lung 14.9%, colorectal 14.3%, breast 13.7%, pancreas 12%, head and neck 11.4%, etc.) have been randomized.
Results: No severe side effects were observed. As for efficacy, the interim analysis showed an improvement in fatigue, REE, IL-6 and TNF-alpha in arm 3, IL-6 in arm 4 and REE and fatigue in arm 5. The ANOVA test demonstrated that arms 1, 3, 4 and 5 were not significantly different statistically and that only patients in arm 3 showed a significant decrease of IL-6. Arm 2 was withdrawn from the study following a first interim analysis performed in February 2007 which showed the worsening of LBM, REE and MFSI-SF in arm 2 versus the others.
Conclusions: The interim results seem to suggest that the most effective treatment for cancer patients with CACS/OS should be either a combination regimen or L-carnitine alone. The study is still in progress up to completion of planned accrual. The final results are expected in the first half of 2008.
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5.72
Disuse atrophy reduces muscle protein synthesis in the fasted and fed state in humans

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Muscle disuse results in a suppression of muscle protein synthesis in the fasted state. Provision of amino acids is known to markedly stimulate muscle protein synthesis. We aimed to determine whether muscle disuse would lessen the normal feeding-induced rise in muscle protein synthesis. 12 healthy young subjects (N=10 men and N=2 women) wore an immobilizing knee brace for 14d. Immobilization resulted in a reduction in muscle CSA (5.0±1.2%, P<0.001). Following immobilization, using the contralateral non-immobilized leg as a control, we infused subjects with [*ring*-¹³C₆]Phe to measure myofibrillar protein synthesis (MPS) in both the fasted and fed states. Subjects received either a low (42.5 mg/kg/h) or a high (262 mg/kg/h) complete amino acid infusion to assess the feeding-induced response of MPS. Immobilization reduced the fasted rate of MPS by 24.2±4.2% (P<0.05). Infusion of AA stimulated MPS in the non-immobilized leg at both 2h and 4h of feeding (P<0.001) in a dose-dependent fashion (P<0.001). Immobilization reduced feeding-induced stimulation of MPS by 35.1±5.3% at the low dose and by 45.8±7.2% at the high dose. The reduction in fasted and fed-state MPS could account, based on conservative calculations, for almost all of the muscle protein lost during our 14d intervention. Our data, in conjunction with previous observations, support the thesis that it is a decline in protein synthesis, and not an increase in protein breakdown, that is primarily responsible for muscle protein loss in disuse. Support from Canadian Natural Science and Engineering Research Council, Canadian Institutes of Health Research, and UK BBSRC

5.73
The effect of weight loss on mobility and frailty in the community-dwelling elderly

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Aim: To evaluate the correlation of BMI (Body Mass Index) with parameters of frailty and mobility in community-dwelling older people according to age.
Methodology: Data from the Belgian Health Interview Surveys of 1997, 2001 and 2004 (n=37,387) are used. Frailty is measured with the VIP (Variable Indicative of Placement)-tool, which gauges living alone, need for assistance with washing and dressing, and mobility outside the own neighbourhood. People are then assigned to a high or low-risk group for frailty. Mobility is assessed by limitation in transfers and walking distance. The relation between BMI, frailty and mobility is examined. To this end, Chi-Square analysis and logistic regression is applied using SPSS for Windows 14.0.
Results: The cohort for evaluation contains 6515 people over 65 years of age out of 37,387. There is a continuous shift of BMI to lower values with increasing age. In the 85+ cohort 9.6% of the participants have a BMI lower than 18.5 compared to 1.8% in the 65-69 yr group (p ≤ 0.001). Mobility problems and risk for frailty score significantly higher in the lower BMI classes (cfr Table: data for all 65+).

BMI	< 17	17 – 18.5	18.5 - 25	25 - 30	30 – 40	> 40
N (total=6515)	75	139	2813	2512	940	36
Severe limitation in mobility: "a few steps" (%)	21.2	23.1	8.6	7.6	14.0	38.0
Normal walking distance: > 200 m (%)	35.9	51.8	75.9	77.5	66.0	42.9
Frailty (%)	30.3	41.8	14.4	11.6	18.5	35.9

Conclusion: There is a progressive loss of weight with aging. This weight loss is significantly correlated with frailty and loss of mobility.

5.74
Muscle quality is maintained in healthy old women: influence of muscle volume optimisation

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Background and aims: Muscle quality (defined here as muscle strength per unit muscle volume) is a potentially important marker of skeletal muscle function and frailty. Our aim was to compare muscle quality in healthy younger and older women and to investigate the effect of subtracting the non-contractile components from the total muscle volume (muscle optimisation).
Methods: We recruited 25 healthy women (n=9 older, median age 80y, range 76-82y; n=16 young, median age 26y, range 19-30y). Maximal voluntary isometric knee extensor strength (N) was measured on each leg. Sequential T1 weighted axial images were acquired using a 1.5T MRI scanner and analysed off-line for determination of quadriceps muscle volume (l). Muscle quality (Nl⁻¹) was derived from volume data both pre- and post-volume optimisation to exclude non-contractile tissue within the quadriceps region of interest.
Results: Older women were 30% weaker than the younger women, 208N (mean); 95% CI 155 to 261 vs 297 N (mean); 256 to 339N) and their muscles were 34% smaller 1.12 l (mean); 95% CI 0.92 to 1.32l vs 1.68 l (mean); 1.51 to 1.85l). Muscle quality was not significantly different between older and younger groups (186 Nl⁻¹ older vs 180 Nl⁻¹ young; p=0.7). The increment in muscle quality following application of the muscle volume

optimisation technique, while greater in the older group (15% vs 9%; $p=0.018$), did not appreciably alter the overall level of significance between groups ($p=0.4$).

Conclusions: Muscle quality in healthy women over 75y appears to be maintained whether non-contractile elements are considered or not.
Supported by Research into Ageing.

