The 110th Abbott Nutrition Research Conference

June 23–25, 2009

Columbus, Ohio

Subject: The Role of Nutrition in Accretion, Retention, and Recovery of Lean Body Mass

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Welcome

We are pleased to provide you with the Proceedings of the 110th Abbott Nutrition Research Conference, entitled “The Role of Nutrition in the Accretion, Retention, and Recovery of Lean Body Mass.”

The 110th Abbott Nutrition Research Conference is one of a series of conferences designed to connect the latest in science and research with the practice of clinical nutrition. We strongly believe that advancing therapeutic nutrition into clinical practice will play a vital role in the future of healthcare.

The subject of the 110th conference was the impact of nutrition on lean body mass and overall health. The purpose of this conference was to explore the underlying mechanisms controlling muscle quality and quantity that could be affected by nutrition. This publication focuses on the clinical consequences of loss of skeletal muscle mass due to sarcopenia, disuse, and the effects of metabolic stress brought on by acute and chronic disease. We hope you find these summaries insightful and that they inspire changes in the way you view the role of nutrition in skeletal muscle health.

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The Role of Nutrition in the Accretion, Retention, and Recovery of Lean Body Mass

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The Role of Nutrition in the Accretion, Retention, and Recovery of Lean Body Mass

The 110th Abbott Nutrition Research Conference was held in Columbus, Ohio, on June 23–25, 2009. This Report contains summaries of presentations given by the following contributors:

Keynote Address

Role of Skeletal Muscle in Health

John E. Morley, MD, St. Louis University, St. Louis, Missouri, USA
Dammert Professor of Gerontology and Director, Division of Geriatric Medicine, St. Louis Medical Center
Director of the Geriatric Research, Education, and Clinical Center, St. Louis Veterans Affairs Medical Center

Loss of muscle is associated with weakness, fatigue, insulin resistance, falls, fear of falling, fractures, frailty, disability, and death. Anorexia, sarcopenia, and cachexia each contribute to the decline in muscle mass in unique ways. This article summarizes the causes and outcomes of skeletal muscle loss as well as current recommended nutritional interventions in the prevention and reversal of muscle loss.

Skeletal Muscle Physiology and Nutrition Parameters

Regulation of Muscle Protein Synthesis and Degradation in Normal and Pathophysiological States

Denis Guttridge, PhD, The Ohio State University, Columbus, OH, USA
Associate Professor, Department of Molecular Virology, Immunology, and Medical Genetics

In normal muscle, hypertrophy and atrophy balance one another. When homeostasis is disrupted and atrophy becomes the dominant response, conditions such as muscular dystrophy, cachexia, and pediatric rhabdomyosarcoma can arise. This presentation explores the role of the Akt/mammalian target of rapamycin (mTOR) pathway in hypertrophy and the FoxO and NF-κB transcription factors in atrophy.

Effects of Nutrition on Muscle Metabolism

Michael Tisdale, PhD, Aston University, Birmingham, UK
Professor of Cancer Biochemistry

Atrophy of skeletal muscle is common in a number of conditions including cancer, sepsis, metabolic acidosis, weightlessness, immobility, diabetes, and AIDS. This leads to weakness (asthenia), and can lead to death through respiratory failure. In cancer patients there is an inverse relationship between weight loss and survival time. The article discusses various nutrients that have the ability to attenuate this condition by increasing protein synthesis and/or decreasing protein degradation in skeletal muscle.

Role of Oxidative Stress in Skeletal Muscle and Strength

Michael Reid, PhD, University of Kentucky, Lexington, KY, USA
Shih-Chun Wang Professor and Chair, Department of Physiology,
Director, Center for Muscle Biology

Current research indicates that reactive oxygen species (ROS) production by muscle fibers is increased in a variety of physiological and pathophysiological conditions, including chronic inflammation, aging, mechanical unloading, and strenuous exercise. Increases in intracellular ROS activity can promote muscle weakness and fatigue via two parallel pathways. This paper describes these pathways and suggests that they appear to be regulated independently and can have separate or additive effects on mechanical function.

Skeletal Mass Retention During Adulthood: Influence of Nutrition and Exercise on Lean Body Mass and Functionality

Role of Protein Absorption and Nutrient Timing on Muscle Mass Accretion

Stuart Phillips, PhD, McMaster University, Hamilton, Ontario, Canada
Professor, Kinesiology
Associate Professor, Medicine, Cell Biology and Metabolism

It is important that clinicians understand the mechanisms of muscle mass accretion if they are to help their athlete patients perform to the best of their abilities and their elderly patients either regain or prevent loss of lean body mass. Diet and exercise both play important roles in protecting lean body mass. This article examines the circumstances under which the greatest muscle protein synthesis occurs.

Impact of Nutrition on Lean Body Mass and Exercise Recovery in Athletes

Jeff Volek, PhD, RD, University of Connecticut, Storrs, CT, USA
Associate Professor and Researcher, Department of Kinesiology

For maximum increase in lean body mass, patients should ingest macronutrients in specific combinations and follow a regimen of resistance exercise. This article investigates the optimal balance of nutrition and exercise.

Role of Vitamin D in Muscle Strength and Function

Michael Holick, MD, PhD, Boston University, MA, USA
Professor of Medicine, Physiology, and Biophysics
Director, General Clinical Research Center
Director, Bone Health Care Clinic and Heliotherapy, Light, and Skin Research Center

Vitamin D deficiency has been linked with many chronic illnesses. Thus, a major effort is needed to re-educate health care professionals and the public about the beneficial effects of sunlight and vitamin D supplementation for health. Adults need at least 1500-2000 IU of vitamin D a day. Children require at least 400 IU of vitamin D a day and preferably 1000 IU of vitamin D a day to improve their overall health and well-being, including muscle strength.

Nutrition, Muscle Mass, and Muscular Performance in Middle Age and Beyond

Catherine Johnson, PhD, RD, LD, Abbott Nutrition, Columbus, OH, USA
Senior Research Scientist, Abbott Nutrition Global Nutrition R&D

Aging is associated with a loss in muscle mass and a gain in fat mass. These changes are related to a decrease in overall health and functional ability. Advancing sarcopenia is associated with increased risk of fall and fractures, decreased ability to complete activities of daily living, and increase in fatigue, all leading to dependency and disability. This paper examines the effects of several factors, including protein consumption and exercise, that promote optimal muscle protein synthesis with age.

Sarcopenia of Aging: Impact of Nutrition and Exercise

Challenges of Defining Sarcopenia: Status Report of the EUGMS Working Group on Sarcopenia

Tommy Cederholm MD, PhD, Uppsala University, Uppsala, Sweden
Professor, Clinical Nutrition
Head, Department of Public Health and Caring Sciences/Clinical Nutrition and Metabolism

Definitions of sarcopenia thus far have been inexact, sharing many characteristics with cachexia, frailty, and starvation. This article discusses the issues and considerations that must be taken in creating an operational definition of sarcopenia.

Lean Body Mass Loss With Age

Douglas Paddon-Jones, PhD, University of Texas, Galveston, USA
Associate Professor, School of Health Professions and Department of Internal Medicine, University of Texas Medical Branch

Sarcopenia is an age-related loss in lean body mass accelerated by poor nutrition and physical inactivity. To counteract this loss of muscle, patients should consume protein in adequate quantities and at proper times. This article investigates the effects of physical inactivity on lean body mass loss and the ingestion of protein on lean body mass accretion.

Effects of Unloading in Old Versus Young Humans

Charlotte Suetta, MD, PhD, Institute of Sports Medicine, Bispebjerg Hospital, Copenhagen, Denmark
Senior Researcher, Institute of Sports Medicine and Centre of Healthy Aging
Faculty, Health Science

Aging affects the response to short-term immobilization and the regenerative capacity of human skeletal muscle. This paper describes research showing that immobilized/retrained old individuals appear to be more affected with respect to neural function while young individuals are more affected in terms of muscle size. These data indicate that aging is accompanied by an impaired ability to recover from disuse muscle atrophy; consequently, old individuals may need a longer time to recover from periods of disuse than young individuals.

Effects of Metabolic Stress on Lean Body Mass

Cachexia Associated With COPD

Annemie Schols, PhD, University Hospitals, Maastrict, Netherlands
Professor, Nutrition and Metabolism in Chronic Diseases
Director, Maastricht University NUTRIM School for Nutrition, Toxicology, and Metabolism

Recent research shows that COPD is not only a chronic inflammatory lung disease but also a metabolic disorder affecting multi-organ systems. Weight loss, skeletal muscle wasting, and a decreased muscle oxidative phenotype are well documented in advanced COPD and have been a target for multimodal intervention strategies. Promising results have been obtained by existing strategies that include exercise and nutritional supplementation with or without anabolic agents. This article describes advances in understanding the molecular mechanisms of altered muscle plasticity in COPD progression, providing new leads for nutritional intervention.

Measurement of Lean Body Mass Using CT Scans

Vickie Baracos, PhD, University of Alberta, Canada
Chair, Alberta Cancer Foundation, Palliative Medicine
Professor, Department of Oncology, University of Alberta

Cachexia is characterized by a loss of muscle with or without a loss of fat mass. With the increasing prevalence of overweight and obesity, emaciation is becoming less common. Even after considerable weight loss from cancer cachexia, many patients are still considered overweight or obese. This article advocates measuring lean body mass, rather than the traditional method of observing weight loss, to better identify cachexia.

Long-Term Outcomes of the Effect of ICU Stay on Lean Body Mass and Strength

Gerald Supinski, MD, University of Kentucky, Lexington, KY, USA
Professor of Medicine
Staff Physician

Critically ill patients often face the challenge of both skeletal and respiratory muscle wasting. The cause of lean body mass loss is multifactorial and this loss results in the activation of several cellular pathways. This paper investigates the mechanisms responsible for increased protein degradation and decreased protein synthesis. By understanding this process, health care professionals can develop better therapies to decrease the morbidity from acquired weakness.

Cachexia in Cancer

Ingvar Bosaeus, MD, Sahlgrenska University Hospital, Gothenberg, Sweden
Consultant Physician and Department Head, Clinical Nutrition
Professor, Clinical Nutrition, University of Gothenberg

Metabolic events surrounding cachexia in cancer differ greatly from those in starvation, with muscle loss reflecting the former and fat loss reflecting the latter. Stand-alone nutrition intervention is unlikely to prevent the development of cachexia as it will restore only body fat. Therefore, treatment should focus on understanding and intervening in the catabolic events leading to muscle loss. This paper discusses the most effective strategies to block catabolism, and thereby increase survival, function, and quality of life in cancer patients.

Contents

Sarcopenia of Aging: Impact of Nutrition and Exercise

Effects of Metabolic Stress on Lean Body Mass

Acronyms and Abbreviations

AA . . . . . .arachidonic acid
ACE . . . . . .acute care for the elderly
ACSM . . . . .American College of Sports Medicine
ang II . . . . . .angiotensin II
AODM . . . . .adult onset diabetes mellitus
ATP . . . . . .adenosine triphosphate
ARDS . . . . .acute respiratory distress syndrome
BMI . . . . . .body mass index
BCAA . . . . .branched chain amino acid
CaBP . . . . .calcium binding protein
CASH . . . . .chemotherapy-associated steatohepatitis
CHD . . . . . .coronary heart disease
COPD . . . . .chronic obstructive pulmonary disease
CRP . . . . . .C reactive protein
CT . . . . . .computed tomography
DHA . . . . . .docosahexaenoic acid
DXA . . . . . .dual energy X-ray absorptiometry
EAA . . . . . .essential amino acid
ECaC . . . . .epithelial calcium channel
eEF2 . . . . . .eukaryotic elongation factor
eIF2 . . . . . .eukaryotic initiation factor 2
EMG . . . . . .electromyogram
EPA . . . . . .eicosapentaenoic acid
ESPEN . . . .European Society of Clinical Nutrition and Metabolism
ESWG . . . . .European Sarcopenia Working Group
EUGMS . . .European Geriatric Medicine Society
FEV . . . . . .forced expiratory volume
FFMI . . . . . .fat-free mass index
FGF . . . . . .fibroblast growth factor
15-LOX 15-lipoxygenase
FMI . . . . . .fat mass index
4EBP1 . . . .4E binding protein
FoxO . . . . . .Forkhead-O
GH . . . . . .growth hormone
GI . . . . . .glycemic index
GPT . . . . . .glutamic pyruvate transaminase
GSK3β . . . .glycogen synthase kinase 3 beta
h . . . . . .hour
HAART . . . .highly active retroviral therapy
HBP . . . . . .high blood pressure
HCl . . . . . .hydrochloric acid
HETE . . . . .hydroxy-eicosatetraenoic acids
HMB . . . . . .β-hydroxy-β methylbutyrate (beta-hydroxy-beta-methylbutyrate)
IAGG-ER . . .International Association of Gerontology and Geriatrics—European Region
IANA . . . . . .International Academy of Nutrition and Aging
ICU . . . . . .intensive care unit
IGF . . . . . .insulin-like growth factor
IFNγ . . . . . .interferon gamma
IKK . . . . . .IkB kinase
IL . . . . . .interleukin (-1, -6, -8)
KIC . . . . . .(α-)ketoisocaproate
LBM . . . . . .lean body mass
LPS . . . . . .lipopolysaccharide
MAFbx/
atrogin-1 . . .muscle-specific F-box protein
MAPK . . . . .mitogen-activated protein kinase
MnSOD . . . .manganese superoxide dismutase
MR . . . . . .magnetic resonance
MRI . . . . . .magnetic resonance imaging

mRNA . . . . .messenger ribonucleic acid
MS . . . . . .multiple sclerosis
MuRF-1 . . .muscle-specific RING finger-1
MyBP-C . . .myosin binding protein C
MyHC . . . . .myosin heavy chain
MyLC . . . . .myosin light chain
NF-κB . . . .nuclear factor kappa B
NMR . . . . . .nuclear magnetic resonance
ns . . . . . .not significant
NSAID . . . . .nonsteroidal anti-inflammatory drug
OM . . . . . .old men
1-OHase . . .D-1α-hydroxylase
1,25(OH)$_2$D .1,25-dihydroxyvitamin D
1RM . . . . . .single repetition maximum
PEM . . . . . .protein-energy malnutrition
PIF . . . . . .proteolysis-inducing factor
PK . . . . . .protein kinase (C, R, etc)
PKR . . . . . .protein kinase R
PLA2 . . . . . .phospholipase A2
PP1 . . . . . .phosphatase 1
PPAR . . . . .peroxisome proliferators-activated receptor
PreD$_3$ . . . . .previtamin D$_3$
PTH . . . . . .parathyroid hormone
PUFA . . . . .polyunsaturated fatty acid
RA . . . . . .rheumatoid arthritis
RANKL . . . .NFκB ligand
RCT . . . . . .randomized control trial
RDA . . . . . .recommended dietary allowance
REE . . . . . .resting energy expenditure
RM . . . . . .repetition maximum
RMR . . . . . .resting metabolic rate
ROS . . . . . .reactive oxygen species
SMI . . . . . .skeletal muscle index
SNAQ . . . .Simplified Nutritional Appetite Questionnaire
SOD . . . . . .super oxide dismutase
SPPB . . . . .short physical performance battery
TB . . . . . .tuberculosis
TNF . . . . . .tumor necrosis factor
TNFR1 . . . .TNF receptor subtype 1
TPN . . . . . .total parenteral nutrition
25(OH)D . . .25-hydroxyvitamin D
24-OHase . .24-hydroxylase
Ub . . . . . .ubiquitin
URI . . . . . .upper respiratory infection
VDR . . . . . .vitamin D receptor
YM . . . . . .young men

Role of Skeletal Muscle in Health

John E. Morley, MB, BCh

Skeletal muscle plays a major role in health. Loss of skeletal muscle has been associated with weakness, fatigue, insulin resistance, falls, fear of falling, fractures, frailty, disability and death. All the components of frailty, as shown in Table 1, are related to skeletal muscle function.[1]

Table 1. Components of Frailty: The Cardiovascular Health Study[1]

- The Cardiovascular Health Study enrolled 5317 men and women ≥65 years of age
- Frailty was defined as a clinical syndrome in which ≥3 of the following were present:
  - Unintentional weight loss (10 lb in past year)
  - Exhaustion (self-reported)
  - Weakness (grip strength, lowest 20%)
  - Walking speed (15 feet, slowest 20%)
  - Low physical activity (kcal/week, lowest 20%)

By this definition, nearly 7% of the participants were frail

This article summarizes the causes and outcomes of skeletal muscle loss, as well as current recommended nutritional interventions in the treatment of muscle loss.

Muscle disease related to aging can lead to loss of mass (sarcopenia), loss of strength, loss of power (dynapenia), fatigue, pain, and cramps. We need to recognize, however, that loss of strength and power are not directly related to loss of muscle mass. In particular, we need to recognize that, with age, collagen infiltration into the tendons leads to loss of the angle of pennation (angle formed by pennate muscle fibers with the line of action of the muscle), which results in a decline in the ability to generate power.[2]

Weight loss can be caused by loss of muscle, fat, and/or bone. Whatever the type of loss, multiple studies have shown that weight loss in people older than 60 years of age increases mortality and morbidity, including patients with diabetes mellitus.[3,4]

One study found that women older than 60 years of age who lost weight, intentionally or unintentionally, had a 2.5 times greater risk for hip fracture than those who had not.[5] Because of the increased morbidity associated with weight loss in older adults, their risk for institutionalization also is increased.

Prevalence of protein energy malnutrition (PEM) also is increased in populations with certain diseases and in certain health care settings. For instance, PEM occurs in 10% to 50% of people with diseases such as renal failure, chronic obstructive pulmonary disease, congestive heart disease, and HIV. Forty percent of people in subacute care are malnourished, as are 5% to 20% of those in nursing homes.[6]

The major causes of muscle wasting are anorexia, sarcopenia (age-associated muscle loss), and cachexia (disease-related muscle loss). Although these conditions all are characterized by loss of muscle, they differ in their impact on several anthropometric and laboratory parameters, as shown in Table 2.

Table 2. Comparison of Three Major Causes of Muscle Wasting

	Anorexia	Sarcopenia	Cachexia
Body mass	––	–	–––
Fat-free mass	–	––	–––
Body fat	–––	0	––
RMR	–	–	++
Physical activity	–	–	–
Food intake	–––	0	––
Proteolysis	–	+	++
Cortisol	+/–	+/–	++
Triglycerides	0	0	++
Cytokines	+/–	+	+++
Anemia	+	0	+++
Insulin resistance	0	0 (+ in sarcopenic obesity)	+

RMR=resting metabolic rate
+ and – symbols indicate the direction and strength of the impact of these conditions on the anthropometric and laboratory parameters listed at left.

Anorexia and Aging

Physiologic anorexia of aging can lead to weight loss. Age-related loss of appetite and reduction of food intake are caused by changes in taste and smell, alterations in the rate of filling of the antrum of the stomach, increased levels of the satiating hormone cholecystokinin, and in males, high leptin levels stemming from declining testosterone. In older people, numerous reversible causes of weight loss occur, as shown in the mnemonic device in Table 3.[7] Depression is the most common cause of weight loss in older adults.[8]

Table 3. Causes of Weight Loss

Medications

Emotions (depression)

Alcoholism, anorexia, abuse (elder)

Late life paranoia

Swallowing problems

Oral problems

Nosocomial infections, no money (poverty)

Wandering/dementia

Hyperthyroidism, hypercalcemia, hypoadrenalism

Enteric problems (malabsorption)

Eating problems (eg, tremor)

Low-salt, low-cholesterol diet

Shopping and meal preparation problems, stones (cholecystitis)

Clinicians can screen patients for anorexia with the Simplified Nutritional Appetite Questionnaire (SNAQ), shown in Table 4.[9]

Table 4. Simplified Nutritional Appetite Questionnaire (SNAQ) To Predict Weight Loss in Older People[9]

1. My appetite is
   A. Very poor
   B. Poor
   C. Average
   D. Good
   E. Very good

2. When I eat
   A. I feel full after eating only a few mouthfuls
   B. I feel full after eating about one third of a meal
   C. I feel full after eating over half a meal
   D. I feel full after eating most of the meal
   E. I hardly ever feel full

3. Food tastes
   A. Very bad
   B. Bad
   C. Average
   D. Good
   E. Very good

4. Normally I eat
   A. Less than one meal a day
   B. One meal a day
   C. Two meals a day
   D. Three meals a day
   E. More than 3 meals a day

Instructions: Complete the questionnaire by circling the correct answers and then tally the results based on the following numerical scale:
A=1, B=2, C=3, D=4, E=5

Scoring: If the score is less than 14, the risk of weight loss is significant

Physiologic anorexia of aging puts older adults at high risk for developing PEM when they develop either psychologic or physical disease processes. Screening for anorexia and early nutritional and/or pharmacologic intervention can reduce this risk.

Sarcopenia

Nearly 3.6 million people in the United States have sarcopenia and are at increased risk for physical disability and frailty. In one study of 4504 adults 60 years of age and older, those with severe sarcopenia had a two to three times greater likelihood of functional impairment and disability than those without sarcopenia.[10] People who are obese but who nonetheless are losing muscle mass (sarcopenic obesity) can develop severe disability and have an increased death rate.[11]

Muscle is normally in a balanced state of anabolism and catabolism. While protein synthesis plays an important role in this process, repair of muscle cells requires the constant generation of satellite cells.[12] Satellite cells are small mononucleated myogenic cells found in skeletal muscle fibers. They are normally quiescent, but they proliferate in response to injury and help in repair and maintenance of skeletal muscle.

Nutrition intervention in sarcopenia typically has focused on dietary supplementation with protein and/or specific amino acids. While a total protein intake of 0.8 g/kg/day is normally recommended, the International Cachexia Society recommends an intake of 1-1.5 g/kg/day for older people to prevent sarcopenia. Protein synthesis is best maintained by a mixture of leucine-enriched essential amino acids. These act not only as building blocks for protein synthesis but also activate mammalian target of rapamycin (mTOR), a serine/threonine protein kinase that drives protein synthesis. Essential amino acids act synergistically with exercise to increase muscle strength. Oral amino acids slow muscle loss that occurs with bed rest, reverses sarcopenia, and increases walk speed.