5.75

Cytokines and their association with functional parameters in older persons – A pilot study using a biochip with high sensitivity for cytokine measurements

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Introduction: Age-associated low-grade inflammation in the elderly may be associated with changes in body composition and a loss of functionality.

Aims of the study: 1. To test a newly developed biochip by Randox® with high sensitivity for the measurement of cytokines. 2. To analyse the association between cytokines and parameters of body composition and functional performance in an older population.

Methods: Recruitment of consecutive patients from the geriatric day clinic Nuremberg. On admission serum measurements of IL-1 α , IL-1 β , IL-2, IL-4, IL-6, IL-8, IL-10, INF γ , TNF α , MCP1, EGF, VEGF with a biochip by Randox® with high sensitivity for cytokines. Functional parameters assessed were Barthel-Index, Timed-up-and-go-test and digital handgrip.

Results: 39 patients (31 females, 8 men) were included, mean age 78.7 ± 4.1 years, mean BMI 28.7 ± 4.6 kg/m², median Barthel-Index 95, mean max. handgrip 18.7 ± 8.5 kg, mean Timed-up-and-go-test 19.8 ± 7.4 sec, mean hsCRP 0.72 ± 1.10 mg/dl. Interleukin-6 (IL-6) levels steadily increased with age and were positively associated with high sensitivity CRP (hsCRP) ($p<0.001$) and BMI ($p=0.007$). Higher BMI values were associated with longer intervals for the Timed-up-and-go-tests. In men, high IL-6 levels were associated with a decrease in maximum handgrip ($p=0.03$).

Conclusions: In this study we show that simultaneous measurement of different cytokines by a Randox biochip® with high sensitivity for cytokines is feasible. Our results indicate that cytokines and body composition may be closely interrelated with regard to their impact on functionality in older persons.

5.76

Cachexias: a 2007 state of the art review of the metabolic and biochemical abnormalities in different clinical models of cachexia

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Background and aims: Cachexia occurs in different chronic diseases such as cancer, tuberculosis, congestive heart failure (CHF), acquired immunodeficiency syndrome (AIDS), end-stage renal disease (ESRD), rheumatoid arthritis (RA), and chronic obstructive pulmonary disease (COPD). We compared cachexia in different clinical settings.

Methods: PubMed/ Medline search was limited to English.

Results: Search results for "cachexia" combined with specific disease found 2394 articles in cancer, compared to only 257 for AIDS, 222 for CHF, 52 for COPD. There is no clear-cut definition of cachexia; a major hurdle for research. It is difficult to build on the present knowledge without strict diagnostic criteria. Loss of both lean body and fat mass is common to most cachexias. All cachexias are multifactorial. Inflammation is a common component. Pro-inflammatory cytokines are involved in different aspects of cachexia. Hypermetabolism is usual (i.e. increase resting energy expenditure). However, the total energy expenditure may be unchanged as a result of a compensatory decrease in physical activity. Increased protein breakdown, glucose turnover, and lipolysis are frequently seen. Anorexia is present in most except RA and is not necessary for the wasting process to happen.

Cachexia is resistant to nutritional support (except in AIDS). Some differences between cachexias are inherent to the disease and its management. For example, AIDS is associated with hypogonadism and lipodystrophy; ESRD with uremic syndrome; cancer has unique tumor-host interactions.

Conclusions: Cachexia research is limited compared to its prevalence in many disorders. Inflammation is pivotal in pathophysiology of all cachexias. A unified definition is needed.

5.77

Natriuretic peptide-and catecholamine-induced lipolysis in cardiac cachectic patients

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Brain and atrial natriuretic peptides (BNP and ANP), as well as norepinephrine (NE) levels increase progressively with the worsening of heart failure. A potential role for both ANP and BNP in lipid metabolism has recently been suggested. The effects of these hormones in the development of cardiac cachexia, which represents one of the worst prognostic parameters in advanced heart failure patients, are less known.

Our study compared a group of non-cachectic advanced heart failure patients (CHF, NYHA III-IV, n=48) with a group of cachectic heart failure patients (CXC, NYHA III-IV, n=19) evaluating the relationship among several plasma neurohormonal parameters such as BNP, ANP, NE and lipid metabolism expressed by plasma free fatty acid (FFA) levels. The patients had no signs of other cachectic states (cancer, thyroid disease, severe liver

disease), diabetes, or severe chronic renal failure. A positive correlation was found between BNP and ANP plasma levels and FFA levels respectively in CHF patients ($R2$ 0.28 for BNP and 0.31 for ANP, $p<0.01$); however, this relationship was lost in the group of CXC patients. A positive correlation between FFA and plasma NE was also found in both CXC and CHF patients ($R2$ 0.53 in CXC and 0.38 in CHF, $p<0.01$). Our results suggest a role for natriuretic peptide in lipolysis modulation in CHF patients which seems to be lost in CXC.

5.78

Differences in body mass changes after the onset of heart failure symptoms. Impact on body composition, biochemical characteristics and prognosis.

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Body mass index (BMI) and body composition are important determinants of exercise performance and prognosis in heart failure (HF). After heart failure onset HF onset these parameters may evolve differently depending on severity of heart injury, type and timing of therapy used. Very little is known on the relations between various scenarios of dry BMI changes after HF onset and biochemical characteristics of HF, body composition and prognosis in prospective observation.

We intended to assess prognostic, biochemical and body composition consequences of three predefined types of dry BMI changes after HF onset.

In 590 HF patients (age: 52 ± 11 years, 15% female, EF: $25\pm 7\%$, NYHA: 2.6 ± 0.9 , ischaemic etiology in 75%) BMI was recorded after optimal therapy was instituted and the patient reached dry body weight (indexBMI). We analyzed dry BMI at 3 time points: highest BMI within one year before HF onset (preHF BMI), minimum BMI after HF onset (minBMI), maximal BMI after HF onset. Using these data we defined 3 scenarios (S) of BMI changes shown in table 1:

S 1	no change or <3% drop of BMI after HF onset
S 2	$\geq 3\%$ drop of BMI after HF onset, followed by $\geq 3\%$ rise from minBMI
S 3	$\geq 3\%$ drop of BMI after HF onset, followed by no change between minBMI and indexBMI, or rise <3% from minBMI

In 407 of patients (69%) body composition was analyzed by DEXA.

During median follow-up of 591 days (8-1660) 147 (25%) patients have died. We have calculated hazard ratio (HR) for each pair of S (Cox model) and compared prognosis using Kaplan-Maier method (log-rank test) (fig 1).

We also compared body composition, clinical and biochemical characteristics of each scenario. Between HF onset and index date BMI have changed according to S1, S2 and S3 category in 136 (23%), 244 (41%), and 210 (36%) patients respectively. As compared to S1, HR of S2 and S3 was 2.85, 95%CI 1.6-5.0, $p<0.000$, and 2.33, 95%CI 1.3-4.2, $p<0.005$ respectively. S3 was associated with nonsignificant change of risk with HR of 0.82, 95%CI 0.24-1.22, $p=0.33$. The probability of death was lowest in S1 and was significantly higher in S2 and S3 (log-rank versus S1: $p=0.003$ and $p=0.005$ respectively). Death probability was not different between S2 and S3 (log-rank $p=0.94$). Despite similar risk in S2 and S3 patients, at index date patients in S2 had lost less body mass after HFO: 7 vs 12%, $p<0.000$, regained more before index date: 12 vs 0.5%, $p<0.000$, had higher BMI: 26.6 vs 24.8 kg/m², $p<0.000$, higher fat: 21.3 vs 19.3kg, $p<0.04$, and lean mass content: 53.5 vs 51.1kg, $p<0.03$. They were trending toward lower NYHA: 2.6 vs 2.7, $p=0.09$, but had no difference between NTproBNP, LFEF, and MVO₂.
Conclusions. BMI changes after HF onset should be used in prognostication.

5.79

Upregulation of STAT-3, SOCS-1, pro-inflammatory and anti-inflammatory circulating cytokine mRNA gene expression in CHF patients with and without cachexia compared to healthy control subjects

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Background: Chronic heart failure (CHF) is a state of chronic inflammation. High serum levels of tumor necrosis factor alpha (TNF) and the presence of cardiac cachexia are known to predict poor survival in CHF. Abnormal balance of pro- and anti-inflammatory circulating cytokine mRNA gene expression is thought to contribute to cachexia in humans, but has never been studied in CHF patients.

Methods and Results: We studied 11 patients with cardiac cachexia (age 70 ± 3 years, LVEF $28\pm 4\%$, peak VO₂ 13.1 ± 1.0 mL/kg/min, all >6% weight loss in previous 6-24 months), 19 non-cachectic CHF patients (age 68 ± 2 years, LVEF $31\pm 2\%$, peak VO₂ 14.7 ± 1.1 mL/kg/min) and 17 control subjects of similar age and gender (LVEF $71\pm 2\%$, peak VO₂ 25.1 ± 1.7 mL/kg/min). The mRNA gene expression of circulating mononuclear blood cells for pro-inflammatory mediators that have been implicated in the wasting process of ageing (TNF, STAT-1, STAT-3, SOCS-1 and SOCS-3) as well as anti-inflammatory cytokines (IL-10, transforming growth factor (TGF) beta) were investigated by real time polymerase chain reaction. Values for the house keeping gene and the genes of interest were calculated from double determinations within 40 cycles. We found a higher TNF expression in cachectic compared to non-cachectic CHF patients (log mean -0.6 ± 0.1 vs. -1.2 ± 0.1 , $p=0.001$) and control subjects (log mean -1.1 ± 0.1 , $p=0.004$, ANOVA $p=0.003$). Furthermore, there was a higher IL-10 gene expression in cachectic patients compared to non-cachectic patients (log mean -2.6 ± 0.2 vs. -3.0 ± 0.09 , $p<0.04$). TNF/IL-10 expression ratio was highest in cachectic patients 269 ± 99 vs. 101 ± 37 in controls ($p=0.08$) vs. 132 ± 58 in non-cachectic CHF patients ($p=0.1$). STAT-3 mRNA expression was higher in cachectic vs non-cachectic patients (0.7 ± 0.1 vs. 0.4 ± 0.1 , $p<0.05$). Cachectic patients showed higher SOCS-1 expression compared to non-cachectic patients and

controls (-0.1±0.09 vs. -0.4±0.07 vs. -0.4±0.08, ANOVA p=0.04). There was a trend for a higher STAT-1 expression in cachectic patients (p=0.07). There were no differences in gene expression of SOCS-3 between cachectic and non-cachectic individuals (all p>0.2). Similarly, no differences were found for gene expression of TGF beta(p>0.3).
Conclusion: High STAT-3 and SOCS-1 m-RNA expression suggest involvement of JAK/STAT pathway in cachexia. Proinflammatory cytokine upregulation in cardiac cachexia may be important for the pathophysiology of body wasting and may represent the activated immune reflex in these patients.

5.80
Direct medical costs of HIV wasting: a retrospective analysis of a national managed care database

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Background: Costs associated with HIV wasting can be substantial. Growth hormone (GH) is an FDA-approved therapy for treatment of HIV wasting. The objective of this study was to evaluate the direct medical costs and the use of GH therapy among commercially insured patients with HIV wasting.

Methods: A national managed care database containing ~14 million lives was queried to identify patients with ≥ 1HIV-wasting diagnosis claim or ≥ 1 pharmacy claim for an HIV wasting-related medication. The index claim was the first identified claim between 2002 and 2004. Patients were required to have continuous enrollment for 1 year following the index claim (study period). Patients with Medigap coverage or no pharmacy claims during the study period were excluded. Annual utilization of healthcare services and mean annual expenditures (in 2005 dollars) were evaluated for HIV wasting-related, general HIV-related and total expenditures.