Supplementation with other nutrients such as creatine and vitamin D have shown some positive effects in people with sarcopenia. Creatine supplementation is shown to increase muscle power, if not mass, in older people, especially when combined with exercise. People with 25(OH) vitamin D levels below 30 ng/mL show declines in muscle strength and increases in disability, falls, hip fracture, and mortality.[13] Levels of 25(OH) vitamin D decline with aging. Vitamin D supplementation can decrease or reverse these changes.

Hormonal treatments also have been used with sarcopenic men. Testosterone replacement increases muscle mass, strength, and function in hypogonadal males. Testosterone in combination with a protein supplement decreased hospitalizations in people living in assisted living.[14] Selective androgen receptor molecules are being developed as potent anabolic steroids for use in treating sarcopenia.

At present, treatment of sarcopenia and frailty consists of supplementation with essential amino acids and creatine, together with resistance exercise. Some studies show that giving essential amino acid and/or calorie supplements between meals results in optimum efficacy.

Cachexia

Cachexia occurs in 10% to 35% of chronically ill older people.[15-17] The International Cachexia Society has defined cachexia as "a complex metabolic syndrome associated with underlying illness and accompanied by loss of muscle with or without [loss of] fat mass. The prominent clinical feature of cachexia is weight loss."[18] Table 5 describes the diagnostic criteria for cachexia.

Table 5. Diagnostic Criteria for Cachexia

- Weight loss (nonedema) of at least 5% in 12 months or less
- BMI (<20 kg/m^2) in the presence of underlying illness
- Plus three of the following:
  - Decreased muscle strength
  - Fatigue
  - Anorexia
  - Low fat-free mass index
  - Abnormal biochemistry
    - Elevated inflammatory markers (eg, CRP, TNF, IL-6)
    - Anemia (<12 g/dL)
    - Low serum albumin (<32 g/L)

BMI=body mass index, CRP=C reactive protein, TNF=tumor necrosis factor, IL-6=interleukin-6

The central pathophysiologic factors in cachexia are cytokines such as tumor necrosis factor alpha, interleukin-1, and interleukin-6.

Some evidence indicates that a balanced calorie supplement improves outcomes in critically ill patients, older hospitalized patients, patients with hip fracture, and those with liver disease. Thus, it is strongly recommended that people with cachexia receive a balanced calorie supplement of 300–600 kcal between meals, given preferably by the enteral route. People with cachexia should receive between 1.5–2.0 g/kg/day of protein. On admission to a hospital, patients with cachexia should receive 50,000 IU of vitamin D.

Omega-3 fatty acids decrease death and hospitalization in cardiac-failure patients and thus, all heart-failure patients should receive them.[19] Omega-3 fatty acids also have positive outcomes and minimal side effects in subgroups with cancer.

Conclusion

Muscle plays a key role in health. The three major causes of muscle wasting—anorexia, sarcopenia, and cachexia—are compared in Table 2. At present, treatment of muscle loss includes resistance exercise, protein supplements (leucine-enriched essential amino acids and creatine) between meals, and vitamin D supplementation (1000-2000 IU/day). Emerging evidence indicates that anabolic steroids also may enhance muscle power. Improving muscle function reverses frailty, increases walking speed, and decreases disability.

References

1. Fried L, Tangen CM, Walston J, et al: Frailty in older adults: Evidence for a phenotype. *J Gerontol A Biol Sci Med Sci* 2001;56:M134-M135.
2. Narici MV, Maffulli N, Maganaris CN: Ageing of human muscles and tendons. *Disabil Rehabil* 2008;30:1548-1554.
3. Kalantar-Zadeh K, Horwich TB, Oreopoulos A, et al: Risk factor paradox in wasting diseases. *Curr Opin Clin Nutr Metab Care* 2007;10:433-442.
4. Morley JE: Is weight loss harmful to older men? *Aging Male* 2006;9:135-137.
5. Ensrud KE, Ewing SK, Stone KL, et al: Intentional and unintentional weight loss increase bone loss and hip fracture risk in older women. *J Am Geriatr Soc* 2003; 51:1740-1747.
6. Thomas DR, Zdrodowski CD, Wilson MM, et al: A prospective, randomized clinical study of adjunctive peripheral parenteral nutrition in adult subacute care patients. *J Nutr Health Aging* 2005;9:321-325.

7. Morley JE, Silver AJ: Nutritional issues in nursing home care. *Ann Intern Med* 1995;123:850-859.
8. Morley JE: Weight loss in the nursing home. *J Am Med Dir Assoc* 2007; 8:201-204.
9. Wilson MM, Thomas DR, Rubenstein LZ, et al: Appetite assessment: Simple appetite questionnaire predicts weight loss in community-dwelling adults and nursing home residents. *Am J Clin Nutr* 2005;82:1074-1081.
10. Janssen I, Heymsfield SB, Ross R: Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability. *J Am Geriatr Soc* 2002;50:889-896.
11. Baumgartner RN, Wayne SJ, Waters DL, et al: Sarcopenic obesity predicts instrumental activities of daily living disability in the elderly. *Obes Res* 2004;12:1995-2004.
12. Morley JE: Weight loss in older persons: New therapeutic approaches. *Curr Pharm Des* 2007;13:3637-3647.
13. Brose A, Parise G, Tarnopolsky MA: Creatine supplementation enhances isometric strength and body composition improvements following strength exercise training in older adults. *J Gerontol A Biol Sci Med Sci* 2003;58:11-19.
14. Chapman IM, Visvanathan R, Hammand AJ, et al: Effect of testosterone and a nutritional supplement, alone and in combination, on hospital admissions in undernourished older men and women. *Am J Clin Nutr* 2009;89:880-889.
15. Yeh SS, Blackwood K, Schuster MW: The cytokine basis of cachexia and its treatment: Are they ready for prime time? *J Am Med Dir Assoc* 2008;9:219-236.
16. Morley JE, Anker SD, Evans WJ: Cachexia and aging: An update based on the Fourth International Cachexia Meeting. *J Nutr Health Aging* 2009;13:47-55.
17. Morley JE, Thomas DR, Wilson MM: Cachexia: Pathophysiology and clinical relevance. *Am J Clin Nutr* 2006;83:735-743.
18. Evans WJ, Morley JE, Argilés J, et al: Cachexia: A new definition. *Clin Nutr* 2008;27:793-799.
19. Gissi-HF Investigators, Tavazzi L, Maggioni AP, Marchioli R: Effect of n-3 polyunsaturated fatty acids in patients with chronic heart failure (the GISSI-HF trial): A randomized, double-blind, placebo-controlled trial. *Lancet* 2008; 372:1223-1230.

Regulation of Muscle Protein Synthesis and Degradation in Normal and Pathophysiological States

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Skeletal muscle is the most abundant tissue in the human body. Its mass is controlled through a delicate balance of signaling pathways that stimulate anabolism or hypertrophy of muscle cells through the protein translation machinery or control catabolism or atrophy by inducing protein breakdown. The main regulator of hypertrophy is the Akt/mammalian target of rapamycin (mTOR) pathway, which both promotes protein synthesis through the activity of p70S6K, and inhibits the activities of Forkhead-O (FoxO) transcription factors that induce the expression of E3 ubiquitin ligases, muscle-specific RING finger-1 (MuRF-1), and atrogin1. In contrast, muscle atrophy is controlled by FoxO and other transcription factors such as NF-κB that stimulate the expression of the E3 ubiquitin ligase genes, as well as other pathways that activate calpain and lysosomal enzymes. Under physiological conditions, hypertrophy and atrophy responses contribute equally to maintain a proper balance of muscle cell size and tissue homeostasis. However, in the event of an atrophy condition, such as that obtained from prolonged inactivity or chronic disease, this delicate balance is compromised, leading to reduced muscle cell size and eventual weakness and fatigue.

Much of what we know regarding the mechanisms regulating protein synthesis and the hypertrophy response in skeletal muscle cells comes from the work of Glass and his colleagues.[1] These researchers elucidated that in response to insulin and insulin-like growth factor (IGF), PI 3-kinase becomes activated, which in turn leads to further activation of Akt to stimulate the protein synthetic machinery. This notion is supported by studies showing that cells from organisms lacking Akt are reduced in size, most likely because of a general decline in protein output. Formal demonstration that Akt is relevant in promoting muscle cell hypertrophy comes from in vitro and in vivo studies from the Glass laboratory.[2] In these studies, investigators showed that transduction of a constitutively active form of Akt in C2C12 myotubes could increase cell size. This finding was confirmed by showing that expression of a dominant negative form of glycogen synthase kinase 3 beta (GSK3β), whose wild-type form is negatively regulated by Akt through phosphorylation, also resulted in larger cultured myotubes. Significantly, expression of the active form of Akt in intact muscles can also rescue against denervated-induced atrophy, establishing the relevance of Akt in vivo.

These initial findings led to the realization that several factors known to promote muscle atrophy, such as angiotensin II, inflammatory cytokines, and tumor factors, mediated their activities in part through the inhibition of the Akt pathway. Important contributions from the Tisdale laboratory[3] showed that these factors signal in common through the double-stranded RNA protein kinase R (PKR), which functions to inhibit the protein synthesis machinery downstream of Akt by phosphorylating and inactivating the translation initiating factor, eIF4B. Thus, their work implies that chronic activation of these factors can tip the balance and induce atrophy by abrogating Akt and the hypertrophic response.

In addition, these same factors promote atrophy through their stimulation of regulatory processes that control muscle protein turnover.[4] The major pathway thought to regulate the catabolism of muscle protein is the ubiquitin proteasome system, defined by the marking of proteins with ubiquitin moieties that signal the 26S proteasome to degrade proteins into small peptides and individual amino acids.

An important question about Akt and the proteasome is whether these muscle protein regulatory factors are mutually exclusive. Independent reports from the groups of Glass and Goldberg[5,6] reveal that these pathways intersect at a critical signaling junction through the activities of FoxO transcription factors. Previous studies in non-muscle cells demonstrate that FoxO activity is tightly regulated by Akt phosphorylation. Upon Akt activation in response to insulin and IGF, protein synthesis is stimulated, but proteasome activity is also suppressed by direct Akt phosphorylation of FoxO protein. In turn, FoxO1 and FoxO3 are inactivated by their inability to translocate to the nucleus. However, in cachectic conditions in which IGF levels are reduced, Akt activity also declines, leading to FoxO1 and FoxO3 nuclear translocation and binding to MuRF-1 and atrogin1/MAFBx promoters, where they function to stimulate proteasome activation.

More recent studies from the laboratories of Goldberg and Sandri also show that FoxO proteins transcriptionally regulate autophagy genes to activate the lysosomal system and promote muscle atrophy.[7,8] Therefore, aside from Akt-stimulating protein synthesis and the hypertrophy response, this kinase also functions to suppress muscle catabolism by blocking proteasome and lysosome activity through the phosphorylation of FoxO.

Another important point that had not been thoroughly investigated until recently are the proteins that are targeted by the proteasome system, leading to a catabolic state. Previously, the assumption was that such targets represented myofibrillar proteins, likely because of their abundance in mature skeletal muscle cells. However, little evidence supported this claim. Using cultured C2C12 myotubes

treated with cytokines tumor necrosis factor alpha (TNFα) and interferon gamma (IFNγ), our laboratory demonstrated that the combination of these factors downregulated expression of myosin heavy chain (MyHC) IIa and IIb, but not the other highly abundant contractile proteins, α-skeletal actin, troponin T, or tropomyosin α/β, myosin light chain (MyLC), and actinin.[9,10] Furthermore, selectivity of MyHC was observed in skeletal muscles from mice bearing colon-26 tumors known to secrete high levels of IL-6. Similar results were found from mice containing CHO tumors expressing a combination of TNFα and IFNγ, or G361 melanoma tumors expressing proteolysis-inducing factor (PIF).[9]

Since this report was published, the Glass laboratory confirmed that MyHC is a bona fide target of MuRF-1.[11] That study nicely showed that in response to dexamethasone treatment, myosin is a predominant muscle protein that is downregulated and can be immunoprecipitated with a MuRF-1 antibody. Importantly, the researchers also provided genetic evidence that silencing MuRF-1 with siRNA or the complete deletion from MuRF-1-deficient mice restored myosin levels in response to dexamethasone treatment in vitro and in vivo, respectively.

This finding was followed up more recently by the Goldberg group, which used a knock in mouse containing a mutant form of MuRF-1 where the RING domain had been deleted.[12] In comparing this to a knock in of a wild-type version of MuRF-1, the group was able to demonstrate conclusively that MuRF-1 activity is required for muscle atrophy in response to denervation. They also elucidated by using a combination of their mouse model and proteomic analysis that in addition to MyHC, other heavy filament proteins such as MyLC and myosin binding protein C (MyBP-C) are downregulated during denervation, and in fact loss of MyLC and MyBP-C precedes the degradation of MyHC.

It had been argued that actinomyosin complexes are insensitive to the ubiquitin proteasome system but can be cleaved by calcium-activated calpain activity, which in turn allows accessibility of the individual filaments to the ubiquitin ligases to mediate their proteasomal degradation. In a revised model, Goldberg and colleagues propose that MuRF-1 is itself capable of ubiquitinating thick filament proteins, MyLC and MyBP-C, thus causing their degradation and leading to the release of the actinmyosin complex and subsequent destruction of MyHC.[12] Since little in vivo evidence indicates that troponin and tropomyosin are susceptible to a similar mechanism of protein turnover, the question remains whether loss of thick filament proteins is sufficient to promote muscle loss in response to an atrophic condition.

The involvement of the NF-κB signaling pathway in skeletal muscle wasting conditions also should be addressed. NF-κB has been increasingly associated with a variety of skeletal muscle disorders, including cachexia, muscular dystrophy, and pediatric muscle cancer rhabdomyosarcoma. Past studies have alluded to the involvement of NF-κB by demonstrating its activation in response to several factors that are themselves tightly linked to muscle wasting. Inflammatory cytokines and tumor factors activate NF-κB through the IkB kinase (IKK) complex that functions to phosphorylate and induce IkB degradation to release NF-κB to the nucleus where it binds its cognate DNA sequences to regulate gene expression. One gene related to the ubiquitin proteasome system that it is thought to be regulated by NF-κB is MuRF-1. Work from the Shoelson group demonstrated that muscle-specific overexpression of a constitutively active form of IKKα leads to severe muscle atrophy.[13] Muscle atrophy was specific to NF-κB because muscles expressing a dominant negative inhibitor of NF-κB were resistant to denervation-induced atrophy. Furthermore, Shoelson and colleagues showed that MuRF-1 was a downstream target of NF-κB since muscle size was restored in IKKβ transgenic mice crossed with MuRF-1 knock out mice.

This work revealed that the classical pathway of NF-κB is a contributing factor in muscle atrophy. In addition to classical signaling, different dimer complexes of NF-κB can be regulated by what is referred to as the noncanonical or alternative pathway. Here, IKKα dimers are activated by different upstream factors (usually related to lymphoid cells), which in turn phosphorylate p100 to cause its proteasomal processing to the mature p52 protein. The resulting p52/RelB complex translocates to the nucleus to regulate a presumably different series of genes than what is controlled through the classical pathway. Deciphering how these respective pathways function in skeletal myogenesis may provide insight for how NF-κB might be involved in various skeletal muscle disorders such as cachexia and muscular dystrophy.[14,15]

Our laboratory recently confirmed by genetic means that the classical pathway functions as a negative regulator of myogenesis.[16,17] Thus, in muscular dystrophy and rhabdomyosarcoma, classical signaling is thought to repress differentiation, thereby limiting the regenerative capacity of satellite cells, or facilitating tumor progression, respectively. In contrast, we find that the alternative pathway is not required for myotube formation, but instead helps maintain homeostasis of mature myotubes. We made this discovery by observing that myotubes expressing the alternative signaling component, IKKα were more resistant to nutrient deprivation than vector control cells. Because myotubes regulate their energy capacity via oxidative phosphorylation, we speculated that the alternative pathway might be a

regulator of mitochondria. Indeed, in recently published findings,[17] we describe that mitochondrial content was increased in C2C12 myotubes expressing IKKα, and remarkably, microarray analysis determined that nearly 50% of the genes regulated by IKKα are related to mitochondrial and metabolic processes.

In more recent unpublished work, we addressed whether alternative pathway regulation of mitochondria is relevant in vivo. First, we found that myogenesis was not affected in differentiating IKKα knockout primary fetal myoblasts, corroborating our results in C2C12 cells. Second, in either primary cells or limb muscles lacking IKKα, we confirmed that mitochondrial markers were markedly reduced. Consistent with these results, we observed that overexpression of IKKa in intact muscles led to an increase in mitochondria content. Taken together, these data support the notion that alternative NF-κB signaling functions in muscle cells in a distinctly different manner from the classical pathway. We believe such information will be vital for increasing our understanding of the mechanisms underlying the regulation of muscle size and balance between the protein synthetic and degradation pathways.

References

1. Glass DJ: Signalling pathways that mediates skeletal muscle hypertrophy and atrophy. *Nature Cell Biol* 2003;5:87-90.

2. Bodine SC, Stitt TN, Gonzalez M, et al: Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. *Nat Cell Biol* 2001;3:1014-1019.

3. Eley HL, Russell ST, Tisdale MJ: Role of the dsRNA-dependent protein kinase (PKR) in the attenuation of protein loss from muscle by insulin and insulin-like growth factor-I (IGF-I). *Mol Cell Biochem* 2008;313:63-69.

4. Tisdale MJ: Mechanisms of cancer cachexia. *Physiol Rev* 2009;89:381-410.

5. Sandri M, Sandri C, Gilbert A, et al: Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy. *Cell* 2004;117:399-412.

6. Stitt TN, Drujan D, Clarke BA, et al: The IGF-1/PI3K/Akt pathway prevents expression of muscle atrophy-induced ubiquitin ligases by inhibiting FOXO transcription factors. *Mol Cell* 2004;14:395-403.

7. Mammucari C, Milan G, Romanello V, et al: FoxO3 controls autophagy in skeletal muscle in vivo. *Cell Metab* 2007;6:458-471.

8. Zhao J, Brault JJ, Schild A, et al: FoxO3 coordinately activates protein degradation by the autophagic/lysosomal and proteasomal pathways in atrophying muscle cells. *Cell Metab* 2007;6:472-483.

9. Acharyya S, Ladner KJ, Nelsen LL, et al: Cancer cachexia is regulated by selective targeting of skeletal muscle gene products. *J Clin Invest* 2004;114:370-378.

10. Guttridge DC, Mayo MW, Madrid LV, et al: NF-κB-induced loss of myoD messenger RNA: Possible role in muscle decay and cachexia. *Science* 2000;289:2363-2366.

11. Clarke BA, Drujan D, Willis MS, et al: The E3 ligase MuRF1 degrades myosin heavy chain protein in dexamethasone-treated skeletal muscle. *Cell Metab* 2007;6:376-385.

12. Cohen S, Brault JJ, Gygi SP, et al: During muscle atrophy, thick, but not thin, filament components are degraded by MuRF1-dependent ubiquitylation. *J Cell Biol* 2009;185:1083-1095.

13. Cai D, Frantz JD, Tawa NE Jr, et al: IKKbeta/NF-κB activation causes severe muscle wasting in mice. *Cell* 2004119:285-298.

14. Peterson JM, Guttridge DC: Skeletal muscle diseases, inflammation, and NF-kappaB signaling: Insights and opportunities for therapeutic intervention. *Int Rev Immunol* 2008;27:375-387.

15. Wang H, Garzon R, Sun H, et al: NF-kappaB-YY1-miR-29 regulatory circuitry in skeletal myogenesis and rhabdomyosarcoma. *Cancer Cell* 2008;14:369-381.

16. Acharyya S, Villalta SA, Bakkar N, et al: Interplay of IKK/NF-kappaB signaling in macrophages and myofibers promotes muscle degeneration in Duchenne muscular dystrophy. *J Clin Invest* 2007;117:889-901.

17. Bakkar N, Wang J, Ladner KJ, Wang H, et al: IKK/NF-kappaB regulates skeletal myogenesis via a signaling switch to inhibit differentiation and promote mitochondrial biogenesis. *J Cell Biol* 2008;180:787-802.

Effects of Nutrition on Muscle Metabolism

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Atrophy of skeletal muscle is common in a number of conditions including cancer sepsis, metabolic acidosis, weightlessness, immobility, diabetes, and AIDS, and can lead to weakness (asthenia) and death through respiratory failure. In cancer patients, an inverse relationship exists between weight loss and survival time. Various nutrients have the ability to attenuate this condition by increasing protein synthesis and/or decreasing protein degradation in skeletal muscle.

Eicosapentaenoic Acid (EPA)

EPA is a 20-carbon (n-3) polyunsaturated fatty acid found in oily fish. EPA was first recognized as a potential treatment for cachexia because of its ability to attenuate weight loss in mice bearing the MAC16 tumor. EPA preserves muscle mass by reducing the increased protein degradation seen in the skeletal muscle of cachectic mice, but it has no effect on the depression of protein synthesis. The increased protein degradation seen in skeletal muscle of both mice and humans with cancer cachexia is due to an increased expression and activity of the ubiquitin proteasome pathway. In this process, myofibrillar proteins, such as myosin, are marked for degradation by proteolytic enzymes in the 20S proteasome by the attachment of a polyubiquitin chain (Fig 1, next page).[1]

In addition to the proteasome, two ubiquitin ligases (E3), muscle-specific RING finger-1 (MuRF-1) and muscle-specific F-box protein (MAFbx/atrogin-1) are important, both in recognition of the target protein and in the transfer of ubiquitin from the ubiquitin-conjugating enzyme (E2). EPA attenuates the enhanced protein degradation seen in skeletal muscle of cachectic mice by decreasing expression of the 20S proteasome and other key components of the pathway down to levels found in noncachectic animals.[2] Thus, EPA is not a proteasome inhibitor but normalizes expression down to basal levels. This is significant because the proteasome has an important function in cellular homeostasis, degrading mutated, misfolded, or oxidized proteins. Proteasome inhibition, therefore, can lead to toxicity, but this is not seen with EPA.