Results: 893 patients met study criteria; most (92.8%) were male and ~ 61% were aged between 25 and 44 years. For HIV wasting-related utilization, 89.1% received outpatient care, 10.5% were hospitalized and 6.4% had at least one ER visit during the study period. About 72% received HIV wasting-related medications; of these, 251 patients (28%) were prescribed GH therapy. The mean annual total expenditure for the study population was \$83,449; GH represented about 18% of total cost.

Conclusions: HIV wasting is associated with significant healthcare costs among patients in a national plan. In our study, only 28% of patients with HIV wasting were receiving GH.

5.81
Hypermuscularity does not abrogate burn injury induced muscle wasting

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Background: Myostatin, a member of the tumor growth factor-beta family, is a potent inhibitor of muscle growth. While over expression of this gene leads to profound muscle and fat loss, interference leads to dramatic increase in muscle mass.

Aim: We sought to determine if an increase in lean body mass (LBM) is protective against burn induced cachexia.

Methods: Hypermuscular transgenic mice having dominant negative myostatin receptor in skeletal muscle (SJL/C57tg), and wild-type littermates (SJL/C57wt) were bred in our colony. Fat and lean body mass were measured by Piximus imaging (GE Medical Systems). Full thickness burns were created using heated brass plates.

Results: After a 20% body surface area (BSA) burn, SJL/C57wt animals had 100% survival and demonstrate 7.7% weight loss at 14 days. SJL/C57tg animals, on the other hand, had 50% survival and demonstrated a 7.6% weight loss at 14 days. Although the percentage of weight loss was similar, SJL/C57tg animals lost 6.7% of their LBM while SJL/C57wt gained 7.0% in LBM (p = 0.0081). Conversely, SJL/C57tg animals gained 4.9% fat body mass (FBM) while SJL/C57wt animals lost 22.68% FBM (p = 0.0501).

Conclusion: Blockade of the myostatin pathway does not change overall weight loss induced by burn injury. However, a change in body composition is observed, highlighted by a loss in LBM while a gain in FBM occurs. Furthermore, myostatin appears to be involved in the metabolic response to burn injury as an increase in mortality is observed when this pathway is interrupted.

5.82
Nutrition management of patients with major burns

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Sepsis, shock, multiple trauma, and burns are often associated with altered metabolism characterized by severe catabolism, wasting of the lean body mass, immune dysfunction, and compromised wound healing. Nutritional support is one of the principal goals in the management of these critically ill patients and is aimed at minimizing these complications. Patients with burns who are unable to meet their nutrient requirements will lose over 20% of lean mass within weeks of injury. This loss will reduce healing and increase infection. A major burn constitutes a major stress and burns patients are under a prolonged increase in energy expenditure as a consequence of the stress response in terms of hormone release and inflammatory mediator activation.

Early and aggressive nutritional support is essential to minimize the breakdown of body proteins, improve metabolic response, reduce infectious complications and support healing. This support is critical to successful therapy and survival.

We studied 2 groups of patients. One group of 22 patients with less than 20% total body surface area injury where nutritional needs were covered by oral diet alone. And the second group by 28 patients with greater than 20% burn injury where we administered enteral supplementation or total enteral support. Our conclusions were that use of total parenteral nutrition is associated with an increased risk of catheter-related infectious, early enteral feeding improves metabolic and clinical response and reduce time to complete healing. Caloric requirement is proportional to the degree of injury, that means increases in metabolic rate can reach twice normal energy expenditure when burn injury exceeds 50% of total body surface area. Increased protein is required for wound healing and for replacement of nitrogen losses from the wound and in the urine, particularly during the first 4 weeks.

5.83
Metabolic and nutritional aspects of traumatic injury in critically traumatic patients

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Cachexia is responsible for the deaths of approximately 15% of the trauma deaths that occur from sepsis-induced organ dysfunction and malnutrition days to weeks after the initial traumatic event. The abnormalities associated with cachexia include anorexia, weight loss, a preferential loss of somatic muscle and fat mass, altered hepatic glucose and lipid metabolism, and anemia. Metabolic alterations in protein, carbohydrate and lipid metabolism contribute to the severe tissue losses because only anorexia cannot explain cachexia.

The degree of hypermetabolism which is common in traumatic injuries can be variable, depending on the type of injury, age, the degree of inflammation, previous conditions and treatment. To estimate metabolic rate in some types of traumatic injury, predictive equations have been studied. Patients with traumatic injuries are usually catabolic, but protein needs after traumatic injury are under discussions. Some authors suggest that 1.5 g protein per kg body weight is adequate and that any additional protein is simply oxidized. Other authors suggest that protein intake >2.0 g/kg body weight increases the absolute rate of body protein synthesis, and achievement of nitrogen balance has been associated with survival. We had in study 35 patients with traumatic injuries with diverse type of injury, age, degree of inflammation and different previous conditions. We determined energy requirements of these patients and we studied which nutrition support has more benefits for the critically traumatic injured patients. Thus, administration of high-protein feeding to achieve nitrogen balance might have more benefits, even if that balance is achieved with additional nitrogen production.

5.84
Reversibility of cachexia and course of exercise capacity over 1 year after heart-, lung- or kidney-transplantation

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Background: Cachexia represents an end stage of heart, lung or renal insufficiency. We investigated the reversibility of cachexia after transplantation (Tx) of the respective organ and the cardiopulmonary exercise capacity (peakVO2) before and after Tx.

Methods: We examined 106 patients before heart (HTx), 46 before lung (LTx) and 26 before renal transplantation (RTx) and 3, 6 and 12 months after Tx (18/25/11 female, median age 54/46/47 years). We divided patients in cachectic (BMI≤21kg/m2, n=15/21/8), normal (BMI21–27kg/m2, n=64/23/9) and overweight (BMI≥27kg/m2, n=27/2/9). 25 healthy volunteers (matched for age and gender) served as control.