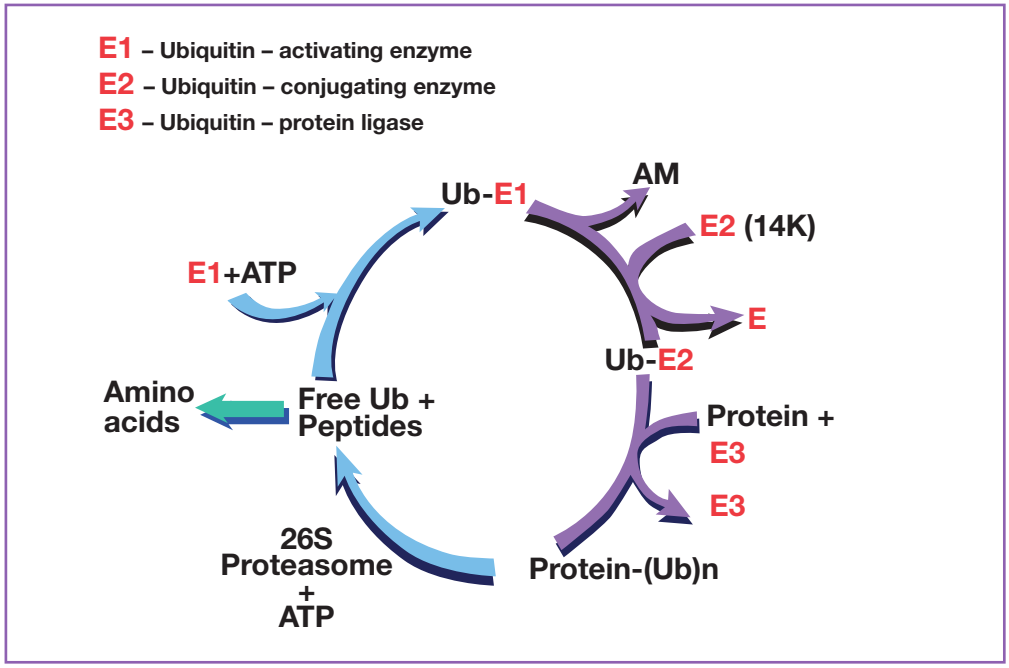


Fig 1. Ubiquitin-proteasome pathway for breakdown of intracellular proteins in skeletal muscle.[1]

From "Loss of skeletal muscle in cancer: Biochemical mechanisms" by Tisdale et al. *Frontiers in Bioscience* 2001;6:164. © 2001 by *Frontiers in Biocscience Publications*. Reprinted with permission.

EPA reduces expression of the ubiquitin-proteasome pathway by interfering with the signaling pathway involved in its upregulation. EPA works by blocking the action of a tumor catabolic factor, proteolysis-inducing factor (PIF), which acts specifically on skeletal muscle to depress protein synthesis and increase degradation. EPA blocks the action of PIF-induced phospholipase A_2, which causes the release of arachidonic acid from membrane phospholipids, and its conversion to prostaglandins and hydroxy-eicosatetraenoic acids (HETE). Only one of these metabolites, 15-HETE, is found to be directly catabolic on muscle by activating nuclear factor-kappaB (NF-κB), which increases expression of both the 20S proteasome and MuRF-1.[3] NF-κB is held as an inactive complex in the cytosol with an inhibitory protein, IκB. On activation of an upstream kinase (IKK) by PIF, possibly through protein kinase C (PKC) or reactive oxygen species (ROS), IκB is phosphorylated and degraded, releasing free NF-κB, which migrates into the nucleus and causes increased gene transcription by binding to its specific sites on DNA (Fig 2). This effect is not seen in the presence of EPA, and NF-κB remains as an inactive complex in the cytosol with IκB.

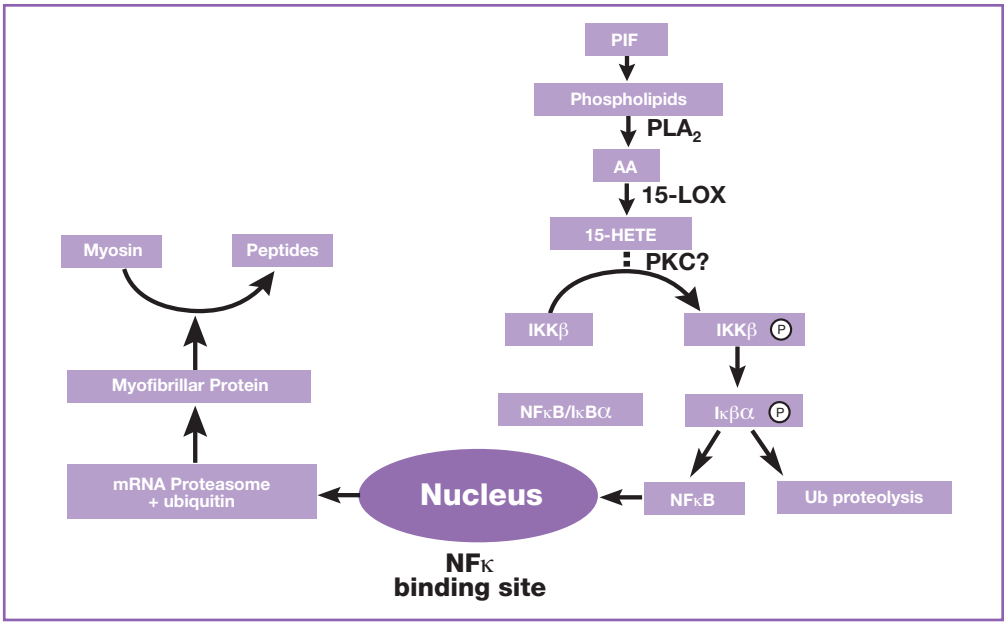


Fig 2. Potential intracellular events in skeletal muscle involved in PIF-induced proteasome activation. PIF=proteolysis-inducing factor, AA=arachidonic acid, PLA_2= phospholipase A2, 15-LOX= 15-lipoxygenase, HETE= hydroxy-eicosatetraenoic acids, PKC= protein kinase C, IKKβ=IkappaB kinase, NF-κB=nuclear factor kappa B, Ub= ubiquitin

Amino Acids

Certain amino acids, namely the branched chain amino acids (BCAA) leucine, isoleucine, and valine are not only substrates for protein synthesis, but also stimulate the process and reduce protein degradation. For this reason, EPA has been combined with a nutritional supplement enriched with both calories and protein that helps prevent muscle atrophy in cachexia, not only by depressing protein degradation, but also by increasing protein synthesis.[4]

Protein synthesis is mainly regulated at the initiation and elongation steps of translation. There are two control points in initiation:

(i) Binding of methionyl tRNA to the 40S ribosomal subunit. This process is regulated by eukaryotic initiation factor 2 (eIF2) and inhibited when eIF2 is phosphorylated on the α-subunit.

(ii) Binding of mRNA to the 43S subunit. This process is stimulated by activation of the mammalian target of rapamycin (mTOR), which phosphorylates the eIF4E binding protein (4EBP1) allowing dissociation of eIF4E, which can then complex with eIF4G to form the active eIF4F complex, allowing binding of the 5'-cap of mRNA.

Leucine and valine, when administered to mice bearing the cachexia-inducing MAC16 tumor, attenuate loss of body weight and increase skeletal muscle mass.[5] Leucine and valine also produce a significant increase in protein synthesis, whereas only leucine produces a decrease in protein degradation in skeletal muscle.[5] Growth of the MAC16 tumor is associated with a significant increase in phosphorylation of eIF2α in gastrocnemius muscle, which reduces protein synthesis. Activation (phosphorylation) of the eIF2α kinase, the dsRNA-dependent protein kinase, PKR, also increases. Treatment with leucine attenuates the increased phosphorylation of both PKR and eIF2α to levels found in nontumor-bearing animals, without affecting total levels.[5] The decreased phosphorylation of PKR is probably due to a 2.5-fold increase in protein phosphatase 1 (PP1). Leucine treatment also increases expression of phospho mTOR, causes hyperphosphorylation of 4E-BP1, allowing the eIF4E to associate with eIF4G to form the active eIF4F complex, which stimulates translation initiation and thus global protein synthesis.[5] Levels of phosphorylation of the eukaryotic elongation factor (eEF2) also increase in skeletal muscle of mice bearing the MAC16 tumor, which results in an inhibition of elongation by decreasing its affinity for the ribosome 10-100 times.[5]

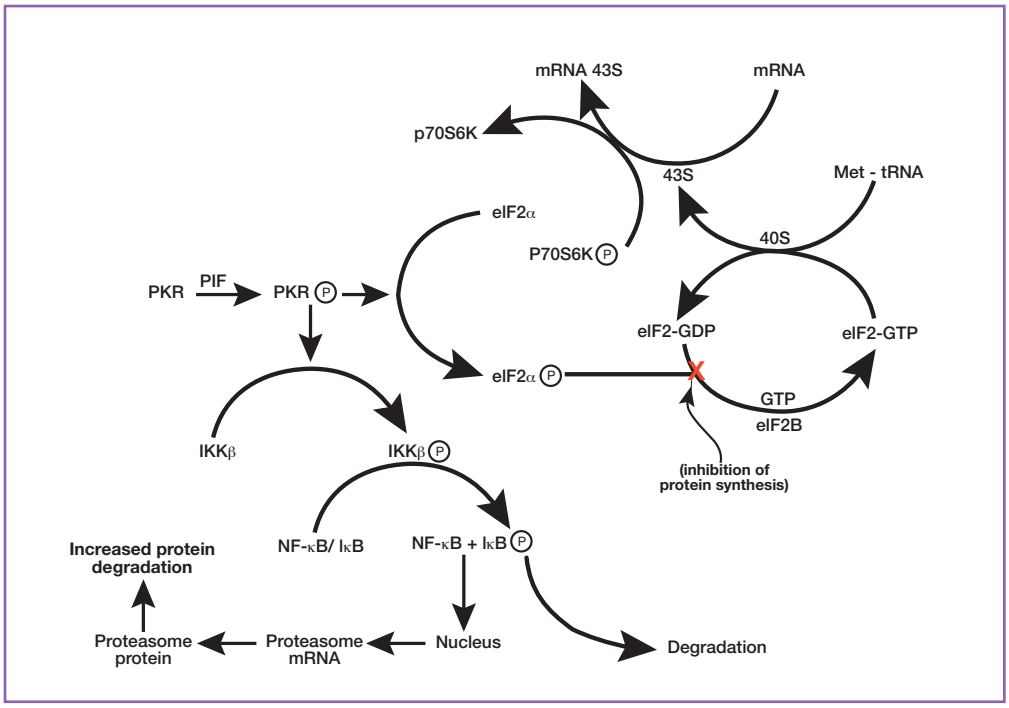


Fig 3. Central role of PKR in the control of protein synthesis and degradation in skeletal muscle.[6] PKR=protein kinase R, PIF=proteolysis-inducing factor, IKKβ=IkappaB kinase, NF-κB=nuclear factor kappa B, eIF2=eukaryotic initiation factor 2, GTP=guanosine-5'-triphosphate

The ability of leucine to attenuate phosphorylation of PKR also explains its ability to reduce protein degradation, since PKR also induces protein degradation through activation of NF-κB by activation of IKK and the subsequent phosphorylation and degradation of I-κB (Fig 3).[6] Increased phosphorylation of both PKR and eIF2α is seen not only in muscles of mice bearing the MAC16 tumor, but also in cancer patients, increasing with increasing weight loss.

β-Hydroxy-β-Methylbutyrate (HMB)

HMB is a metabolite of leucine formed by transamination to α-ketoisocaproate (KIC) in the muscle followed by oxidation of KIC to HMB in the cytosol of the liver (Fig 4).

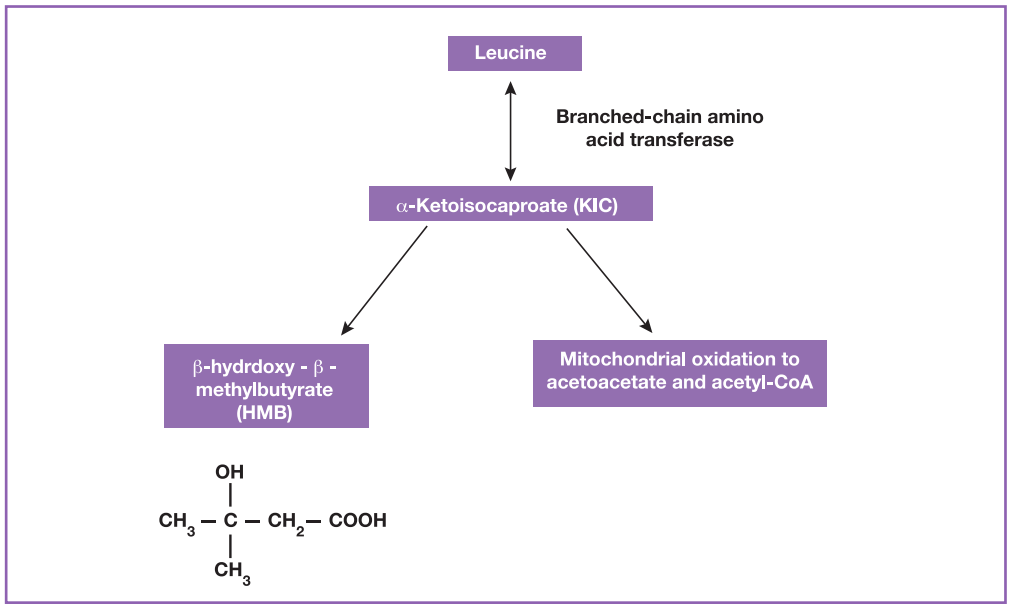


Fig 4. Formation of HMB.

The effect of HMB on muscle metabolism closely resembles that of leucine. Thus, HMB at doses >0.125 g/kg in mice bearing the MAC16 tumor causes a significant reduction in weight loss caused by increased lean body mass without an effect on adipose mass.[7] The increase in muscle mass is caused by both a depression in protein degradation and a significant increase in protein synthesis. As with leucine, HMB attenuates the increased phosphorylation of both PKR and eIF2α in skeletal muscle, supporting its ability to suppress protein degradation, and at the same time increasing protein synthesis. In vitro studies using murine myotubes show that HMB attenuates both the depression of protein synthesis and an increase in protein degradation in response to a number of catabolic stimuli including PIF, angiotensin II (ang II), tumor necrosis factor-α (TNF-α), and lipopolysaccharide (LPS), essentially

by the same mechanism.[8-12] As observed in vivo, all stimuli increase phosphorylation of both PKR and eIF2α, which is completely attenuated by HMB.[8-12] This process is shown to be responsible for the depression of protein synthesis using myotubes transfected with a catalytically inactive variant of PKR called PKRΔ6, which lacks 6 amino acids (361-366) between catalytic domains IV and V. Thus, while LPS and TNF-α depresses protein synthesis on myotubes transfected with empty plasmid (pcDNA) and wild-type PKR, there is no effect in myotubes transfected with PKRΔ6. Protein degradation by all catabolic stimuli also is attenuated by HMB. This is shown to involve the caspase-3 and caspase-8 activation of PKR and the subsequent formation of ROS, which leads to activation of NF-κB (Fig 5).[11]

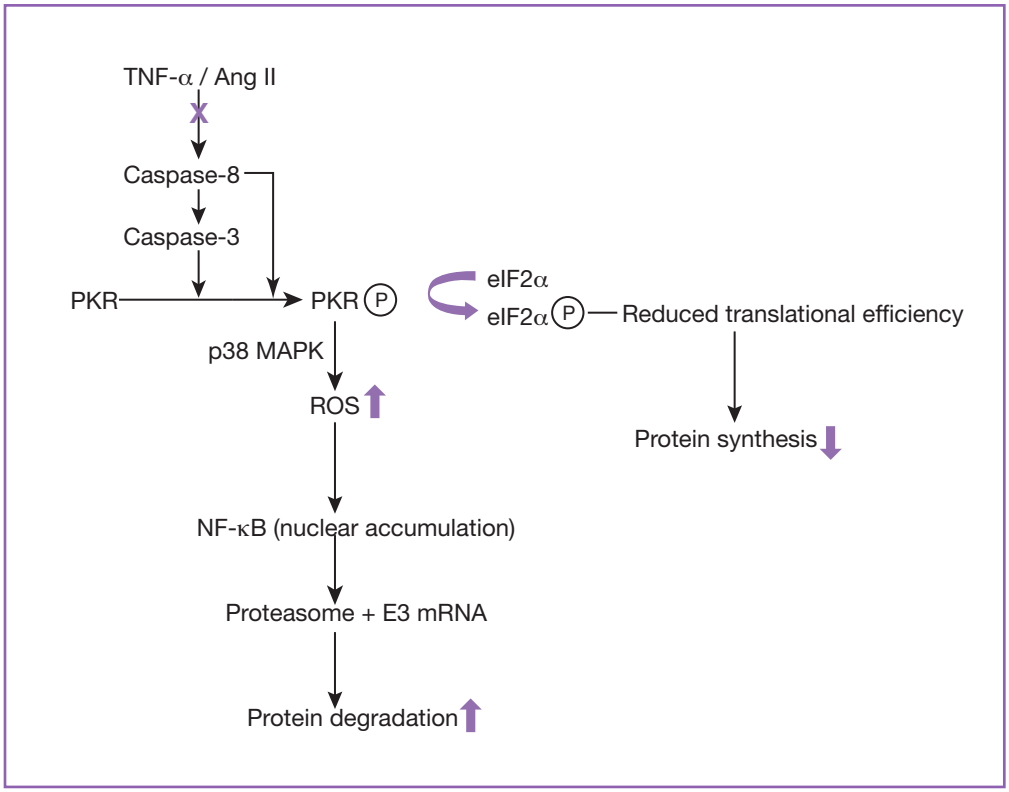


Fig 5. Signaling cascade initiated by LPS/TNF-α/ang II and the effect of HMB on this process.[11] TNF=tumor necrosis factor, ang II=angiotensin II, PKR=protein kinase R, eIF2=eukaryotic initiation factor 2, MAPK=mitogen-activated protein kinase, ROS= reactive oxygen species, NF-κB=nuclear factor kappa B, LPS= lipopolysaccharide.

From "Mechanism of attenuation of muscle protein degradation by tumour necosis factor-a and angiotensin II by β-hydroxy-β-methylbutyrate" by Eley et al. *American Journal of Physiology – Endocrinology and Metabolism* 2008;295(6): E1417. © 2008 by American Physiological Society. Reprinted with permission.

Thus, all stimuli increase activity of both caspase-3 and caspase-8, and this is attenuated by HMB. This attenuation is important in protein degradation because both the caspase-3 and caspase-8 inhibitors attenuate protein degradation and autophosphorylation of PKR. Activation of PKR is also important in protein degradation, because, as with protein synthesis, it is not seen in myotubes transfected with PKR 6. Thus, HMB blocks the depression of protein synthesis and increased protein degradation induced by catabolic stimuli by inhibiting an upstream signaling pathway (activation of caspase-3/-8) leading to activation of PKR. The similarity in the signaling pathway employed by a range of catabolic stimuli suggests that EPA, BCAAs, and HMB may be effective in the treatment of other catabolic disorders in addition to cancer cachexia.

References

1. Tisdale MJ: Loss of skeletal muscle in cancer: Biochemical mechanisms. *Front Biosci* 2001;6:164-174.
2. Whitehouse AS, Smith HJ, Drake JL, Tisdale MJ: Mechanism of attenuation of skeletal muscle protein catabolism in cancer cachexia by eicosapentaenoic acid. *Cancer Res* 2001;61:3604-3609.
3. Cai D, Frantz JD, Tawa NE Jr, et al: IKK/NF-κB activation causes severe muscle wasting in mice. *Cell* 2004;119:285-298.
4. Smith HJ, Greenberg NA, Tisdale MJ: Effect of eicosapentaenoic acid, protein and amino acids on protein synthesis and degradation in skeletal muscle of cachectic mice. *Br J Cancer* 2004;91:408-412.
5. Eley HL, Russell ST, Tisdale MJ: Effect of branched-chain amino acids on muscle atrophy in cancer cachexia. *Biochem J* 2007;407:113-120.
6. Eley HL, Skipworth RJE, Deans DAC, et al: Increased expression of phosphorylated forms of RNA-dependent protein kinase and eukaryotic initiation factor 2alpha may signal skeletal muscle atrophy in weight-losing cancer patients. *Br J Cancer* 2008;98:443-449.
7. Smith HJ, Mukherji P, Tisdale MJ: Attenuation of proteasome-induced proteolysis in skeletal muscle by β-hydroxy-β-methyl-butyrate in cancer-induced muscle loss. *Cancer Res* 2005;65:277-283.
8. Eley HL, Russell ST, Baxter JH, et al: Signaling pathways initiated by β-hydroxy-β–methylbutyrate to attenuate the depression of protein synthesis in skeletal muscle in response to cachectic stimuli. *Am J Physiol Endocrinol Metab* 2007;293:E923-E931.
9. Smith HJ, Wyke SM, Tisdale MJ: Mechanism of the attenuation of proteolysis-inducing factor stimulated protein degradation in muscle by β-hydroxy-β methylbutyrate. *Cancer Res* 2004;64:8731-8735.
10. Eley HL, Russell ST, Tisdale MJ: Attenuation of depression of muscle protein synthesis induced by lipopolysaccharide, tumor necrosis factor, and angiotensin

II by β-hydroxy-β-methylbutyrate of muscle protein degradation induced by lipopolysaccharide. *Am J Physiol Endocrinol Metab* 2008;295:E1409-E1416.

11. Eley HL, Russell ST, Tisdale MJ: Mechanism of attenuation of muscle proteindegradation by tumour necrosis factor-a and angiotensin II by β-hydroxy-β-methylbutyrate. *Am J Physiol Endocrinol Metab* 2008;295:E1417-E1426.
12. Russell ST, Tisdale MJ: Mechanism of attenuation by β-hydroxy-β-methylbutyrate of muscle protein degradation induced by lipopolysaccharide. *Mol Cell Biochem* 2009;330:171-179.
13 Barber MD, Ross JA, Voss AC, et al: The effect of an oral nutritional supplement enriched with fish oil on weight loss in patients with pancreatic cancer. *Br J Cancer* 1991;81:80-86.
14. Fearon KCH, von Meyenfeldt MF, Moses AGW, et al: Effect of a protein and energy dense n-3 fatty acid enriched oral supplement on loss of weight and lean tissue in cancer cachexia: A randomized double-blind trial. *Gut* 2003;52:1479-1486.
15. Tisdale MJ, Beck SA: Inhibition of tumour-induced lipolysis in vitro and cachexia and tumour growth in vivo by eicosapentaenoic acid. *Biochem Pharmacol* 1991;41:103-107.
16. Wigmore SJ, Barber MD, Ross JA, et al: Effect of oral eicosapentaenoic acid on weight loss in patients with pancreatic cancer. *Nutr Cancer* 2000;36:177-184.

Q & A

Q: You gave a good presentation using the mouse model. Have you used EPA in human cancer patients and if so, have you shown that protein degradation decreases?

Dr Tisdale: We have done several studies in collaboration with Abbott Nutrition in patients with cancer cachexia [Barber MD et al: *Br J Cancer* 1991;81:80-86; Fearon KCH et al: *Gut* 2003;52:1479-1486]. We know that lean body mass is directly related to the amount of EPA in the serum of the patient. There is a linear relationship suggesting that EPA increases lean body mass [Fearon KCH et al: *Gut* 2003;52:1479-1486]. However, we did not measure protein synthesis and degradation in cancer patients, mainly because this was a large multicenter study. It also was difficult to do those measurements on patients who do not have long to live. However, it would be good to use these agents for such a study in the future.

Q: You probably are aware that a major class of chemotherapy drugs targets mTOR. Sorafenib is the model compound. I am interested in the obvious prediction that this class of drug would cause muscle wasting because it has a powerful catabolic effect on muscle. I am writing up a randomized placebo-controlled study of nutritional intervention in patients with renal cell carcinoma who are on sorafenib. If mTOR is important, these patients are not going to respond to any intervention. They have a fundamental block in their ability to utilize those nutrients and to direct amino acids toward protein synthesis. I think this is a critical point: Do we not need to know whether there are instances in which these stimuli cannot produce muscle anabolism?

Dr Tisdale: I think you are correct. If you are treating a patient with cancer who is losing muscle but increasing tumor, then the two processes are diametrically opposite. Therefore, if you have an agent that prevents the tumor from growing, you might have an agent that also stimulates muscle loss because of a hypotrophy pathway. Treatment in that condition depends on several factors. Leucine, for instance, has an effect on PKR phosphorylation, which would be part of the process. But we do not know the relative weightings of mTOR and PKR. If that pathway is still operative, it may be possible to treat these patients even when they are being treated with a drug that inhibits mTOR. I cannot guess whether it will work, but it might be worth looking into.

Q: Going back to the EPA question, do you think there is a specific effect on muscle mass from the EPA, or would you have a similar effect if you gave docosahexaenoic

acid (DHA) instead? Do you think that the replacement of arachidonic acid produces that effect, or is it just an effect from EPA or omega-3 fatty acids?

Dr Tisdale: In our mouse studies [Tisdale MJ, Beck SA: *Biochem Pharmacol* 1991;41:103-107], we initially found that only EPA had that effect. DHA did not. Although there has not been a clinical study with DHA alone, a study with EPA alone showed it had the same anticachectic effect as fish oil [Wigmore SJ et al: *Nutr Cancer* 2000;36:177-184]. So either DHA did not have an effect on patients, or it did not have any more effect than you might expect from the EPA alone.

The effect of EPA is quite complex, and like any signaling pathway, there is crossover. I tried to make the story as simple as possible by saying that EPA inhibits formation of 15-HETE. However, it also inhibits the 15-HETE-induced activation of NF-κB, so it seems to act separately on the IKK cascade as well. So it probably has more than one point within the signaling pathway in which it can act. The signaling pathway is also a lot more complicated than the process I first described, so it is possible to connect by multiple mechanisms, ie, between the NF-κB and FoxO pathways. This may explain the efficacy of EPA. If the effect was just the result of replacement of arachidonic acid, DHA would be equally effective, but in our studies it was not.

Role of Oxidative Stress in Skeletal Muscle Weakness

Michael Reid, PhD, University of Kentucky Medical Center, Lexington, KY

Introduction

Our laboratory has a longstanding interest in the weakness of respiratory and limb skeletal muscle caused by reactive oxygen species (ROS). Our current experimental model (Fig 1) proposes that ROS production by muscle fibers is increased in a variety of physiological and pathophysiological conditions. These include chronic inflammation, aging, mechanical unloading, and strenuous exercise.

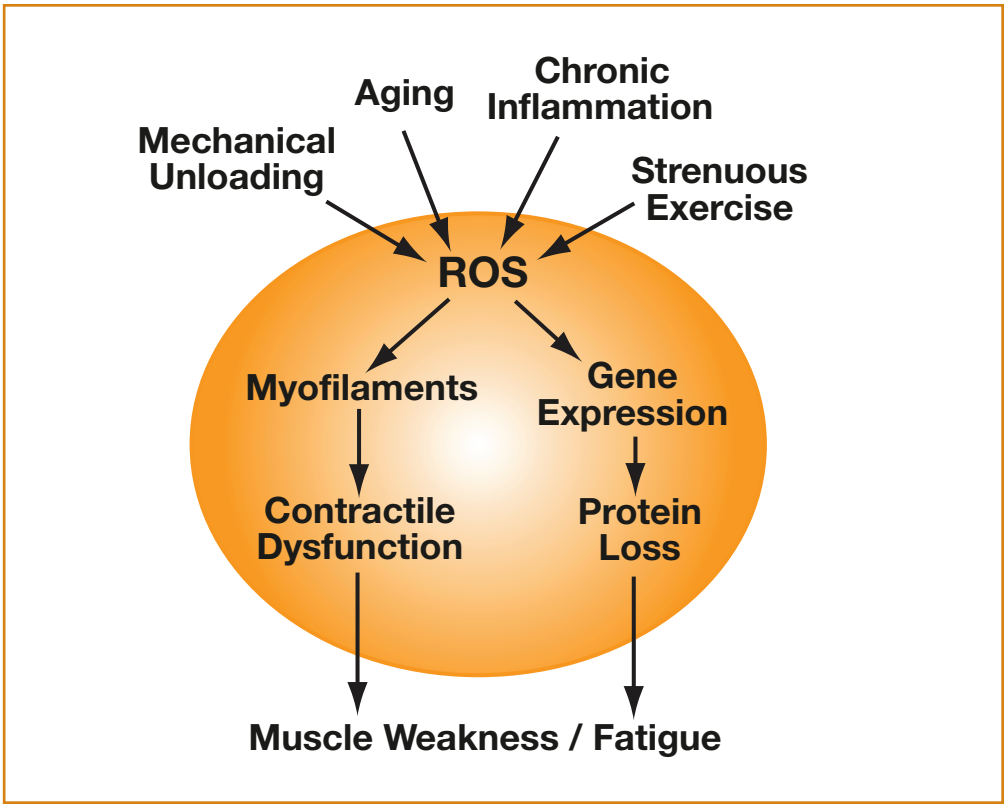


Fig 1. General model of ROS-induced weakness. ROS=reactive oxygen species

Increases in intracellular ROS activity can promote muscle weakness and fatigue via two parallel pathways. First, long-term elevation of ROS activity can act via redox signaling mechanisms to alter muscle gene expression, causing protein loss that diminishes muscle mass ("atrophy"). Second, ROS also can act via post-translational mechanisms to modify constitutively expressed proteins, causing contractile dysfunction that decreases force per cross-sectional area ("specific force"). These two pathways appear to be regulated independently and can have separate or additive effects on mechanical function.

Atrophy-Induced Weakness

As reviewed elsewhere,[1] chronic inflammatory diseases increase ROS activity, causing oxidative stress. This is documented clinically in diseases that range from Duchenne's dystrophy to chronic obstructive pulmonary disease, and from rheumatoid arthritis to cancer. Oxidative stress is commonly associated with changes in antioxidant capacity of the affected tissues and is linked to elevated levels of cytokines, chemokines, and other circulating markers of inflammation. Patients experience muscle wasting in almost all chronic inflammatory conditions. The prevalence approaches 100% in many diseases and inevitably causes weakness.

Proinflammatory cytokines are likely to function as endocrine mediators in chronic disease, stimulating ROS production and muscle atrophy via redox-activated mechanisms. Cytokines in this category include interferon-γ (IFNγ), interleukin-1 (IL-1), and tumor necrosis factor (TNF). Among these, TNF is most likely to trigger ROS-mediated atrophy. Cell culture studies show that TNF acts directly on differentiated muscle cells to stimulate cytosolic ROS activity.[2] In contrast, oxidant activity is unaffected by exposure to IFN, IL-1, interleukin-6, or c-reactive protein.[3,4] TNF administration also stimulates loss of muscle protein, both in cultured muscle cells[2] and in experimental animals.[5] In patients with chronic disease, elevated serum TNF levels are strongly correlated with muscle atrophy and peripheral weakness. Muscle atrophy appears to be caused by the rise in oxidant activity since administration of exogenous antioxidants can blunt muscle loss in TNF-treated animals.[5]

Studies of muscle wasting during chronic inflammation have focused largely on regulation of proteolysis. Muscle proteins are disassembled and degraded via the coordinated actions of multiple parallel mechanisms. Dissociation and release of proteins from the myofibrillar lattice likely are caused by the actions of selective proteolytic enzymes, ie, calpains and caspases. Degradation of muscle protein occurs through autophagy and, to a greater extent, the ubiquitin-proteasome

pathway. The latter pathway (Fig 2) includes three classes of regulatory E-proteins that interact to sequentially activate and conjugate ubiquitin to specific substrate proteins. Ubiquitin accumulation targets the doomed substrate for proteolysis via the 26S-proteasome.

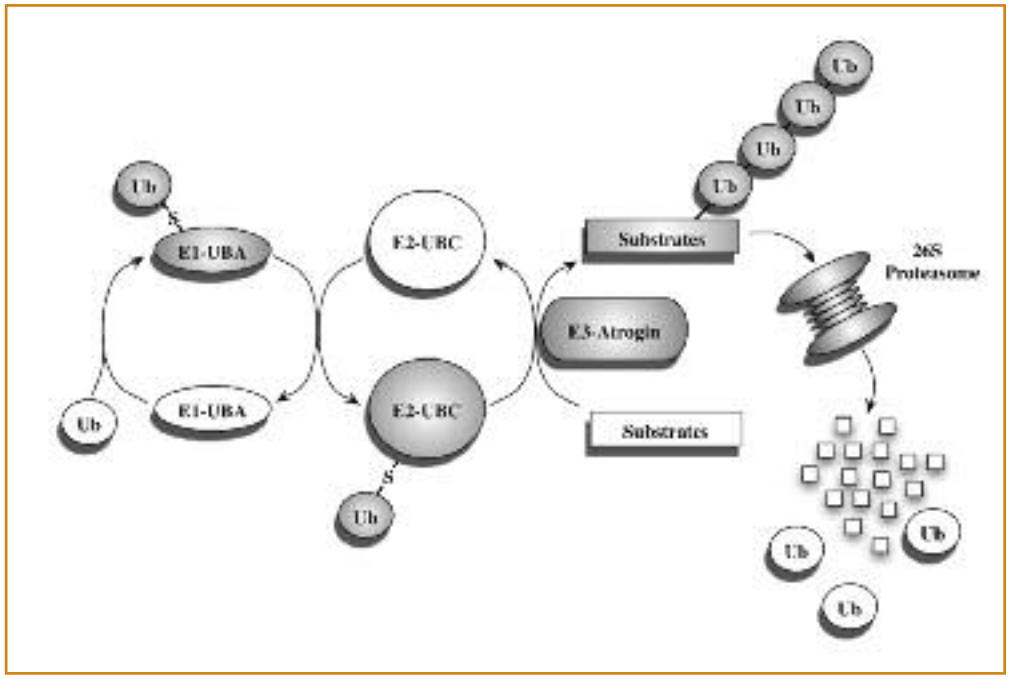


Fig 2. The ubiquitin proteasome pathway.[6] Ubiquitin monomers (Ub) are activated by the E1 or ubiquitin-activating protein (E1-UBA), transferred to the E2 or ubiquitin-conjugating protein (E2-UBC), and attached to substrate proteins in cooperation with E3 proteins such as atrogin1/MAFbx (E3-atrogin); repetition of this process creates ubiquitin polymers that trigger substrate degradation by the 26S proteasome.

From "Response of the ubiquitin-proteasome pathway to changes in muscle activity" by Reid MB. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology* 2005;288:R1423.

Muscle exposure to either TNF or ROS increases activity of the ubiquitin-proteasome pathway. This rise in pathway activity is regulated primarily at the transcriptional level. Genes that code for ubiquitin, E-proteins, and proteasome subunits are upregulated in response to TNF or ROS exposure. These include genes for muscle-specific E2 proteins (E2-14k and UbcH2/E2-20k) and E3 proteins (E3α, atrogin1/MAFbx, and muscle-specific RING finger-1 [MuRF-1]) that are strongly associated with muscle catabolism. E2 and E3 proteins determine specificity of the ubiquitin-proteasome pathway. They are rate-limiting elements of the pathway, determining the maximal rate of ubiquitin conjugation to substrates. Increased expression of E2 and E3 proteins can influence the composition of proteolyzed

substrates and can accelerate the overall rate of proteasomal degradation, favoring net loss of muscle protein.

The signal transduction mechanisms that mediate this response are best understood for two muscle-specific E3s that strongly influence muscle atrophy. Atrogin/MAFbx expression is regulated positively by the Forkhead-O (FoxO) family of transcription factors. FoxO is tonically inhibited by Akt in muscle and other cell types. FoxO signaling is stimulated by TNF or ROS exposure and by other metabolism-associated stimuli. Another positive regulator of atrogin1/MAFbx is p38 mitogen-activated protein kinase (p38 MAPK), an essential mediator of gene expression in response to TNF, which also is activated by ROS exposure.[7] MuRF-1 is a second atrophy-associated E3 in muscle. Like atrogin1/MAFbx, MuRF-1 expression is positively regulated by FoxO. MuRF-1 is also sensitive to nuclear factor- B (NF-κB), a transcription factor that is activated by inflammatory mediators, including TNF and ROS.[8]

Contractile Dysfunction

The weakness experienced by individuals with chronic inflammatory disease is not a simple function of muscle atrophy. Loss of force can exceed loss of muscle. This reflects contractile dysfunction of the remaining muscle, which generates less specific force than healthy muscle. This is observed in pathophysiological states that include chronic heart failure, cancer, COPD, and the sarcopenia of aging.

Serum TNF levels are elevated in these same diseases, a common denominator that may be responsible for contractile dysfunction. TNF depresses specific force in a variety of experimental preparations. These include animals injected with TNF,[9] transgenic mice engineered for cardiac overexpression of TNF,[10] and wild-type muscle preparations incubated with TNF in vitro.[10] This is associated with a rise in cytosolic oxidant activity[10] that appears to be essential for the fall in specific force. Pretreatment with antioxidants preserves specific force of muscle exposed to TNF either in vitro[10] or in vivo.[9] Interestingly, post-hoc incubation with an antioxidant can partially reverse dysfunction after prolonged TNF exposure.[10]

The intracellular target of TNF-stimulated oxidants appears to be myofibrillar proteins. In intact muscle fibers, TNF depresses specific force of tetanic contractions without altering cytosolic calcium transients or resting calcium concentration.[11] More directly, specific force is depressed in skinned fibers isolated from muscles of TNF-treated animals.[9] Activating calcium levels are controlled under these experimental conditions, clearly demonstrating dysfunction of the myofibrillar lattice.

The cellular mechanism by which TNF stimulates contractile dysfunction is beginning to emerge. We know the response is mediated via the TNF receptor subtype 1 (TNFR1) because TNF administration does not cause dysfunction in TNFR1-deficient mice.[9] Fig 3 illustrates the major post-receptor events that remain undefined.

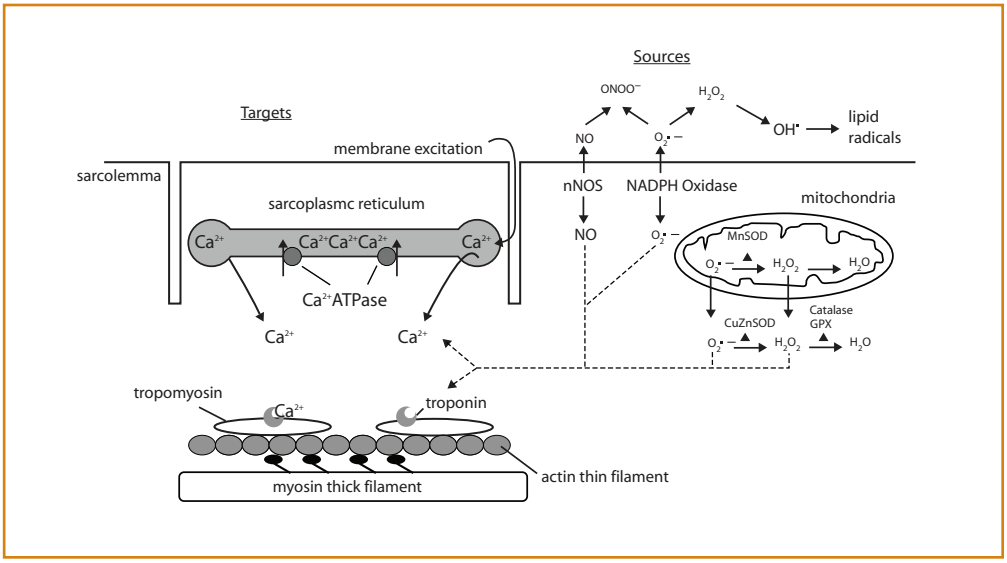


Fig 3. Oxidant sources and targets in muscle fibers.[12] ONOO– =peroxynitrite, H2O2=hydrogen peroxide, NO=nitric oxide, H2O=water, OH•=hydroxyl radical, O2•– =superoxide anion, NADPH=nicotinamide adenine dinucleotide phosphate, Ca2+=calcium, MnSOD=manganese superoxide dismutase, CuZnSOD=copper-zinc superoxide dismutase, ATP= adenosine triphosphatase, GPX=glutathione peroxidase.

One issue is the composition and source of TNF-stimulated oxidants. Skeletal muscle fibers contain multiple ROS sources, including the mitochondrial electron transport chain and a sarcolemmal NADPH oxidase complex. The source of oxidants stimulated via TNFR1 activation has not been tested in skeletal muscle. Further, fast-type muscle fibers constitutively express both the neuronal and endothelial isoforms of nitric oxide (NO) synthase, ie, nNOS and eNOS. NO derivatives are detectable in the sarcoplasm of rodent muscle fibers and have not been ruled out as possible co-mediators of TNF-stimulated dysfunction.

A second issue is the protein chemistry of myofibrillar dysfunction. What proteins are affected and what modifications compromise function? Regulatory proteins of the myofibrillar lattice that influence force production include actin, myosin heavy chain, myosin light chain, tropomyosin, and the troponin complex. Each is subject

to post-translational modifications that diminish force and is a potential target of TNF-stimulated oxidants. Oxidants may act directly on proteins to stimulate carbonylation, nitration, sulfhydryl oxidation, and formation of nitrotyrosine or 4-hydroxy-2-nonenol adducts. Oxidants also may act indirectly via redox-sensitive regulatory proteins. For example, oxidizing conditions might alter the activity of a specific kinase or phosphatase and thereby disrupt the phosphorylation state of myofibrillar proteins. These issues remain unresolved, emphasizing the potential value of future research into the cellular mechanism of inflammation-induced dysfunction.

Summary

The weakness that commonly occurs in chronic inflammatory disease appears to be mediated by circulating proinflammatory mediators. The best recognized is TNF, which acts via the TNFR1 complex to increase cytosolic ROS activity in muscle fibers and cause weakness via two processes. Muscle atrophy is linked to increases in procatabolic signaling, E3 upregulation, ubiquitin conjugation, and protein loss. Contractile dysfunction is caused by ROS-mediated modifications to myofibrillar proteins that decrease specific force. Atrophy and dysfunction are parallel processes that appear to be largely independent. Both are attractive targets for nutritional or pharmacologic interventions to preserve muscle function in patients with chronic inflammatory disease.