Results: All 3 groups of patients lost weight early postoperatively (at 3 months -2.9 to -6.5%; p<0.05) and gained weight in follow-up (+1.0 to 6.1%). Among cachectic patients, only those after HTx or LTx gained weight: 3 months +6.8% (HTx) resp. +2.0% (LTx), 6 months +11.3/+5.9%, and 12 months +15.6/+8.5% (all p<0.05). In contrast to that, cachectic RTx-recipients showed no significant changes in weight (-4.0 to +2.4%; p=n.s.). All groups of patients had reduced peakVO2 before Tx: HTx 11.6±3.8, LTx 9.7±2.2, RTx 23.2±5.9 ml/min/kg. PeakVO2 improved after HTx or LTx only (+44.4 to +74.0%; all p<0.001). After RTx, peakVO2 decreased over 6 months (-21.0%; p<0.05) and increased even at 12 months (+5.6%; p<0.01).

Conclusions: Cachectic patients gain weight after HTx or LTx, but not after RTx. Comparably, exercise capacity (peakVO2) increases early after HTx and LTx, but not after RTx. Thus, the reconstitution of weight and exercise capacity follows different mechanisms after HTx or LTx compared to RTx.

5.85
Exercise capacity and body composition after renal transplantation of living donors: changes over time

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Background: Cardiovascular morbidity improves after renal transplantation (RTx) despite an increase in fat mass and constant lean tissue mass. Little is known about these effects in patients receiving renal grafts from living donors (RTx-LD). This study was to investigate changes in exercise capacity (as a parameter of cardiovascular morbidity) and body composition in RTx-LD.

Methods: 25 Patients (14 male, age 46±18 years; weight 72±19 kg, BMI 23.7±4.3 kg/m²) were prospectively examined before and serially 1, 3 and 12 months after transplantation. At each examination, every patient underwent exercise testing with measurement of peak oxygen uptake (peakVO₂) and body composition analysis using dual energy X-ray absorptiometry (DEXA).

Results: At 1 month after RTx peakVO₂ and peakVO₂/lean mass decreased compared to before RTx (p<0.0001) and recovered to baseline values after 1 year (p<0.0001). Body lean mass decreased at 1, 3 and 12 months (all p<0.0001 vs baseline; see table), whereas whole body weight remained unchanged and body fat increased (all p<0.0001). The decline in peakVO₂/lean mass at 1 month suggests worse quality of muscle function which may be due to post-operative steroid therapy and post-operative stress. Re-increase of peakVO₂/lean mass to baseline values at 1 year indicates on recovery and improvement of muscle function.

Table (all data mean±SEM)	Baseline	1 month	3 month	1 year
weight, kg	72.0±19.3	69.9±18.7	71.0±18.4	73.4±19.7
body fat content, %	26.2±12.6	27.6±11.9	29.5±10.5	31.2±11.5
body lean mass content, %	71.0±12.0	69.5±11.4	67.9±10.0	66.4±11.0
peak VO ₂ , mL/min/kg	23.2±5.9	17.5±4.8	19.6±4.8	23.4±6.1
peak VO ₂ /lean mass, mL/min/kg	33.4±9.5	25.4±6.5	28.9±6.4	35.1±8.0

Table: PeakVO₂ and body composition at baseline, 1, 3 and 12 months after RTx-LD. All p<0.005 (ANOVA).

Conclusion: Exercise capacity declined up to 1 month after RTx-LD, but recovered after 1 year. Body composition changed towards increased fat tissue and less lean mass. Changes in exercise capacity were independent of changes in muscle mass, thus indicating improved muscle quality.

5.86

Plasma high- but not low-molecular weight adiponectin is positively associated with resting energy expenditure in male non-diabetic hemodialysis patients

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Background and Aims: Adiponectin upregulates oxidative substrate utilization in lean tissues (skeletal muscle and liver) in experimental models, and these effects could contribute to its clinical association with weight reduction and higher insulin sensitivity. High- (HMW) rather than low-molecular weight (LMW) circulating hormone appears to be responsible for adiponectin metabolic effects. The role of adiponectin and its forms in regulating oxygen consumption and resting energy expenditure (REE) in vivo is however largely undefined. No studies are available on potential relationships between adiponectin and REE in individuals prone to negative energy balance and cachexia such as chronic kidney disease (CKD) patients undergoing maintenance hemodialysis (MHD). We studied associations between total, HMW- and LMW-adiponectin and REE in MHD.

Methods: REE (indirect calorimetry), body composition (multifrequency bioimpedance analysis), plasma adiponectin forms were measured in male non-obese, non-diabetic MHD patients (n=15, age= 58±12 years, BMI= 23.8±2 kg/m²).

Results: Lean and fat mass were positively associated with REE (r=0.47/0.54; 0.03>P>0.08). After adjusting for age, REE was associated positively with T- and HMW-adiponectin (r=0.53/0.58, P<0.05). Both adiponectin forms were also associated positively with REE normalized per kg fat mass (r=0.54, P><0.05) but not per kg lean mass (P=NS). No associations were observed between LMW-adiponectin and REE (P>0.2).

Conclusions: Higher HMW but not LMW adiponectin is associated with higher REE in non-obese, non-diabetic male MHD patients. This association could potentially involve adiponectin metabolic effects in lean tissues. The results suggest a potential negative impact of higher plasma HMW adiponectin on patient nutritional state in CKD.

5.87

Pathogenic mechanisms of muscle loss in patients undergoing liver transplantation

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Background and aims: Chronic diseases, including chronic liver failure (CLF), are often accompanied by malnutrition and muscle loss (ML), which in turn negatively affect quality of life, morbidity and mortality. Unlike other chronic conditions little is known about the molecular mechanism underlying ML in CLF.