References

1. Moylan JS, Reid MB: Oxidative stress, chronic disease, and muscle wasting. *Muscle Nerve* 2007;35:411-429.
2. Li YP, Schwartz RJ, Waddell ID, et al: Skeletal muscle myocytes undergo protein loss and reactive oxygen-mediated NF-kappaB activation in response to tumor necrosis factor alpha. *Faseb J* 1998;12:871-880.
3. Li W, Moylan JS, Chambers MA, et al: Interleukin-1 stimulates catabolism in C2C12 myotubes. *Am J Physiol Cell Physiol* 2009;297:706-714.
4. Smith MA, Moylan JS, Smith JD, et al: IFN-gamma does not mimic the catabolic effects of TNF-alpha. *Am J Physiol Cell Physiol* 2007;293:1947-1952.
5. Buck M, Chojkier M: Muscle wasting and dedifferentiation induced by oxidative stress in a murine model of cachexia is prevented by inhibitors of nitric oxide synthesis and antioxidants. *EMBO J* 1996;15:1753-1765.
6. Reid MB: Response of the ubiquitin-proteasome pathway to changes in muscle activity. *Am J Physiol Regul Integr Comp Physiol* 2005;288:1423-1431.
7. Li YP, Chen Y, John J, et al: TNF-alpha acts via p38 MAPK to stimulate expression of the ubiquitin ligase atrogin1/MAFbx in skeletal muscle. *Faseb J* 2005;19:362-370.

8. Moylan JS, Smith JD, Chambers MA, et al: TNF induction of atrogin-1/MAFbx mRNA depends on Foxo4 expression but not AKT-Foxo1/3 signaling. *Am J Physiol Cell Physiol* 2008;295:986-993.
9. Hardin BJ, Campbell KS, Smith JD, et al: TNF-alpha acts via TNFR1 and muscle-derived oxidants to depress myofibrillar force in murine skeletal muscle. *J Appl Physiol* 2008;104:694-699.
10. Li X, Moody MR, Engel D, et al: Cardiac-specific overexpression of tumor necrosis factor-alpha causes oxidative stress and contractile dysfunction in mouse diaphragm. *Circulation* 2000;102:1690-1696.
11. Reid MB, Lannergren J, Westerblad H: Respiratory and limb muscle weakness induced by tumor necrosis factor-alpha: Involvement of muscle myofilaments. *Am J Respir Crit Care Med* 2002;166:479-484.
12. Smith MA, Reid MB: Redox modulation of contractile function in respiratory and limb skeletal muscle. *Respir Physiol Neurobiol* 2006;151:229-241.

Q & A

Q: Interestingly, you shared the results of Akt activation by TNF. Two years ago, we showed that PIF had the same effect. However, we had problems getting the referees to accept that we could get activation of Akt. I think they forgot that this is up to about 2 hours: Within the first 2 hours of adding the catabolic stimulus to muscle, a whole new set of proteins was synthesized. That is, we showed that if we added cycloheximide, we did not get protein degradation because a lot of protein is synthesized. So it is not surprising that anabolic signals are increased at the same time as catabolic signals. Can you comment?

Dr Reid: I was fascinated by your data because with PIF the responses are hours out. The signaling events we are looking at occur within minutes. We see an increase in Akt phosphorylation within 15 minutes or so of our TNF stimulus. In our hands, the MAPKs, Akt, Fox0 responses are a little bit later, but NF-κB activation happens within the first 5 to 30 minutes.

Q: We see most of those, including ROS production, within 15 minutes, but Akt lasted longer.

Discussion

Leader: Jeffrey Baxter, PhD, Abbott Nutrition, Columbus, OH

Dr Baxter: I think everyone in this room would agree that skeletal muscle is important for a lot of reasons that have nothing to do with moving around. Since skeletal muscle status plays a role in the pathology, morbidity, and mortality of a number of different diseases, it is a key target for Abbott Nutrition. That is why we sponsored this conference, and why we invited you to be here. Skeletal muscle status is probably amenable to alterations of diet as well as exercise, and so I will open the floor to any questions or comments on this topic.

Dr Supinski: Dr Guttridge, two hypotheses were proposed to account for force loss in muscle in response to activation of proteolytic pathways. The first hypothesis is that caspase and calpain are initially activated and play a key role in dissembling the myofibrillar lattice. The second proposal, from Goldberg's lab, is that E3 components of the proteasomal system (eg, atrogin) enter the myofibrillar lattice and attach to contractile proteins, including myosin, initiating lattice disruption.

Do you think that, if the Goldberg hypothesis is true, it would be consistent with the finding that you do not see much loss of actin in your model? On the other hand, other researchers report that there is actin degradation in a variety of common chronic diseases, including diabetes and uremia. Which of these theories is correct? Is it also possible that these two processes work in combination? How do you fit all of this together?

Dr Guttridge: First, back to your point about actin. I am often confused about actin because I see the 14 kilodalton cleavage, but that does not really explain its total degradation. That could explain a dysfunction of actin. That is why I ask, what about the other sarcomeric proteins? Does actin get degraded later on in either a denervation model or a cancer cachexia model? We do not know. As in Goldberg's model, perhaps you first degrade the thick filament proteins, and then over time, maybe out of default, actin, tropomyosin, and troponin also become substrates and become degraded. But I do not consider that complete actin degradation in those models you suggested. I consider it as a cleavage product that may cause actin dysfunction but does not completely clear actin out of the cell.

I hope that your research group and other groups can continue on with this because I was fascinated by the work of Goldberg's lab. I think it opens up the door to

understanding that both play a role. Or perhaps there is a greater role for the calpain system in sepsis, or for the caspase system versus a denervation model.

Dr Supinski: My research team is interested in this. We find that at 2 days after the induction of sepsis, diaphragm muscle force has gone down a great deal. Administration of an inhibitor of calpain or caspase prevents force loss, but we have found that administration of proteasomal inhibitor does not at all prevent reductions in muscle-specific force generation. We have tested virtually every proteasomal inhibitor we could get our hands on, and none of them have prevented loss of specific muscle force generation. One limitation to our measurements is that they are performed early after the induction of sepsis, at which point calpain and caspase activation may be the major factors influencing contractile-protein-lattice degradation.

It is possible that in more chronic inflammatory states (eg, prolonged infections or other inflammatory stimuli) that selective myosin loss may become progressively a more important issue, and this latter phenomenon may represent a targeted proteasomally mediated selective degradation of myosin.

Dr Guttridge: Right. The reason I brought this up is because of examples from your publication, because I do think we are trying to understand whether there are common themes in different types of catabolic states. It would be nice if these mechanisms overlapped. Then we could find the nodal points, target those, and maybe have an effect on sepsis, cancer, or diabetes. However, I think your paper points out that specificities may exist such that, in a very acute condition, this may be regulated better by the caspase activity, and that in that short window, a role for the proteasome may not exist in a denervation model over 10 to 14 days. The Goldberg study shows that there may be a role for the ubiquitin-proteasome system, or they may be acting in concert in some way.

Dr Supinski: I should mention that we recently have found that caspase does degrade myosin and degrades it fairly well. I do not think anyone else has reported that.

Dr Baracos: I would like to throw out a couple of thoughts. One is that the animal models some of us use could be described as caricatural, or at least very specific scenarios. One person here prefers to work in cell culture because these models are so complicated. I wonder how we can get from that generalized view to where we might predict the clinical efficacy of a new nutritional formulation. I see us as being a great distance from that.

Another thought is that I have heard about three classes of essential nutrients just now as implicated in muscle wasting. One of them is n-3 polyunsaturated fatty acids, which are required in the dietary supply. Another one is branched-chain amino acids, which are essential nutrients of which leucine is prominently discussed. The last one is antioxidant nutrients. I do not see a convenient way to go forward, because I do not believe I have an answer to the question of which one of these nutrients is likely to be first-limiting. If I am going to start feeding someone n-3 polyunsaturated fatty acids, do I know whether that person has sufficient branched-chain amino acids and antioxidants to permit the anabolism to happen? Is there a credible basis for tossing all three of those things into a cocktail and saying, one is good, two are good, three are good, but all three of them are better.

Dr Tisdale: Without doing the requisite experiments, it is difficult to say, but the n-3 fatty acids only inhibit protein degradation in skeletal muscle and have no effect on protein synthesis. If you want a combination that promotes protein synthesis, you have to have something like a branched-chain amino acid combination. You already have two of your three.

Regarding antioxidants, we found that production of reactive oxygen species (ROS) is part of the signaling cascade leading to activation of nuclear factor kappa B (NF-κB) and, therefore, protein degradation. Eicosapentaenoic acid (EPA) may have the same effect. We may be able to have an antioxidant that is superior to that. We need either two or three in various combinations. But until we do the requisite experiments using the most appropriate animal model for the condition we are studying, then it is impossible to say.

Dr Johnson: Dr Tisdale, you described some nice data on reversal of muscle wasting with EPA and β-hydroxy-β-methylbutyrate (HMB). Can you hypothesize what the effect of those nutrients on the model of sarcopenia, disuse, or injury would be?

Dr Tisdale: The catabolic stimuli we have used—tumor necrosis factor (TNF), lipopolysaccharide (LPS), angiotensin II, and proteolysis-inducing factor (PIF)—all seem to go down the same pathway. If you can tell me which of those four stimuli are involved in the catabolic stimulus in those disease models, I can answer your question. At the moment, however, I do not know which, if any, of them are important in the conditions you mentioned. Do you know?

Dr Johnson: I do not know right now, but we will hear more about sarcopenia and some of its etiology later.

Discussion

Dr Tisdale: The more I work in the film field, the more I think that one central pathway is involved in all catabolic stimuli. Therefore, agents that are effective in one type of muscle wasting will probably be effective in all of them. At the moment, that is more of a hypothesis than a fact. I can say that we have never done any experiments that showed a signaling pathway that was not inhibited by the same type of inhibitors that we had previously used. That is not to say that we will not find one.

Dr Supinski: A previous report said that the researchers used a proteasome inhibitor in an animal with heart failure, and it improved the animal's condition. We used the same agent in sepsis, and it made things worse.

Dr Tisdale: The problem with proteasome inhibitors is that they tend to be toxic. Therefore, if you are comparing two conditions, you should compare doses and agents, because if you knock out all of the proteasome you are likely to get a very toxic effect.

Dr Supinski: We used a variety of doses. None of our doses were beneficial, and the higher the dose the more toxicity we observed. In contrast, the team that demonstrated a beneficial effect of proteasomal inhibitor used relatively high doses and found they were beneficial.

Ultimately, I think we may come up with a variety of treatments, such as antioxidants, EPA, and other drugs, that we could reasonably give to people when they are first sick to try to stop muscle damage. I wonder, however, whether the same agents will reverse this damage after it has occurred, or whether other agents will be needed to reverse dysfunction. So I wonder whether in the long run, we will have two classes of therapeutic agents—one that prevents dysfunction, such as EPA in an inflammatory state, and another that restores muscle function.

Dr Tisdale: Clinical data support what you are saying because bortezomib has been tested in patients with cancer cachexia and shown to have no beneficial effect. With regard to regaining muscle mass, we do not know what happens to the anabolic signaling pathways, because those can be switched off mainly by phosphorylation and switched on by dephosphorylation. We do not know in these patients what the state regulation of these pathways is. Until we know that, we do not know whether we can build up muscle mass. But we could consider a process of catabolism to start, followed by defective anabolism. We may need to use agents that can stimulate anabolism in patients, whether they be branched-chain amino acids, exercise, or something else. It would be interesting to have biopsies from these patients to see the state of regulation of the pathways and whether this is a permanent or self-perpetuating change.

Dr Supinski: From what we know, it seems that it is self-perpetuating, but I have no idea what that mechanism could be. We hope that mechanism will be determined soon. I think this is a major issue. I know that ARDSnet, which is a national organization looking at outcomes in patients with acute respiratory distress syndrome (ARDS), has come to the same conclusion. The association is trying to figure out what to do next. ARDSnet thinks mortality from the acute events is reduced fairly well, but the problem is that the long-term morbidity in weak patients is tremendous. We can understand how TNF, LPS, or PIF acutely injures a muscle, but we do not understand why these people are still weak 1 year later.

Dr Reid: Dr Baracos, I would like to go back to your question about the systems and experimental approaches we use and how we recruit patients. It worries me because we live by the mantra that we do mechanistic research in culture and in vitro, and then we take it to animal models. If all the results are consistent and we have a probe that can go to humans, then we try human trials. It sounds as though that general gestalt is not satisfactory for you. Do you think the animal models are inadequate or that they are inconsistent with what you are seeing in patients?

Dr Baracos: The patients at my center and others are heterogeneous. Their ages range from 40 to 90 years. They have solid tumors and other kinds of malignancies at different sites. They have something that people often ignore—a whole host of comorbid conditions. Their nutritional statuses are all over the map. This is because of their disease condition or conditions, as well as their lifelong previous dietary patterns and the dietary patterns they may have adopted after cancer diagnosis when they start to eat some unusual things.

I am disappointed by a number of randomized clinical trials of nutritional support and drug therapy directed at cancer cachexia, in which the clinical entity was not clearly defined and which generated results that were negative, inconclusive, or equivocal. These results seem to offer an opportunity for people to conclude that nutrition does not work or that nutrition does work. I am aware that a great deal of money, time, and energy of researchers and participation of patients goes into these clinical trials. They are onerous to conduct, and I would like to be able to better predict the right therapies to apply.

Dr Reid: In the 1980s, there was a lot of interest in free radical biology and free radicals as mediators of fatigue. People did exactly what you described—conducted well-intended, carefully controlled trials with nutritional antioxidants in a variety of subject populations under a variety of conditions, and it never altered performance. By the early 1990s, researchers had generally concluded that it was an epiphenomenon, a result of fatiguing exercise, not a cause of fatigue. Then the

seminal experiment came from Dr Supinski's lab. Instead of using vitamins and nutritional supplements, they used a drug, N-acetyl-cysteine, and they inhibited fatigue dramatically. Then other people were able to reproduce those findings, which opened the door for the field. Sometimes a seminal experiment in the right animal model can be what it takes to move us forward. That is not an answer to your question, but it is a tiny ray of hope from another field.

Dr Phillips: I want to make one comment about the animal model. If you compare animal model data on simple muscle disuse with human data, you find a drastic difference. If you hind-limb suspend a rat for just 12 hours, it will lose about 5% of its muscle mass. Suspended for a couple of days, it will lose 15%. There is no human equivalent of that. We see drastic upregulation of the proteasomal genes in the rat, and yet no evidence of that in humans. We also do not see measured increase in protein breakdown when we measure it in vivo in humans. So some important differences should be considered in these models of disease when there is a component of disuse.

In answer to one of Dr Tisdale's questions, I will share data later showing that fish oil supplementation actually stimulates protein synthesis in humans. We can see the effects.

Dr Morley: One problem I have when I look at animal scientists and cell scientists—I am basically a human doctor in the end—is that people study only one thing. There is this concept that if you study one thing and you study it often and to excess, it miraculously will solve your problem. If I want a grant, I have to write it that way because that is how we get grants. But if we look at normal physiology in animals or anywhere else, we see homeotic mechanisms in which some doses will produce one effect and other doses will produce a different effect within the system. Dr Reid, you have shown us wonderful data indicating that more or less nitric oxide is bad for you. In muscular dystrophy, we see that if there is no nitric oxide, muscles will fatigue quickly.

Our problem is that we all are looking at different parts of the elephant. Until we put the elephant together and realize that the tail and the trunk are not the same part of that elephant, we will continue not knowing where to go. In the end, we will have to find combination treatment for people who have severe disease, whether it is cachexia or end-stage sarcopenia. This is why I push for diagnosing and treating early.

I will add that we do not have nutritional studies worth anything, to be quite honest, because the numbers of subjects are too small. Scientists, including myself, believe that if we study 12 or 14 people in each group, the study is pretty good. We expect

a nutritional study of 20 or 30 people to be able to show a major effect. Then we do statistics and the results are not significant. Even studies of cholesterol-lowering agents involving thousands of people have found only a small change. Weiskopf's research, for instance, showed no change in mortality at 6 years. I am trying to say to big nutrition companies that it is time to do some reasonable studies of 1000, 2000, or more people using the agents we think will work, because otherwise we are going to keep on doing what Dr Baracos says—rejecting results. I think we have to be more realistic about what we are doing if we want to go forward in this field. The big studies in humans will finally answer questions about where we should be with nutrition.

Dr Bosaeus: We also should think about the role of muscle in this. Could that role be different in different situations? In inflammatory disease, for instance, is muscle the innocent bystander in the "crossfire" between immune systems and other things, or is it the taxpayer that should contribute to the war effort? The role also depends on the person's nutritional status from the beginning or during the course of the disease. I am a bit doubtful that it helps to multiply the trial size before sorting that out.

Dr Refaat Hegazi [Abbott Nutrition]: Dr Morley, I agree that nutrition research has a way to go, but let me comment on the effect size and the sample size based on the effect size. If we have a homogenous sample and the effect size is tremendous, we do not need a thousand patients, correct? Cholesterol-lowering studies need a lot of people because of heterogeneity of the studied patients and a smaller anticipated effect size.

Dr Reid, I want to ask about TNF. You showed it looks selective in the pathogenesis of cachexia. Do you think biological therapy will prevent or at least ameliorate the anti-TNF?

Dr Reid: Our philosophical approach to the problem is to not inhibit TNF per se. Arthritis patients are already getting soluble TNF receptors or monoclonal antibodies to TNF as a therapy. It is possible to give TNF both to animals and humans to try to block cachexia. To my knowledge, that has not been very successful because TNF is part of an integrated inflammatory response that may be important, as Dr Bosaeus points out, for other processes going on elsewhere in the body. We do not want to compromise the inflammatory response. For that reason, we may not want to give antioxidants either. However, if we can identify a receptor-mediated response causing the cachexia and then a muscle-specific target in the cell to inhibit, we may be able to preserve the muscle and allow the inflammation to proceed for the greater benefit of the body.

Discussion

Dr Baxter: I think we all agree on one thing: This is an extremely complicated set of conditions. We have all kinds of stressors that can affect lean muscle mass and functionality, and once the functionality or mass is gone, it is difficult to replace it. Nutrition can play a dramatic role, not only in prevention, but also in the restoration of not only muscle mass but also muscle functionality. However, if a cachectic, fatigued patient is cured of cancer, he or she could stay in that state for years.

Dr Tisdale: If you cure the cancer, the muscle mass comes back.

Dr Baxter: If we do the nutritional interventions necessary to replete the energetic systems and make sure that the patient has plenty of protein to rebuild muscle and so on, the patient may not stay in that state. For whatever reason, however, the nutritional follow-up may not take place.

Role of Protein Absorption and Nutrient Timing on Muscle Mass Accretion

Stuart Phillips, PhD, McMaster University, Hamilton, ON, Canada

In the years beyond those in which humans are growing there is normally no net new accretion of skeletal muscle mass. In fact, beginning in the 4th or 5th decade of life the mass of skeletal muscle begins to slowly decline, a condition referred to as sarcopenia.[1-3] Regardless of our age, however, muscle proteins are constantly and simultaneously being synthesized and degraded. Thus, maintenance of the mass of skeletal muscle is through the net balance between the processes of synthesis and breakdown of muscle proteins. This balance is maintained by ingestion of protein-containing meals, which results in a systemic increase in amino acids, which are stimulatory for the synthesis of new muscle proteins.[4-7]

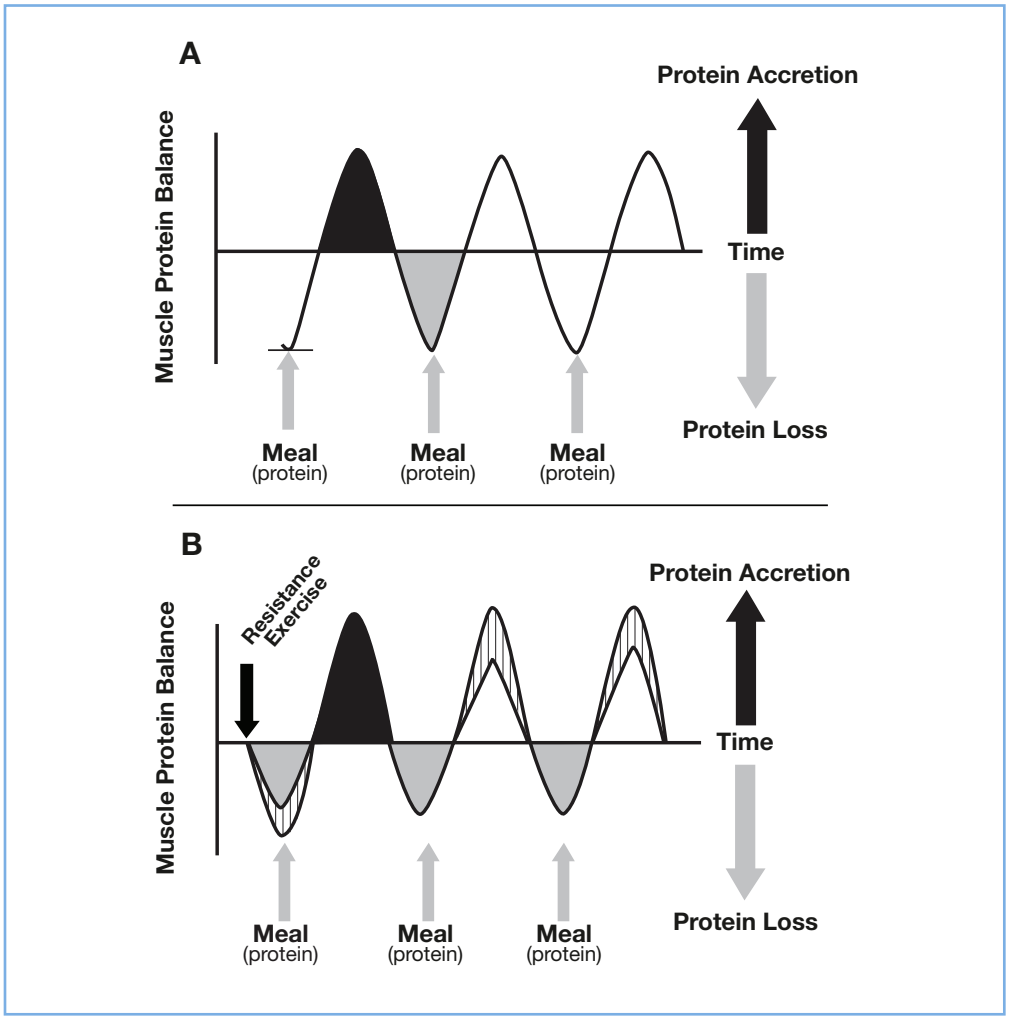
Skeletal muscle is a plastic tissue with the ability to respond to a variety of external stimuli such as exercise. Regular performance of dynamic endurance-type exercise results in a muscle phenotype that is more fatigue resistant, highly oxidative, and has a high capacity for lipid oxidation.[8-10] By contrast, regular performance of resistance exercise induces an increase in muscle-fiber cross-sectional area, greater force generating capacity, and is less stimulatory for changes in oxidative capacity.[11-15] The potency of resistance exercise as a stimulator of muscle protein synthesis (MPS) is evident in the fact that the acute increase in MPS is of greater magnitude and, especially, of far longer duration than the change after feeding.[16-18]

Feeding protein or amino acids stimulates MPS, an effect that appears to be due almost exclusively to the amino acids themselves.[7,19-22] It appears that only the indispensable amino acids are required to manifest this effect.[22,23] The amino acid leucine, in particular, occupies a position of prominence in that it alone can act as a stimulatory signal for MPS.[24,25] In humans, the ability of leucine alone to act as a signal for activating MPS has been tested only once[26]; however, many lines of evidence point to the ability of leucine to act in stimulatory manner for feeding-induced increases in MPS.[27-29] It should be noted, however, that even if leucine alone were to stimulate a rise in MPS by activating proteins in the mammalian target of rapamycin (mTOR) pathway, in the absence of substrate (ie, a full compliment of indispensable amino acids), MPS would ultimately slow and eventually revert to basal levels. Thus, complete mixtures of amino acids, both infused[5,6,19] and ingested,[30] or ingestion of intact proteins[31-35] have all reported increases in MPS.

Role of Protein Absorption and Nutrient Timing on Muscle Mass Accretion

Fig 1 shows our general understanding of how muscle protein is accrued both with feeding, resistance exercise, and with the two stimuli together.

Fig 1. (A) Normal fed-state gains and fasted-state losses in skeletal muscle protein



balance (synthesis minus breakdown). The area under the curve in the fed state (black area) would be equivalent to the fasted loss area under the curve (grey area); hence, skeletal muscle mass is maintained by feeding. (**B) Fed-state gains and fasted-state losses in skeletal muscle protein balance with performance of resistance exercise.** In this scenario, fasted-state gains are enhanced by an amount equivalent to the stimulation (striped area) of protein synthesis brought about by exercise (black area). In addition, fasted-state losses (striped area) appear to be less (grey area) due to persistent stimulation of protein synthesis in the fasted state.[36]

Interestingly, the process of MPS is saturable and appears to be a function of the extracellular amino acid concentrations rather than the intracellular.[5] The response of MPS in both young and older people is curvilinear, with an approximate plateau at 10 g of indispensable amino acids.[37] Recently, we reported on the dose-response of MPS following resistance exercise using intact isolated egg protein as a dietary source.[38] What we observed, similar to what was reported previously,[37] was that at 20 g of ingested protein (~8.5 g of essential amino acids) MPS plateaued. Fig 2 shows a plot of the response of MPS as well as a corresponding plot of leucine oxidation as an index of amino acid catabolism, as percentage of the basal (ie, 0 g) protein dose.

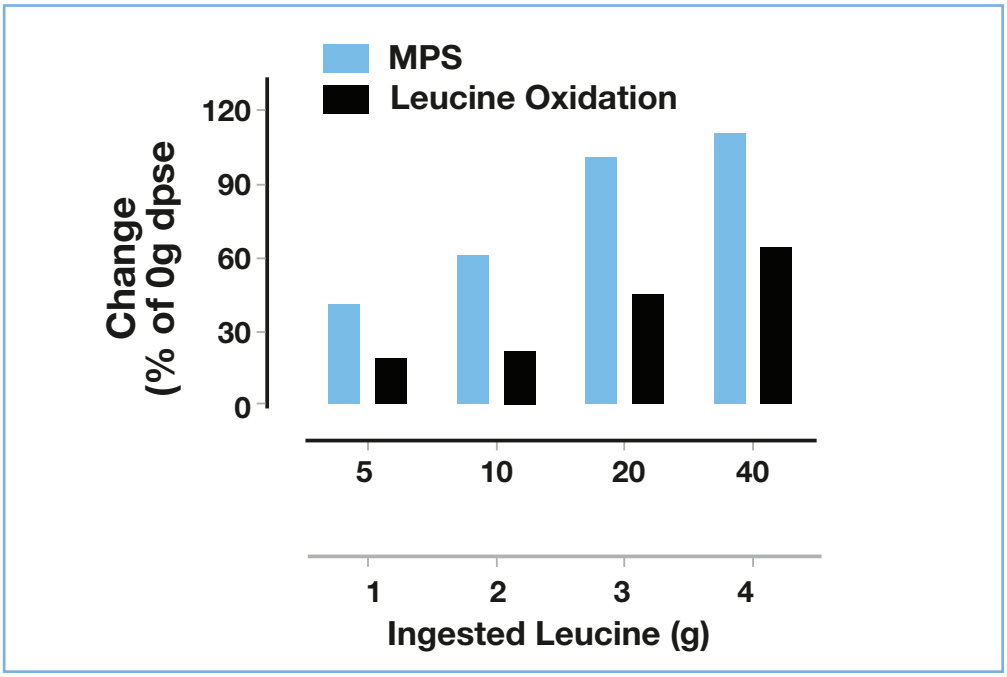


Fig 2. Percentage increases (from basal or 0g) in muscle protein synthesis (MPS) and leucine oxidation after resistance exercise in young men as a function of ingested protein and leucine dose.[38] The ingested protein was isolated egg protein.

Data adapted from Moore et al. "Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. *American Journal of Clinical Nutrition* 2009;89:161.

What becomes evident is that as MPS plateaus the extra amino acids are burned for fuel. Thus, the consumption of massive quantities of amino acids and/or protein by many resistance-trained athletes is clearly unnecessary when a mere 20 g of high-quality protein will suffice to maximally stimulate the process (MPS) that underpins changes in muscle mass.

Role of Protein Absorption and Nutrient Timing on Muscle Mass Accretion

Resistance-exercise stimulation of muscle protein synthesis lasts at least 48 hours.[17] Hence, resistance exercise and protein ingestion should interact synergistically to stimulate protein synthesis at any time within 48 hours following exercise cessation, and ultimately lead to protein accretion. However, evidence exists to support the contention that consumption of protein (or amino acids), and not simply energy as carbohydrate, in close temporal proximity, both before and/or after, to resistance exercise is important to support greater hypertrophy.[39-43] These chronic training studies suggest that, in younger people, the window during which consumption of protein or amino acids should be consumed is likely 30-45 minutes before and/or less than 2 hours after exercise to support greater increases in lean body mass and muscle hypertrophy. In the elderly, it is possible that the window for nutrition may be as little as 1 hour after exercise.[41] Notably, one acute study in young people has shown that a full anabolic response can be mounted by skeletal muscle at both 1 hour and 3 hours post-exercise with crystalline amino acid consumption[44]; however, it has not been investigated whether this feeding pattern would translate into similar increases in muscle hypertrophy with training. Therefore, to support greater hypertrophy with resistance training at any age, consuming a source of protein within 1 hour after exercise cessation would be beneficial.

Researchers who study the phenomenon of resistance-training-induced hypertrophy have long held that a contraction intensity threshold exists that induces hypertrophy. This belief dates back to the classic work of DeLorme,[45] who in 1945 made the following conclusions, "…Low-repetition, high-resistance exercises produces power. High-repetition, low-resistance exercises produce endurance. Each of these two types of exercise is incapable of producing results obtained by the other. In order to obtain rapid hypertrophy in weakened, atrophied muscle, the muscle should be subjected to strenuous exercise and, at regular intervals, to the point of maximum exertion." In fact, advocating the practice of lifting heavier loads to induce hypertrophy and strength still is inherent in even the most up-to-date reviews on this topic.[46] Acute studies appear to support the "lift heavier" paradigm, at least in part,[47] inasmuch as a rise in MPS is seen only when intensities of load lifted exceed 60% of the single repetition maximum (1RM). Of note, however, is that at intensities beyond 60% and up to 90% of 1RM stimulation of MPS is similar. This finding may indicate that chronic performance of resistance exercise at intensities greater than 60% of 1RM has little additional value for stimulating MPS and possibly hypertrophy. This supposition is predicated on the assumption that acute changes are meaningful in terms of predicting long-term hypertrophic changes, which is a concept that does have support.[35,42]

Other studies offer credence to the concept that it may not be the intensity of the lift that is the active variable in determining the response of MPS. Fujita et al[48] reported that even low intensity exercise (20% of 1RM) stimulated MPS when blood flow was occluded. This acute finding is perhaps not surprising when one considers that a number of training studies have shown that this practice of blood flow occlusion can result, when practiced chronically and even when lifting at low intensities (30%-40% of 1RM), in substantial hypertrophy and strength gains equivalent to those seen at 80% of 1RM.[49] Why occlusion has this effect is not known, but the most likely explanation is that it induces a local fatigue that forces recruitment of type II muscle fibers, which would not normally be recruited at such low intensities. An alternative explanation is that the acute rise in growth hormone is somehow responsible for increases in skeletal muscle growth. Evidence to support that growth hormone is in any way anabolic for skeletal muscle and even affects MPS is lacking, however, with a lot of evidence to support the contrary position.[50-52] Thus, the possibility that the recruitment of type II fibers per se, independent of the exercise intensity, is the prime variable affecting the stimulation of MPS and ultimately training-induced hypertrophy provides an interesting avenue for future study.

We have known for some time that in response to resistance training the larger type II fibers display a greater degree of hypertrophy than the smaller type I.[13,53,54] Moreover, recent evidence demonstrates that following resistance exercise, primarily type II fibers activate many of the critical signaling proteins involved in the regulation of MPS.[55] This is notable because the phosphorylation of this protein is reported to predict hypertrophy in rats,[56] humans,[57] and, at least in young people, the intensity-dependent rise in MPS.[47] Collectively, therefore, these data suggest that, regardless of exercise intensity, a prerequisite condition to maximizing the anabolic effect of resistance exercise may be the activation of these highly trainable type II muscle fibers. This effectively means that one needs to work to fatigue, even at a low load, to see effective gains in muscle mass.

The effect of the timing of amino acid delivery after resistance exercise has been examined acutely[44,58,59] and long term.[40-43,60] It appears to make little difference whether a protein-plus-carbohydrate supplement (6 g of amino acids plus 35 g of sucrose) is consumed 1 hour or 3 hours post-exercise because the same positive net protein balance results at both times.[44] In another investigation by Tipton et al,[59] pre-exercise consumption of the same protein plus carbohydrate supplement used previously[44] did augment muscle protein balance. However, this finding was not reproduced with pre- and post-exercise ingestion of whey protein.[58] In longer-term training studies, the effects of immediate (or at least temporally close) provision of nutrition appears to enhance muscle mass gains.[40-43,60] As Fig 3 summarizes, it

appears that consuming protein sometime following the performance of resistance exercise, say 1-2 hours following, does appear to additively stimulate MPS and enhance gains in muscle and, presumably, strength.

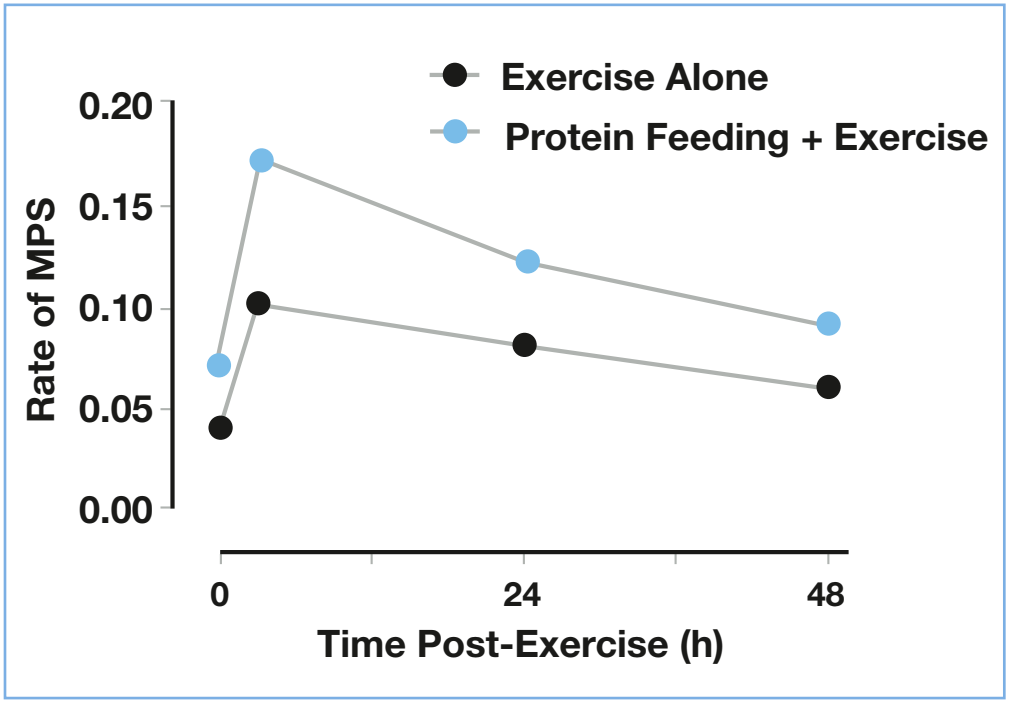


Fig 3. Schematic figure illustrating the effect of an isolated bout of resistance exercise alone on the rate of muscle protein synthesis (MPS) from rest (0h) and at 4h, 24h, and 48h post-exercise. The additive effect of feeding is shown at the same times. Note that the additive effect of feeding is greatest at 4h post-exercise, which is proposed to occur after ingesting protein immediately post-exercise[37] and is diminished with time post-exercise. Thus, it appears that consuming protein in close temporal proximity to exercise (ie, at least within 1-2h after) is advantageous from the standpoint of promoting gains in muscle mass.

In summary, we are now beginning to understand the factors that regulate gains in muscle mass, and MPS is the primary locus of control. As such, studies examining acute and chronic changes in MPS are of primary importance to understanding what factors regulate gains in muscle mass with feeding and resistance exercise. Perhaps more important are studies of situations in which muscle mass is lost, such as with aging. With aging, it is also likely important that declines in the feeding-induced sensitivity to protein feeding, and as such the declines in the feeding-induced rise in protein balance, are also underpinning the decline in muscle mass. Clearly, more work needs to be done and acute mechanistic studies will ultimately inform, at least qualitatively, how chronic interventions may be able to

help athletes gain the muscle they need to compete or help the elderly reclaim the muscle they lose with aging. Regardless of the situation, an enormous capacity for good exercise planning, good overall nutrition, and specialized nutritional products exists that can enhance these processes and provide benefit to athletes and the elderly alike.

Acknowledgements

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References

1. Evans WJ: What is sarcopenia? *J Gerontol A Biol Sci Med Sci* 1995;50:5-8.
2. Frontera WR, Hughes VA, Lutz KJ, Evans WJ: A cross-sectional study of muscle strength and mass in 45- to 78-yr-old men and women. *J Appl Physiol* 1991;71:644-650.
3. Hughes VA, Frontera WR, Wood M, et al: Longitudinal muscle strength changes in older adults: Influence of muscle mass, physical activity, and health. *J Gerontol A Biol Sci Med Sci* 2001;56:209-217.
4. Bennet WM, Connacher AA, Scrimgeour CM, et al: Increase in anterior tibialis muscle protein synthesis in healthy man during mixed amino acid infusion: Studies of incorporation of [1-13C]leucine. *Clin Sci (Lond)* 1989;76:447-454.
5. Bohe J, Low A, Wolfe RR, Rennie MJ: Human muscle protein synthesis is modulated by extracellular, not intramuscular amino acid availability: A dose-response study. *J Physio*l 2003;552:315-324.
6. Bohe J, Low JF, Wolfe RR, Rennie MJ: Latency and duration of stimulation of human muscle protein synthesis during continuous infusion of amino acids. *J Physiol* 2001;532:575-579.
7. Fujita S, Dreyer HC, Drummond MJ, et al: Nutrient signalling in the regulation of human muscle protein synthesis. *J Physiol* 2007;582:813-823.
8. Holloszy JO: Cellular adaptations to endurance exercise: Master athletes. *Int J Sport Nutr Exerc Metab* 2001;11 Suppl:S186-S188.
9. Holloszy JO, Booth FW: Biochemical adaptations to endurance exercise in muscle. *Annu Rev Physiol* 1976;38:273-291.
10. Holloszy JO, Coyle EF: Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. *J Appl Physio*l 1984;56:831-838.
11. Häkkinen K, Newton RU, Gordon SE, et al: Changes in muscle morphology, electromyographic activity, and force production characteristics during progressive strength training in young and older men. *J Gerontol A Biol Sci Med Sci* 1998;53:B415-B423.

12. Staron RS, Hikida RS, Hagerman FC, et al: Human skeletal muscle fiber type adaptability to various workloads. *J Histochem Cytochem* 1984;32:146-152.
13. Staron RS, Karapondo DL, Kraemer WJ, et al: Skeletal muscle adaptations during early phase of heavy-resistance training in men and women. *J Appl Physiol* 1994;76:1247-1255.
14. Staron RS, Leonardi MJ, Karapondo DL, et al: Strength and skeletal muscle adaptations in heavy-resistance-trained women after detraining and retraining. *J Appl Physiol* 1991;70:631-640.
15. Tang JE, Hartman JW, Phillips SM: Increased muscle oxidative potential following resistance training induced fibre hypertrophy in young men. *Appl Physiol Nutr Metab* 2006;31:495-501.
16. Miller BF, Olesen JL, Hansen M, et al: Coordinated collagen and muscle protein synthesis in human patella tendon and quadriceps muscle after exercise. *J Physiol* 2005;567:1021-1033.
17. Phillips SM, Tipton KD, Aarsland A, et al: Mixed muscle protein synthesis and breakdown after resistance exercise in humans. *Am J Physiol* 1997;273:99-107.
18. Tang JE, Perco JG, Moore DR, et al: Resistance training alters the response of fed state mixed muscle protein synthesis in young men. *Am J Physiol Regul Integr Comp Physiol* 2008;294:172-178.
19. Biolo G, Tipton KD, Klein S, Wolfe RR: An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. *Am J Physiol* 1997;273:122-129.
20. Greenhaff PL, Karagounis L, Peirce N, et al: Disassociation between the effects of amino acids and insulin on signaling, ubiquitin ligases and protein turnover in human muscle. *Am J Physiol Endocrinol Metab* 2008;295:E595-604.
21. Paddon-Jones D, Sheffield-Moore M, Zhang XJ, et al: Amino acid ingestion improves muscle protein synthesis in the young and elderly. *Am J Physiol Endocrinol Metab* 2004;286:321-328.
22. Volpi E, Kobayashi H, Sheffield-Moore M, et al: Essential amino acids are primarily responsible for the amino acid stimulation of muscle protein anabolism in healthy elderly adults. *Am J Clin Nutr* 2003;78:250-258.
23. Tipton KD, Gurkin BE, Matin S, Wolfe RR: Nonessential amino acids are not necessary to stimulate net muscle protein synthesis in healthy volunteers. *J Nutr Biochem* 1999;10:89-95.
24. Anthony JC, Reiter AK, Anthony TG, et al: Orally administered leucine enhances protein synthesis in skeletal muscle of diabetic rats in the absence of increases in 4E-BP1 or S6K1 phosphorylation. *Diabetes* 2002;51:928-936.
25. Anthony JC, Yoshizawa F, Anthony TG, et al: Leucine stimulates translation initiation in skeletal muscle of postabsorptive rats via a rapamycin-sensitive pathway. *J Nutr* 2000;130:2413-2419.
26. Smith K, Barua JM, Watt PW, et al: Flooding with L-[1-13C]leucine stimulates human muscle protein incorporation of continuously infused L-[1-13C]valine. *Am J Physiol* 1992;262:372-376.

27. Greiwe JS, Kwon G, McDaniel ML, Semenkovich CF: Leucine and insulin activate p70 S6 kinase through different pathways in human skeletal muscle. *Am J Physiol Endocrinol Metab* 2001;281:466-471.

28. Karlsson HK, Nilsson PA, Nilsson J, et al: Branched-chain amino acids increase p70S6k phosphorylation in human skeletal muscle after resistance exercise. *Am J Physiol Endocrinol Metab* 2004;287: 1-7.

29. Katsanos CS, Kobayashi H, Sheffield-Moore M, et al: A high proportion of leucine is required for optimal stimulation of the rate of muscle protein synthesis by essential amino acids in the elderly. *Am J Physiol Endocrinol Metab* 2006;291:381-387.

30. Tipton KD, Ferrando AA, Phillips SM, et al: Postexercise net protein synthesis in human muscle from orally administered amino acids. *Am J Physiol* 1999;276:628-634.

31. Katsanos CS, Chinkes DL, Paddon-Jones D, et al: Whey protein ingestion in elderly persons results in greater muscle protein accrual than ingestion of its constituent essential amino acid content. *Nutr Res* 2008;28:651-658.

32. Paddon-Jones D, Sheffield-Moore M, Katsanos CS, et al: Differential stimulation of muscle protein synthesis in elderly humans following isocaloric ingestion of amino acids or whey protein. *Exp Gerontol* 2006;41:215-219.

33. Symons TB, Schutzler SE, Cocke TL, et al: Aging does not impair the anabolic response to a protein-rich meal. *Am J Clin Nutr* 2007;86:451-456.

34. Tipton KD, Elliott TA, Cree MG, et al: Ingestion of casein and whey proteins result in muscle anabolism after resistance exercise. *Med Sci Sports Exerc* 2004;36:2073-2081.