Methods: rectus abdominis biopsies were obtained intraoperatively in 12 patients undergoing orthotopic liver transplantation for CLF and 4 well-nourished subjects undergoing elective surgery for non-neoplastic conditions. Total RNA was extracted and m-RNA for atrogens (MuRF-1, MAFbx), myostatin (MSTN), and IGF-1 was assayed (arbitrary units) by semiquantitative PCR.

Results: Overall, no differences were observed between CLF and controls in m-RNA levels. Moreover, no differences in m-RNA expression were found when CLF patients were stratified according to impaired nutritional status (subjective global assessment [SGA] vs SGA 1-2) or ML (Mid Arm Muscle Area [MAMA]<5th vs MAMA>5th percentile) (Table).

Conclusion: These preliminary data suggest that the molecular pathways underlying ML in CLF might be different from those involved in other chronic diseases, such as cancer, and deserve further research.

	MuRF	MAFbx	MSTN	IGF-1
CONTROLS (n=4)	0.20±0.00	0.38±0	0.40±0.002	0.41±0.05
CLF MAMA >5th (n=9)	0.29±0.00	0.35±0.13	0.40±0.05	0.51±0.05
CLF MAMA <5th (n=3)	0.21±0.00	0.36±0.05	0.38±0.07	0.44±0.14
SGA 0 (n=6)	0.17±0.00	0.35±0.05	0.36±0.08	0.40±0.13
SGA 1-2 (n=6)	0.29±0.00	0.38±0.09	0.40±0.05	0.50±0.09
TOTAL CLF (n=12)	0.23±0.00	0.36±0.07	0.39±0.06	0.46±0.12

6.88

Animal models for cancer cachexia: What are the options?

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Background and aims: Cancer cachexia, a complex wasting syndrome, is common in palliative medicine. Animal models expand our understanding of its mechanisms. A review of cancer cachexia animal models will help investigators make an informed choice of the appropriate study model.

Methods: PubMed/ Medline search was performed. MeSH terms used: ("biologic model" OR "animal model") AND "neoplasm" AND "cachexia"

Results: A preliminary search found 267 articles with 23 review articles. 96% were in English, 43% published during the last 10 years. Animal literature is extensive. MCG101 is a good anorexia model. MAC16 is excellent to study cachexia-related metabolic effects. Yoshida AH-130 and Lewis lung carcinoma cause severe wasting at low tumor burden. Animal models used to study cancer cachexia offer the advantage of tumor and host genetic homogeneity, allow controlled studies and have less confounding variables such as food intake, comorbidities, variable tumor burden, multiple primary site, and heterogeneous cancer responses. However no animal model explains inter-individual and inter-tumor differences in clinical cachexia; none reproduces clinical settings (e.g. polymedicated patients); cachexia in these models often occurs at a high tumor burden; many models lack human inflammatory responses; and most tumors do not metastasize.

Conclusions: Animal models help study mechanisms of cancer cachexia. However, no model reproduces human cancer cachexia as a whole and none mimics the spectrum of tumor-host interactions.

6.89

Effect of cancer cachexia upon the balance pro and anti-inflammatory cytokines in the adipose tissue of trained rats

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The adipose tissue (AT) is severely affected by cancer-cachexia. Moderate endurance training modulates the content of cytokines in this tissue. We tested the effect of a moderate intensity training running program (6 weeks) upon the balance of anti and pro-inflammatory cytokine synthesis in different depots of white adipose tissue of tumour-bearing rats. Animals Wistar rats male were randomly assigned to a sedentary control (SC, n=7), sedentary (Walker-256) tumour-bearing (ST, n=7), sedentary pair-fed (SPF, n=7) or trained control (TC, n=6), trained (Walker-256) tumour-bearing (TT, n=6) and trained pair-fed (TPF, n=6) groups. Trained rats ran on a treadmill (60%VO₂max) for 60min/day, 5 days/week, for 6 weeks. Retroperitoneal (TARP) and mesenteric (TAME) adipose tissue mRNA expression was analyzed for TNF-alpha, IL-1beta, IL-10 and PPAR-gamma by RT-PCR. Tissue cytokine levels (TNF-alpha, IL-1beta and IL-10) were assessed by ELISA. In TAME, TNF-alpha mRNA was increased in ST (p<0.05) in relation to SC, with no changes in protein level. Cytokines (TNF-alpha, IL-1beta and IL-10) levels assessed in TARP were increased in ST in relation to SC and SPF (p<0.05). These parameters were restored to control values when the animals were submitted to the training protocol. Walker-256 tumour cachexia affected heterogeneously adipose tissue depots studies inducing a pro-inflammatory state. Endurance training prevented the installation of these changes.

6.90

New insights into adipose atrophy in cancer cachexia

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Background: Profound loss of adipose tissue is a hallmark of cancer cachexia but the underlying mechanisms remain elusive. We examined cellular and molecular characteristics of white fat in mice with cancer cachexia.

Methods: MAC16 tumour was transplanted into the flank of NMRI mice to induce cachexia. Adipose tissue morphology was examined using light and electron microscopy. The mRNA levels of the key adipogenic factors were quantified by real-time PCR, and protein analysed by western blotting.

Results: Adiposity was markedly reduced in MAC16 mice compared with pair-fed and freely-fed controls. Adipose tissue from MAC16 mice contained adipocytes that were shrunken and heterogeneous in size. Increased fibrosis was evident by strong collagen-fibril staining in the tissue matrix. Ultrastructure of 'slimmed' adipocytes revealed severe delipidation and modifications in cell membrane conformation. Neither apparent infiltration of mononuclear cells nor increased expression of cytokines was found. There were major reductions in mRNA levels of adipogenic transcription factors including CCAAT/enhancer

binding protein alpha (C/EBPalpha), C/EBPbeta, peroxisome proliferator-activated receptor gamma, and sterol regulatory element binding protein-1c (SREBP-1c) in adipose tissue, which was accompanied by reduced protein content of C/EBPalpha and SREBP-1. mRNA levels of SREBP-1c targets, fatty acid synthase, acetyl CoA carboxylase, stearoyl CoA desaturase-1 and glycerol-3-phosphate acyl transferase, also fell as did glucose transporter-4 and leptin.