35. Wilkinson SB, Tarnopolsky MA, MacDonald MJ, et al: Consumption of fluid skim milk promotes greater muscle protein accretion following resistance exercise than an isonitrogenous and isoenergetic soy protein beverage. *Am J Clin Nutr* 2007;85:1031-1040.

36. Phillips SM: Protein requirements and supplementation in strength sports. *Nutrition* 2004;20:689-695.

37. Cuthbertson D, Smith K, Babraj J, et al: Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. *FASEB J* 2005;19:422-424.

38. Moore DR, Robinson MJ, Fry JL, et al: Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. *Am J Clin Nutr* 2009;89:161-168.

39. Andersen LL, Tufekovic G, Zebis MK, et al: The effect of resistance training combined with timed ingestion of protein on muscle fiber size and muscle strength. *Metabolism* 2005;54:151-156.

40. Cribb PJ, Hayes A: Effects of supplement timing and resistance exercise on skeletal muscle hypertrophy. *Med Sci Sports Exerc* 2006;38:1918-1925.

41. Esmarck B, Andersen JL, Olsen S, et al: Timing of postexercise protein intake is important for muscle hypertrophy with resistance training in elderly humans. *J Physiol* 2001;535:301-311.

42. Hartman JW, Tang JE, Wilkinson SB, et al: Consumption of fat-free fluid milk after resistance exercise promotes greater lean mass accretion than does consumption of soy or carbohydrate in young, novice, male weightlifters. *Am J Clin Nutr* 2007;86:373-381.

43. Holm L, Esmarck B, Mizuno M, et al: The effect of protein and carbohydrate supplementation on strength training outcome of rehabilitation in ACL patients. *J Orthop Res* 2006;24:2114-2123.

44. Rasmussen BB, Tipton KD, Miller SL, et al: An oral essential amino acid-carbohydrate supplement enhances muscle protein anabolism after resistance exercise. *J Appl Physiol* 2000;88:386-392.

45. Delorme TL: Restoration of muscle power by heavy-resistance exercises. *J Bone Joint Surg Am* 1945;27:645-667.

46. ACSM Position Stand: Progression models in resistance training for healthy adults. *Med Sci Sports Exerc* 2009;41:687-708.

47. Kumar V, Selby A, Rankin D, et al: Age-related differences in the dose-response relationship of muscle protein synthesis to resistance exercise in young and old men. *J Physiol* 2009;587:211-217.

48. Fujita S, Abe T, Drummond MJ, Cadenas JG, et al: Blood flow restriction during low-intensity resistance exercise increases S6K1 phosphorylation and muscle protein synthesis. *J Appl Physiol* 2007;103:903-910.

49. Abe T, Kearns CF, Sato Y: Muscle size and strength are increased following walk training with restricted venous blood flow from the leg muscle, Kaatsu-walk training. *J Appl Physiol* 2006;100:1460-1466.

50. Liu H, Bravata DM, Olkin I, et al: Systematic review: The effects of growth hormone on athletic performance. *Ann Intern Med* 2008;148:747-758.

51. Rennie MJ: Claims for the anabolic effects of growth hormone: A case of the emperor's new clothes? *Br J Sports Med* 2003;37:100-105.

52. Yarasheski KE, Zachwieja JJ, Campbell JA, Bier DM: Effect of growth hormone and resistance exercise on muscle growth and strength in older men. *Am J Physiol* 1995;268:268-276.

53. McCall GE, Byrnes WC, Dickinson A, et al: Muscle fiber hypertrophy, hyperplasia, and capillary density in college men after resistance training. *J Appl Physiol* 1996;81:2004-2012.

54. Staron RS, Malicky ES, Leonardi MJ, et al: Muscle hypertrophy and fast fiber type conversions in heavy resistance-trained women. *Eur J Appl Physiol Occup Physiol* 1989;60:71-79.

55. Tannerstedt J, Apro W, Blomstrand E: Maximal lengthening contractions induce different signaling response in the type I and type II fibers of human skeletal muscle. *J Appl Physiol* 2008;106:1412-1418.

56. Baar K, Esser K: Phosphorylation of p70(S6k) correlates with increased skeletal muscle mass following resistance exercise. *Am J Physiol* 1999;276:120-127.

57. Terzis G, Georgiadis G, Stratakos G, et al: Resistance exercise-induced increase in muscle mass correlates with p70S6 kinase phosphorylation in human subjects. *Eur J Appl Physiol* 2008;102:145-152.

58. Tipton KD, Elliott TA, Cree MG, et al: Stimulation of net muscle protein synthesis by whey protein ingestionbefore and after exercise. *Am J Physiol Endocrinol Metab* 2006;292:71-76.

59. Tipton KD, Rasmussen BB, Miller SL, et al: Timing of amino acid-carbohydrate ingestion alters anabolic response of muscle to resistance exercise. *Am J Physiol Endocrinol Metab* 2001;281:197-206.

60. Holm L, Olesen JL, Matsumoto K, et al: Protein-containing nutrient supplementation following strength training enhances the effect on muscle mass, strength, and bone formation in postmenopausal women. *J Appl Physiol* 2008;105:274-281.

Q & A

Q: Dr Phillips, did you use essential amino acids throughout your studies or essential and nonessential amino acids? If both kinds, what ratio did you target? Did you vary that ratio or was it constant?

Dr Phillips: All the studies I have shown you are using high-quality whole proteins, either whey protein or milk protein. All I have reported has been the rise in blood leucine concentration or the rise in essential amino acid concentration. At no point have we manipulated using crystalline amino acids or anything like that—the essential amino acid composition.

Q: You performed a nice series of experiments. As far as I remember, they were all performed in males. Do you expect any gender-specific anabolic responses?

Dr Phillips: We are about to submit a paper describing research using the same resistance-exercise protocol—milk drinking vs control—in women. The women who drank the milk responded similarly to the men; acutely, of course, we see no rise in testosterone in those women. We do see a small rise in growth hormone, but it is entirely consistent between groups, indicating that the differential gain in muscle mass has nothing to do with hormones. Of course, women have the capacity to hypertrophy, but it has very little to do with a change in testosterone. It is all driven internally within the muscle itself.

Impact of Nutrition on Lean Body Mass and Exercise Recovery in Athletes

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Improving body composition by increasing the amount of lean body mass relative to fat mass is a goal of many people for the purpose of increasing physical performance and general health. Greater muscle mass is associated with increased strength and power, as well as better metabolic health and reduced risk for chronic disease. Lifestyle approaches aimed at improving body composition focus on nutrition and exercise, but the type of diet and exercise program and their interaction are important factors that affect responses in percent body fat. Reduced-calorie weight loss approaches usually result in considerable loss of both fat and lean body mass.[1,2] Maximizing fat loss while preserving or building lean body mass requires consideration of macronutrient composition and the inclusion of resistance training.

Low-Carbohydrate Diets and Body Composition

Decreased energy intake is required to induce weight loss, but the composition of macronutrients (carbohydrate, protein, and fat) has a significant effect on the composition of weight loss. According to a recent comprehensive review of weight loss diets, lower carbohydrate intake was associated with greater fat loss and higher protein intake was associated with better retention of lean body mass independent of energy intake.[3] Several recent studies have shown that carbohydrate-restricted diets result in greater weight and fat loss compared to low-fat diets,[4,5] and in fact, lean body mass may actually increase in response to a very low-carbohydrate intake in normal weight men.[6] Even severely hypocaloric very low-carbohydrate diets spare lean tissue as evidenced by studies showing positive nitrogen balance in subjects consuming <800 kcal/day.[7]

The central role of the glucose-insulin axis in the control of metabolic processes is the basis for the use of carbohydrate-restricted diets. Insulin has anabolic functions that inhibit breakdown and promote storage of nutrients. Adipose tissue lipolysis and fat oxidation are exquisitely sensitive to changes in insulin within the physiological range of concentrations.[8] Small reductions in insulin levels, such as those easily achieved with dietary carbohydrate restriction, remove the normal inhibition on access to and oxidation of fat for fuel. Thus, low-carbohydrate diets are associated with significant changes in lipid metabolism favoring decreased

storage and increased breakdown and oxidation of fat, as well as improved atherogenic dyslipemia (decreased triglycerides, increased high-density lipoprotein cholesterol [HDL-C], and increased low-density lipoprotein [LDL] particle size).[4] The ability of low-carbohydrate intake to inhibit lipogenesis and to bias lipid metabolism toward oxidation was demonstrated in a recent experiment in which we showed a significant decrease in plasma saturated fatty acids despite greater intake of saturated fat on a very low-carbohydrate diet.[9]

Diet in Combination With Resistance Training

Resistance training is a potent stimulus for increasing muscle size and strength, and when combined with dietary caloric restriction helps preserve lean body mass. For example, we showed that overweight men and women consuming a low-fat, high-fiber diet for 12 weeks lost about 10 kg of body weight, of which 69% was from fat.[2] A separate group who followed the same diet and performed resistance exercise workouts three times a week had the same weight loss that was almost exclusively from fat (97%).

The effects of low-carbohydrate diets in combination with resistance training was addressed by Layman and colleagues.[10] They reported that the combination of a low-carbohydrate diet and resistance exercise had the most favorable response for both fat loss and preservation of lean body mass in middle-aged women.

We performed a similar experiment in overweight/obese men who were placed in a low-fat-diet group that restricted fat to less than 25% of energy, or in a very low-carbohydrate-diet group that reduced carbohydrate to less than 15% energy.[11] Both groups also participated in a resistance training program (3 or 4 times a week). Body composition was assessed using dual energy x-ray absorptiometry before and after the 12-week program. The results were compared to non-training diet-only groups. As expected, the low-carbohydrate-diet group lost more fat mass, which was associated with greater decreases in insulin. Resistance training, independent of diet, resulted in increased lean body mass without compromising fat loss in both diet groups. The most dramatic reduction in percent body fat was in the low-carbohydrate-diet resistance-training group (-5.3%), followed by the low-fat resistance-training (-3.5%), low-carbohydrate-diet only (-3.4%), and low-fat-diet only (-2.0%) groups. These studies show that low-carbohydrate diets promote greater fat loss independent of training, whereas resistance training promotes increased lean body mass independent of diet. The combination of a low-carbohydrate diet and resistance training is therefore additive, promoting the largest decreases in percent body fat (Fig 1).

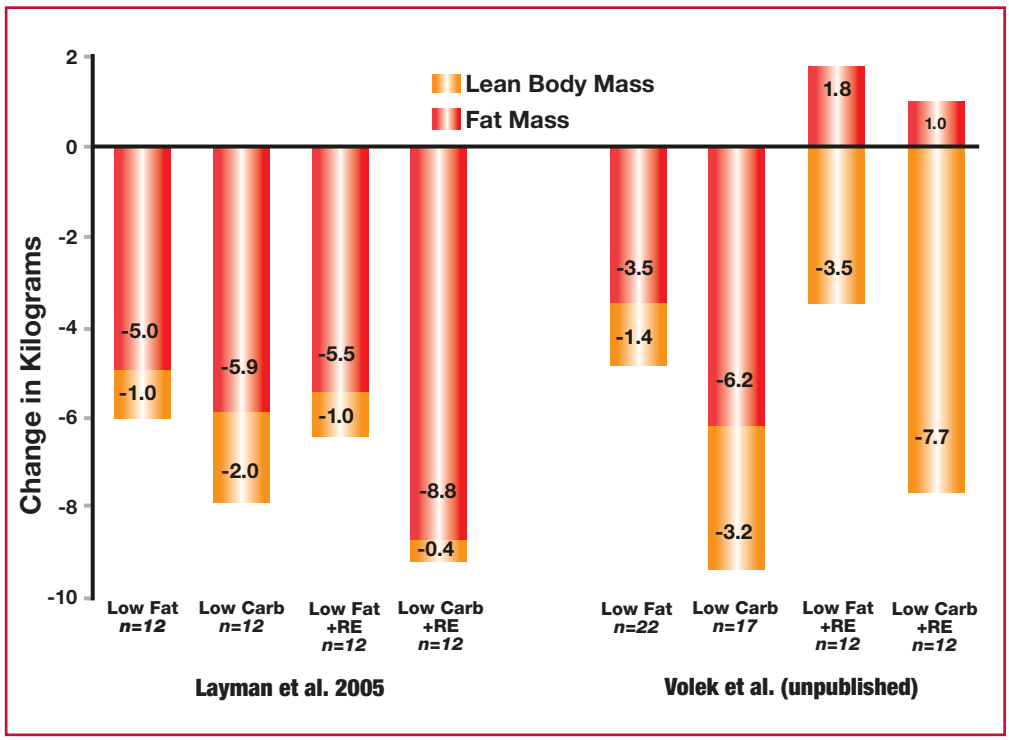

Fig 1. Effects of diet composition with and without resistance training on change in lean body mass and fat mass after 16 wk in untrained women[10] and 12 wk in untrained men.[11] RE=resistance exercise

Creatine Supplementation and Lean Body Mass

Creatine is one of the most extensively studied dietary supplements over the last 15 years. Short-term studies involve a loading phase of creatine (15-25 g/day for 5 to 7 days) have shown significant improvement in muscle strength and power during short-burst high-intensity exercise tasks. Creatine loading increases the muscle content of creatine (and phosphocreatine) and accelerates the rate of resynthesis of phosphocreatine, a high-energy compound in muscle, during recovery so that muscle phosphocreatine levels are higher at the start of the next exercise bout. We showed that 7 days of creatine supplementation (25 g/day) allowed subjects to perform a total of eight more repetitions during a bench press workout consisting of 5 sets,[12] translating into a better training stimulus for inducing gains in muscle.

Chronic studies that examined the effects of taking creatine while engaged in a resistance-training program have found consistent benefits on gains in strength and muscle mass.[13] In a recent review, we concluded that the average increase in muscle strength following creatine supplementation plus resistance training was

24% compared to 18% in subjects training and taking a placebo.[14] Similarly, the average increase in maximal repetitions at a given percent of maximal strength following creatine supplementation plus resistance training was 34%, compared to 13% in the placebo groups. In terms of gains in lean body mass, creatine supplementation plus resistance training results, on average, in 2-3 kg of additional muscle over a 12-week training period, and this is associated with significant muscle fiber hypertrophy in all fiber types (Fig 2).[15]

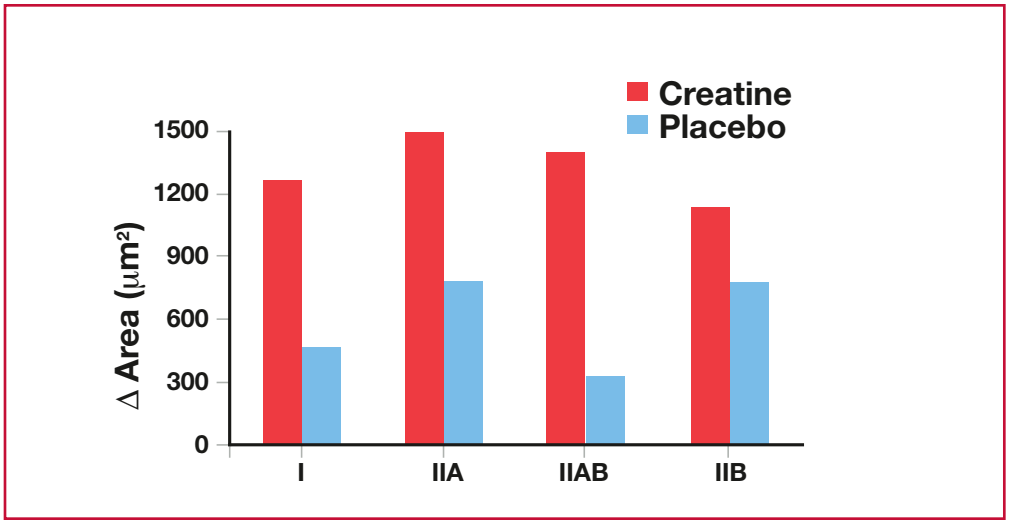

Fig 2. Effects of 12 weeks of resistance training in subjects consuming creatine or placebo on muscle fiber hypertrophy.[15]

These outcomes represent average expected improvements in strength and muscle mass with creatine. Many individuals exceed these average responses, and some do not respond to creatine. People who have low muscle creatine levels tend to have the largest increases in muscle creatine after a loading period, and this translates into better gains in performance.

HMB Supplementation and Lean Body Mass

A number of studies have also investigated the effects of β-hydroxy-β-methylbutyrate (HMB) supplementation on lean body mass and muscular performance. Some, but not all, have reported that gains in muscle strength and lean body mass from resistance training are augmented by HMB.[16] We recently reported that a formula consisting of HMB and amino acids taken during 12 weeks of resistance training resulted in significantly greater increases in lean body mass (5.3 kg) compared to placebo.[17] Thus, HMB may work best when combined with amino acids.

Conclusion

In summary, a primary concern with conventional weight loss approaches is the loss of lean body mass that occurs when fat mass is decreased. Consuming moderate protein while restricting carbohydrate and increasing fat allows for greater preservation of lean body mass. A low-carbohydrate diet in conjunction with resistance training results in greater fat loss while preserving lean body mass and improving metabolic health. Considerable scientific work has shown that creatine and HMB supplementation augment gains in muscle size and strength in response to resistance training.

References

1. Garrow JS, Summerbell CD: Meta-analysis: Effect of exercise, with or without dieting, on the body composition of overweight subjects. *Eur J Clin Nutr* 1995;49:1-10.
2. Kraemer W J, Volek JS, Clark KL, et al: Influence of exercise training on physiological and performance changes with weight loss in men. *Med Sci Sports Exerc* 1999;31:1320-1329.
3. Krieger JW, Sitren HS, Daniels MJ, Langkamp-Henken B: Effects of variation in protein and carbohydrate intake on body mass and composition during energy restriction: A meta-regression. *Am J Clin Nutr* 2006;83:260-274.
4. Volek JS, Fernandez ML, Feinman RD, Phinney SD: Dietary carbohydrate restriction induces a unique metabolic state positively affecting atherogenic dyslipidemia, fatty acid partitioning, and metabolic syndrome. *Prog Lipid Res* 2008;47:307-318.
5. Volek JS, Sharman MJ, Gomez AL, et al: Comparison of energy-restricted very low-carbohydrate and low-fat diets on weight loss and body composition in overweight men and women. *Nutr Metab* (Lond) 2004;1:13.
6. Volek JS, Sharman MJ, Love DM, et al: Body composition and hormonal responses to a carbohydrate-restricted diet. *Metabolism* 2002;51:864-870.
7. Hoffer LJ, Bistrian BR, Young VR, et al: Metabolic effects of very low calorie weight reduction diets. *J Clin Invest* 1984;73:750-758.
8. Jensen MD, Caruso M, Heiling V, Miles JM: Insulin regulation of lipolysis in nondiabetic and IDDM subjects. *Diabetes* 1989;38:1595-1601.
9. Forsythe CE, Phinney SD, Fernandez ML, et al: Comparison of low fat and low carbohydrate diets on circulating fatty acid composition and markers of inflammation. *Lipids* 2008;43:65-77.
10. Layman DK, Evans E, Baum JI, et al: Dietary protein and exercise have additive effects on body composition during weight loss in adult women. *J Nutr* 2005;135:1903-1910.
11. Quann EE, Scheett TP, Ballard KD, et al: Carbohydrate restriction and resistance training have additive effects on body composition during weight loss in men. American Dietetic Association, Philadelphia, PA. (abstract) April, 2007.

12. Volek JS, Kraemer WJ, Bush JA, et al: Creatine supplementation enhances muscular performance during high-intensity resistance exercise. *J Am Diet Assoc* 1997;97:765-770.

13. Volek JS, Rawson ES: Scientific basis and practical aspects of creatine supplementation for athletes. *Nutrition* 2004;20:609-614.

14. Rawson ES, Volek JS: Effects of creatine supplementation and resistance training on muscle strength and weightlifting performance. *J Strength Cond Res* 2003;17:822-831.

15. Volek JS, Duncan ND, Mazzetti SA, et al: Performance and muscle fiber adaptations to creatine supplementation and heavy resistance training. *Med Sci Sports Exerc* 1999;31:1147-1156.

16. Rowlands DS, Thomson JS: Effects of β-hydroxy-β-methylbutyrate supplementation during resistance training on strength, body composition, and muscle damage in trained and untrained young men: A meta-analysis. *J Strength Cond Res* 2009;23:836-846.

17. Kraemer WJ, Hatfield DL, Volek JS, et al: Effects of amino acids supplement on physiological adaptations to resistance training. *Med Sci Sports Exerc* 2009;41:1111-1121.

Q & A

Q: Can you speak to the benefit of creatine and then creatine plus HMB in the elderly?

Dr Volek: Several studies have been done with creatine in the elderly. We have done a couple of studies in which we acutely load elderly patients with creatine and have shown improved functional capacity and strength and ability to sit up and get out of a chair—activities of daily living [Gotshalk LA et al: *Eur J Appl Physiol* 2008;102:223-231. Epub 2007, Oct 18; Gotshalk LA et al: *Med Sci Sports Exerc* 2002;34(3):537-543]. So elderly people respond similarly to younger adults.

Q: I was interested in your studies with the low-carbohydrate/high-fat diet. I was confused about the mechanism because you attributed it solely to a reduction in insulin. You did not measure catacholamine or glucagon in the subjects. I understand that, if you have a low-carbohydrate diet, you might get increased lipolysis because gluconeogenesis from amino acids is needed for energy, but I was not sure about the increase in lean body mass because insulin would stimulate that. If gluconeogenesis increases, you have to decrease precursor population for muscle protein synthesis.