Conclusions: MAC16 tumour induces significant remodelling of adipose tissue and suppression of the adipogenic factors. These findings suggest that the tumour has an inhibitory effect on adipocyte development and lipid storing capability which may underlie adipose atrophy in cancer cachexia.

6.91

Serine-protease activity is increased in experimental cancer cachexia

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Background and aims: Muscle wasting, a prominent feature of cancer cachexia (CC), is mainly brought about by hypercatabolism, but perturbations in the anabolic pathways may also contribute. The ATP-ubiquitin-dependent system plays a pivotal role in catabolic response, although the lysosomal compartment, Ca²⁺-dependent proteolysis and caspases may be involved as well.

Recent data show the involvement of a serine protease in normal myofibrillar protein turnover in a murine model, suggesting that upregulation of these systems might occur in pathological conditions and play a role in muscle wasting. Aim of this study was to investigate if serine protease activity is increased in experimental CC.

Methods: Male Wistar rats were randomized into tumor bearers (TB) and controls (C) (n=8 each). TB were inoculated i.p. with 10⁸ AH-130 cells. At day 7 after transplantation rats were sacrificed and gastrocnemius was excised. Muscle protein concentration were determined by Lowry assay. Serine protease activity was determined by measuring the rate of Boc -VPR-MCA hydrolysis in muscle extracts. Release of free MCA was measured after 90' incubation by fluorometric assay. Student's t test was used for statistical analysis.

Results: After 90' serine-protease activity (nKatal x 10⁻⁷ /mg prot) was significantly increased in TB versus C (150,87 ±18,50 vs 102 ±23,16 respectively, p<0.001).

Conclusions: These results first demonstrate that muscle serine protease activity is markedly increased in CC. Further studies are needed to clarify the role of serine proteases in muscle protein degradation in CC.

6.92

The IGF-1 signaling pathway is not down-regulated in 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. Enhanced protein breakdown mainly accounts for muscle wasting. Recent data from our laboratory showed that muscle wasting in tumor bearing rats was associated with a marked reduction of both circulating and gastrocnemius mRNA levels of IGF-1 and with increased mRNA encoding the Ub-ligases atrogin-1 and MuRF1. Aim of this study was to investigate the IGF-1 signal transduction pathway in two different experimental models of cancer cachexia.

Methods: Tumor-bearing rats received 10⁸ Yoshida AH-130 cells i.p. and 7 days after transplantation were sacrificed. Tumor-bearing mice received 5*10⁵ Colon 26 adenocarcinoma cells s.c. and were sacrificed 13 days later. Phosphorylation and total content of Akt, FOXO, and p70s6k in the gastrocnemius muscle were assayed by western blotting.

Results: A marked loss of body and muscle weight was detected at the end of the experimental period in both models. No significant differences could be appreciated in the phosphorylation of Akt in both Ser473 and Thr308 residues. The levels of P-p70s6k in C26 bearing mice were unaffected, while an unexpected increase was found in the AH-130 bearing rats. Finally, phosphorylated FOXO was increased in both models, suggesting a reduced nuclear translocation.

Conclusions: These results demonstrate that the IGF-1 pathway is not down-regulated in experimental cancer cachexia. In addition, these data suggest the lack of a causative relation among low IGF-1, increased Ub-ligases and muscle wasting, at least in the experimental models considered.

6.93

Genetic and pharmacologic inhibition of myostatin for muscle preservation in cancer cachexia

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Background and aims: Myostatin inhibits muscle growth. Animals genetically deficient in myostatin show 2-5 fold increased skeletal muscle. Conversely, we have shown that excess myostatin causes wasting. Myostatin activity is inhibited by Follistatin, expression of which can be induced with Trichostatin A (TSA), a histone deacetylase inhibitor that reduces degeneration in muscular dystrophy. We sought to determine how genetic or pharmacological inhibition of myostatin activity might promote muscle preservation in cancer cachexia.

Methods: Myostatin null mice and matched wild-type controls were injected with B16.F10 melanoma cells and euthanized at 25d. CD2F1 mice were injected with colon-26 (C26) adenocarcinoma cells. TSA was administered daily (0.6 mg/kg i.p. in DMSO) from d9 to d16 after C26 injection. Control C26 mice received DMSO only, while non-tumor bearing mice received either TSA or DMSO on the same schedule.

Results: B16.F10 tumor size was not different in myostatin null versus wild-type mice; however, reductions in total body mass, muscle and fat mass were all proportionately greater in myostatin null mice than controls. TSA induced muscle follistatin mRNA 2.5-fold in non-tumor mice and 1.5 fold in C26 mice. Consistent with reduced myostatin, gastrocnemius muscles from TSA-only mice were 22% larger than controls. Percent body weight, skeletal muscle and fat loss were statistically indistinguishable in TSA- versus DMSO-treated C26 mice, however.

Conclusions: Neither genetic nor pharmacological inhibition of myostatin activity was sufficient to prevent or reduce muscle wasting in two models of cancer cachexia. Indeed, hypermuscular myostatin null mice appeared more sensitive to cancer-induced wasting.

6.94

Impaired immune function in an animal model for cancer cachexia prior to weight loss

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Fifty percent of cancer patients have significant weight loss prior to treatment and many of them are pre-cachectic or already suffer from cachexia. Malnutrition and inflammation occurring during cachexia directly affect immune function. Therefore, these patients have a higher susceptibility towards infections which influences survival significantly.

Several studies show the cachectic features of the C26-tumor model in mice in which pro-inflammatory cytokines are denoted as pivotal mediators. In contrast, fewer studies have investigated effects on immune function. Present study aims to investigate immune function in (pre-)cachectic mice.

Murine colon adenocarcinoma (C26) cells were inoculated in syngenic CD2F1 mice to induce cachexia. This resulted in a significant loss of body weight and wasting of skeletal muscles and adipose tissue. In addition, pro-cachectic cytokines IL-6 and TNF-alpha in plasma increased, whereas the anti-cachectic cytokine IL-4 was significant lower in tumor-bearing mice.

Contact hypersensitivity (CHS) against oxazolone, an in vivo model for cellular Th1 immune response, was already significantly decreased by 31% (P<0.001) in tumor-bearing animals before onset of weight loss. Furthermore, ConA-induced splenocyte proliferation capacity and Th1/Th2 cytokine production were significantly reduced in cachectic animals compared to controls. Similarly, immune cells in these mice showed a lower capacity to react to whole blood LPS stimulation.

Present data not only demonstrate an impaired immune function in tumor-bearing mice suffering from cachexia, but show a significantly reduced in vivo Th1-response already prior to the onset of weight loss. Therefore it is important to identify early risk factors and provide supportive care to patients in time.

6.95

Impaired daily activity and muscle function in cachectic C26 adenocarcinoma bearing mice

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Cancer cachexia is characterized by metabolic alterations leading to loss of adipose tissue and lean body mass, resulting in weight loss. This directly compromises physical performance and the quality of life of cancer patients. The present study aims to validate the C26 tumor bearing mouse model for the study of cancer cachexia related muscle dysfunction and the resulting consequences on daily physical activity.

Male CD2F1 mice, 6-7 weeks of age, were divided in 2 groups: Tumor-Bearing mice (TB; n=17), tumor induced by s.c. inoculation with murine colon adenocarcinoma (C26) cells, and Control mice (Ctr; n=10). Food intake, body weight (BW), tumor size and 24h activity were recorded. At 20 days after tumor/vehicle inoculation, animals were sacrificed and muscle function was tested ex-vivo.

Body weight of TB mice was lower than of Ctr mice from day 13 onwards (P<0.05). Tumor mass on day 20 was 2.1±0.1g. TB mice showed a reduced carcass weight (17.7±0.4g; -33%), epididymal fat mass (-80%) and muscle weight (-23%) vs Ctr mice on day 20, while food intake was still comparable between groups. EDL muscle contraction and relaxation force during single and repeated contractions was lower in the TB mice (P<0.05). Total daily activity was lower in TB mice vs Ctr mice (day 20: -50%; P<0.05 for TB vs Ctr).

C26 tumor bearing mice show clear symptoms of cachexia and related muscle dysfunction with muscle fatigue. This mouse model therefore seems valid for further mechanistic and/or intervention studies, as it resembles clinical characteristics of cancer cachexia.

6.96

Metabolomics of cancer cachexia

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Background: Cancer cachexia remains a challenging clinical problem with a complex pathophysiology and unreliable diagnostic tools. Metabolomics, the study of metabolic profiles in biologic samples, was applied to define a metabolic fingerprint and identify metabolites in an established murine model of cachexia.

Methods: Fifteen, male CD2F1 mice that received subcutaneous flank injections of C26 adenocarcinoma cells and 10 controls were studied. Serum was collected for metabolomic NMR spectrometer analysis at baseline, when the tumor was palpable, and when the mice had cachexia. Weight loss and tumor size were recorded and molecular markers, E3 ubiquitin ligase Muscle Ring Finger 1 (MuRF1) and β -dystroglycan (β -DG), were used to confirm atrophy in the tumor-bearing group.

Results: Experimental mice lost weight with the mean final body weight (19.1 ± 2.3 g) being significantly less (19.1% ; $p < 0.001$) than the control group (23.6 ± 0.6 g). Cachectic mice had less hind limb weight (0.69 ± 0.10 g) showing significant muscle atrophy (41% ; $p < 0.001$) compared to the control group (1.16 ± 0.12 g). MuRF1 and β -DG confirmed atrophy in the tumor group. Metabolomic NMR spectrometry demonstrated distinct clusters based on a unique set of metabolites, discovering a metabolic fingerprint for cachexia. Metabolite identification revealed that very low density lipoproteins, low density lipoproteins, valine, and leucine were increased while glucose, lactate, and glycerol were decreased.

Conclusions: This study demonstrates that metabolomics has potential as a diagnostic tool in cancer cachexia and it shows proof of principle that metabolomics will simultaneously elucidate multiple pathways involved in this syndrome.

6.97

The obesity paradox in dogs with spontaneously occurring heart failure

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Background: Although cachexia is associated with impaired survival in heart failure, overweight or obese human 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 dogs with spontaneously occurring heart failure.

Methods: All dogs with moderate to severe congestive heart failure secondary to idiopathic dilated cardiomyopathy or chronic mitral valve regurgitation (endocardiosis) 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 dogs that were discharged from the hospital and that lived >1 day.

Results: 111 dogs were enrolled in the study. Survival was significantly different between dogs that gained, lost, or maintained body weight over the course of their disease, with dogs that gained weight surviving the longest ($P=0.049$). Body condition score ($P=0.07$) and medications were not significantly associated with survival time, but dogs receiving n-3 fatty acid supplementation did have a longer survival time ($P=0.006$).

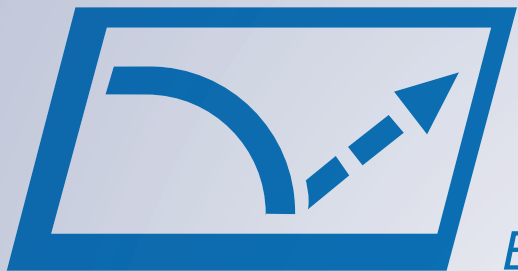
Conclusions: These results suggest that changes in body weight and, possibly, body condition may be important considerations in the survival of dogs with heart failure, as in people. N-3 fatty acid supplementation also was associated with longer survival times, an effect that warrants further evaluation.

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