Dr Volek: You are right—insulin would promote protein synthesis. Insulin is not that important for maintaining protein balance and has a much more potent effect on fat balance, so the ability to use fat more efficiently and use alternative fuels would spare lean tissue during negative caloric intake. The preponderance of evidence indicates that low-carbohydrate diets that promote increased reliance on fat for fuel result in better preservation of lean body mass during weight loss. If resistance training is added, we can actually build lean body mass while insulin is very low and fat breakdown is accelerated. I do not want to oversimplify the situation. Certainly insulin is not the only mechanism here, but it is a key variable that regulates metabolism, and in some ways is a switch to allow access to body fat stores. A lot of people are insulin-resistant and have problems with hyperinsulinemia. Getting that condition under control is important, and restricting carbohydrates is probably the most direct way to do that.

Q: In the nutrition field, we struggle with the placebo effect, and the HMB study you showed was mixed with arginine and glutamine. Have you controlled for arginine and glutamine? Do you think that the additive effect of three nutrients is better than just HMB?

Dr Volek: We do not know from that experiment. It is not, however, a reductionist approach. We are looking at a formula. The placebo was isonitrogenous, so we can rule out an effect of nitrogen, but the nitrogen was coming from non-essential amino acids. I tend to think that, looking at pure HMB studies, the magnitude of the effect was much greater. My hunch is that amino acids are playing a synergistic role with HMB in this case and at bioactive levels that could affect protein synthesis.

Q: Is there any downside to increasing creatine intake at these levels?

Dr Volek: There does not appear to be. These levels are relatively benign; a person excretes the extra creatine in urine. There is no need for high doses; a person just needs to maintain a normal breakdown. Increased muscle creatine levels are resilient and slowly return to baseline over a period of 4-6 weeks. Previously, someone alluded to cramping issues. There probably are some water shifts as creatine moves into cells, so creatine is an osmolyte. It will accumulate in muscle and bring some water into the muscle, so there could be cramping because this may alter electrolyte balance. A steady amount of research has been conducted with creatine for nearly 20 years with some long-term follow-ups, and all this research has found no remarkable side effects.

Q: Two studies have found that creatine improved cramping, and another study in athletes said it does not affect cramping.

Dr Volek: Most of the information about cramping is anecdotal.

Q: With respect to your low-carbohydrate diet effects, do you think that you would get the same effects with very low glycemic carbohydrates?

Dr Volek: A study by Eric Westman at Duke University [Westman EC et al: *Nutr Metab* (Lond) 2008;19:36] compared a low glycemic index (GI) diet to a low-carbohydrate diet in diabetics. The low GI diet was beneficial, but the low-carbohydrate diet was more beneficial. At any given level of carbohydrate intake, a low GI intake probably will have beneficial effects, but restricting the total amount of carbohydrates has a more direct effect because of the reduction of the supply of glucose.

Role of Vitamin D in Muscle Strength and Function

Michael Holick, PhD, MD

It was well documented at the turn of last century that one of the physical findings associated with rickets was severe muscle weakness. Children with rickets had a difficult time standing and were at higher risk for upper respiratory tract infections due in part to poor muscle tone of their diaphragm and accessory muscles for breathing.[1] In the 1930s exposure to ultraviolet radiation was used by some Olympic teams to improve the performance of their athletes. At this time it was thought that because severe vitamin D deficiency caused hypocalcemia, which increased neuromuscular irritability, that it was vitamin D's effect on calcium metabolism that was important for maximizing muscle strength.[1]

In the early 1970s it was realized that vitamin D that was made in the skin or came from the diet required two obligate hydroxylations before it became active on regulating calcium and phosphorus metabolism and maintaining bone health (Fig 1).[2]

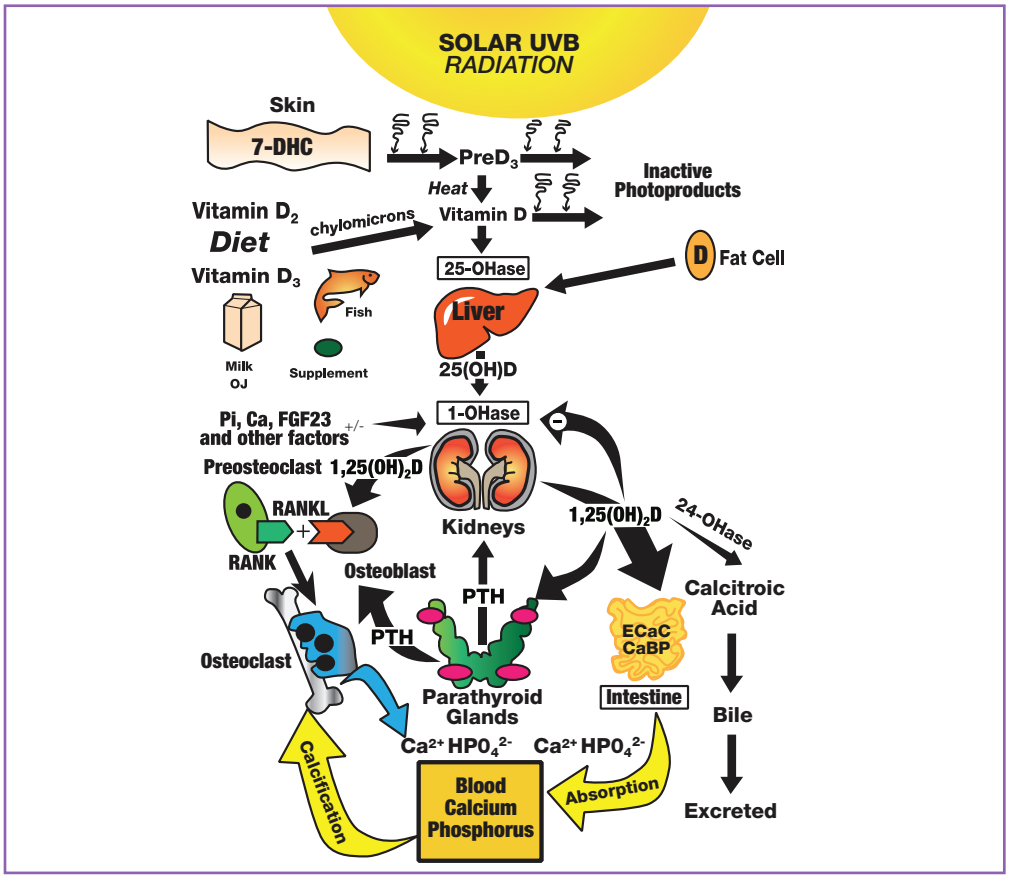

Fig 1. Schematic representation of the synthesis and metabolism of vitamin D for regulating calcium, phosphorus, and bone metabolism. During exposure to sunlight 7-dehydrocholesterol in the skin is converted to previtamin D_3. Previtamin D_3 immediately converts by a heat dependent process to vitamin D_3. Excessive exposure to sunlight degrades previtamin D_3 and vitamin D_3 into inactive photoproducts. Vitamin D_2 and vitamin D_3 from dietary sources are incorporated into chylomicrons and transported by the lymphatic system into the venous circulation. Vitamin D (D represents D_2 or D_3) made in the skin or ingested in the diet can be stored in and then released from fat cells. Vitamin D in the circulation is bound to the vitamin D binding protein, which transports it to the liver where vitamin D is converted by the vitamin D-25-hydroxylase to 25-hydroxyvitamin D [25(OH)D]. This is the major circulating form of vitamin D that is used by clinicians to measure vitamin D status. (Although most reference laboratories report the normal range to be 20-100 ng/mL, the preferred healthful range is 30-60 ng/mL.) It is biologically inactive and must be converted in the kidneys by the 25-hydroxyvitamin D-1α-hydroxylase (1-OHase) to its biologically active form 1,25-dihydroxyvitamin D [$1,25(OH)_2D$]. Serum phosphorus, calcium fibroblast growth factors (FGF-23), and other factors can either increase (+) or decrease (-) the renal production of $1,25(OH)_2D$. $1,25(OH)_2D$ feedback regulates its own synthesis and decreases the synthesis and secretion of parathyroid hormone (PTH) in the parathyroid glands. $1,25(OH)_2D$ increases the expression of the 25-hydroxyvitamin D-24-hydroxylase (24-OHase) to catabolize $1,25(OH)_2D$ to the water soluble biologically inactive calcitroic acid, which is excreted in the bile. $1,25(OH)_2D$ enhances intestinal calcium absorption in the small intestine by stimulating the expression of the epithelial calcium channel (ECaC) and the calbindin 9K (calcium binding

protein; CaBP). 1,25$(OH)_2$D is recognized by its receptor in osteoblasts, causing an increase in the expression of receptor activator of NF-κB ligand (RANKL). Its receptor RANK on the preosteoclast binds RANKL, which induces the preosteoclast to become a mature osteoclast. The mature osteoclast removes calcium and phosphorus from the bone to maintain blood calcium and phosphorus levels. Adequate calcium and phosphorus levels promote the mineralization of the skeleton.

Vitamin D first enters the liver where it is hydroxylated to form the major circulating form of vitamin D, 25-hydroxyvitamin D [25(OH)D]. 25(OH)D travels to the kidneys where it is converted to its active form, 1,25-dihydroxyvitamin D [1,25$(OH)_2$D].[2-4] 1,25$(OH)_2$D enters the bloodstream and travels to its target tissues, where it interacts with a nuclear vitamin D receptor (VDR) to alter gene expression. In the small intestine 1,25$(OH)_2$D enhances the efficiency of intestinal calcium absorption to about 30% to 40% and phosphorus absorption to ~80%.[2] In the skeleton 1,25$(OH)_2$D interacts with its nuclear receptor in osteoblasts increasing the expression of receptor activator of nuclear factor kappa B (NF-κB) ligand (RANKL), which binds to the preosteoclast's RANK, which in turn induces the formation of mature osteoclasts (Fig 1).[2,5] Once formed osteoclasts release enzymes and hydrochloric acid (HCl) to dissolve the matrix and mineral, resulting in the release of calcium and phosphorus into the circulation.

Vitamin D and Muscle Function

In the 1980s it was recognized that embryonic chick skeletal muscle had a vitamin D receptor (VDR).[6] Immunohistochemical evaluation of human skeletal muscle revealed the presence of a VDR[7] and researchers observed a significant reduction in the VDR with increased age from 21 to 91 years.[8] Mice that were genetically engineered to have no VDR had small and variable muscle fibers that were demonstrated to be independent of calcium and phosphorus levels.[9] The muscle phenotype in these mice that lacked VDR also had a persistence of immature muscle gene expression during adult life.[10] These abnormalities persisted even when the mice were placed on a high-calcium diet so that their calcium metabolism was corrected. VDR genotype was associated with muscle strength in non-obese older women, with a 23% difference in quadriceps strength between bb and BB genotype for the VDR.[11] These observations collectively suggested that vitamin D played an important role in the maintenance of skeletal muscle function that was independent of its effect on regulating calcium and phosphorus metabolism. El-Hajj Fuleihan et al[12] reported on bone health and lean body mass in 170 girls ages 10-17 years who were randomized to receive weekly oral vitamin D_3 doses of 1400 IU or 14,000 IU in a double-blind placebo-controlled 1-year study. In the overall group of

girls lean body mass increased significantly but not grip strength. The blood levels of 25(OH)D reached 38 ± 31 ng/mL in the group that received an equivalent of 2000 IU of vitamin D3 a day compared to 17 ± 6 ng/mL in the group that received an equivalent of 200 IU of vitamin D3 a day. The researchers concluded that vitamin D supplementation for 1 year resulted in substantial increases in lean body mass as well as bone area and bone mass in girls ages 10-17 years without any toxicity. An evaluation of serum 25(OH)D in 99 post-menarchal 12-14 year old females revealed a positive relationship with jump velocity, jump height, and power. A 75% reduction in power was observed in girls who had a blood level of 25(OH)D <15 ng/mL (Fig 2).[13]

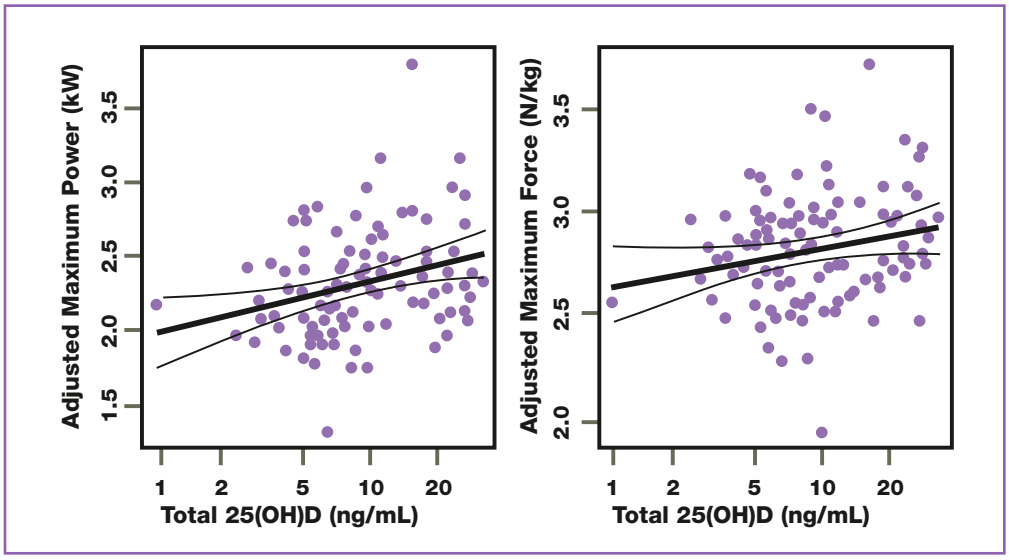

Fig 2.[13] The positive relationship between 25(OH)D status and muscle power (left panel, P = 0.003) and force (right panel, P = 0.05), after adjustment for weight as quadratic term. 25(OH)D=25-hydroxy vitamin D

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These data are consistent with a longitudinal aging study from Amsterdam that reported a 30%-50% reduction in grip strength and loss of appendicular skeletal muscle mass in elders who had a serum 25(OH)D <20ng/mL.[14] Stewart et al[15] evaluated 231 healthy postmenopausal women ages 45-65 years on the relationship between serum 25(OH)D levels and overall fitness. They found that 19% and 44% of the women were vitamin D deficient and insufficient respectively, consistent with what has been previously observed in children and adults throughout the United States.[2] They observed that 25(OH)D was a common

contributor to physical fitness indices including androidal fat mass, whole body lean mass, and balance and in grip strength in healthy postmenopausal women.

The observations that human skeletal muscle had a VDR and that it decreased with age[8] set the stage for observational studies demonstrating that higher blood levels of 25(OH)D were associated with improved muscle strength and lower extremity function. An evaluation of NHANES III revealed a dose response relationship between serum 25(OH)D levels and improvement in the ability to walk 8 feet or to get from sitting to standing position.[16] In the 4100 ambulatory adults 60 years and older the poorest muscle function was observed when their 25(OH)D was <20 ng/mL. Improvement in muscle function was observed in the reference range of 9-37 ng/mL.[7] These association studies were followed by several double-blind randomized controlled trials demonstrating that increased vitamin D intake to 800 IU/d improved muscle strength and balance and reduced risk of falling by as much as 72%.[8,17] A meta-analysis of five high-quality trials demonstrated that 400 IU of vitamin D a day did not appear to have any benefit and that the threshold for improvement of skeletal health was observed when the vitamin D intake was at least 800 IU of vitamin D a day.[16] An evaluation of physical performance in older people revealed a more than 200% improvement in physical performance when 25(OH)D levels were between 28-32 ng/mL (Fig 3).[18]

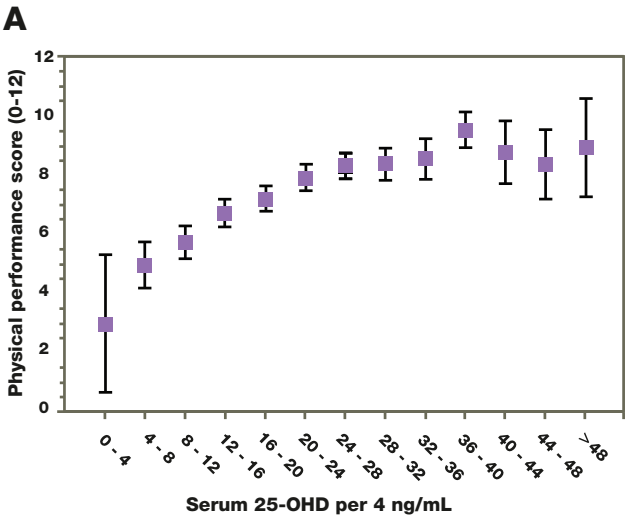

Fig 3.[14] Physical performance in 1234 older people in relation to 25(OH)D. Shown are CI for the mean. Adjusted for age, gender, number of chronic diseases, degree of urbanization, BMI, and alcohol consumption. CI=confidence interval, BMI=body mass index, 25(OH)D=25-hydroxy vitamin D

Vitamin D Deficiency Pandemic and Other Health Consequences

Vitamin D deficiency is now being recognized as one of the most common if not most common medical conditions worldwide. It has been estimated that 30%-100% of children, young, middle-aged, and older adults are vitamin D deficient [25(OH)D <20 ng/mL] or insufficient [25(OH)D = 21-29 ng/mL].[2]

Fifty million teenagers in the United States were found to be vitamin D deficient or insufficient[19] and have a 240% increased risk of having high blood pressure, high blood sugar, and blood biochemistries consistent with metabolic syndrome (pre type 2 diabetes).[20] Vitamin D deficiency has been associated with a 50% increase risk of developing and dying from prostate, colon, breast, and other deadly cancers.[21-24] Vitamin D deficiency and insufficiency have also been linked to hypertension and heart disease.[25-27] There is a 50% increased risk of having a heart attack and more than 100% increase risk of dying from a heart attack if the patient is vitamin D deficient.[28,29] An evaluation of peripheral vascular disease revealed an 80% reduced risk when the 25(OH)D was >29 ng/mL. Autoimmune diseases including multiple sclerosis, rheumatoid arthritis, Crohn's disease, and type I diabetes as well as type II diabetes also have been associated with vitamin D deficiency.[30-34] Men and women who ingested more than 800 IU of vitamin D and 1000 mg of calcium a day reduced their risk of type 2 diabetes by 33%.[34]

Treatment and Prevention of Vitamin D Deficiency

There are a multitude of causes of vitamin D deficiency with devastating health consequences (Fig 4).[2]

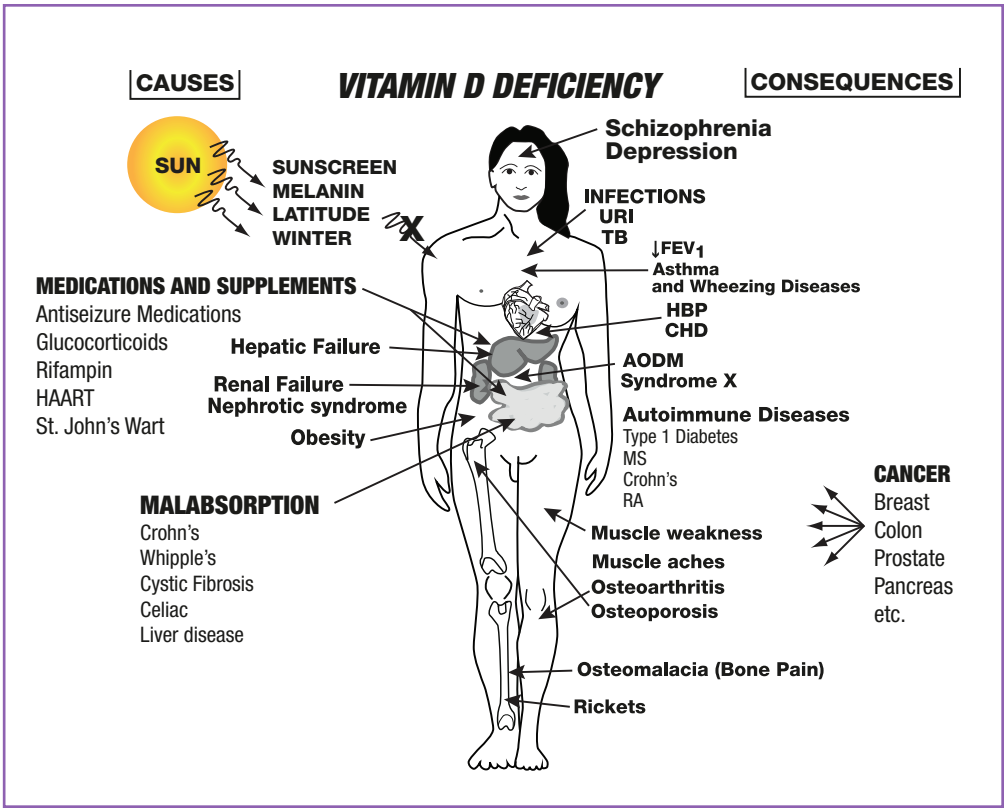

Fig 4. Schematic representation of the major causes of vitamin D deficiency and potential health consequences. HAART=highly active retroviral therapy, URI=upper respiratory (tract) infection, TB=tuberculosis, FEV=forced expiratory volume, HBP=high blood pressure, CHD=coronary heart disease, AODM=adult onset diabetes mellitus, MS=multiple sclerosis, RA=rheumatoid arthritis

It has been estimated that for every 100 IU of vitamin D/d ingested the serum level of 25(OH)D increases by 1 ng/mL.[35] Vitamin D_2 and vitamin D_3 are equally effective in raising and maintaining blood levels of 25(OH)D when given to children and adults.[36,37]

To treat vitamin D deficiency 50,000 IU of vitamin D_2 once a week for 8 weeks is effective in raising the blood levels to >30 ng/mL in non-obese children and adults.[36-39] Recurrence of vitamin D deficiency can be prevented by giving 50,000 IU

of vitamin D_2 once every 2 weeks. We observed after 6 years on this medical regimen that blood levels were maintained between 40 and 60 ng/mL without any toxicity.[39]

Conclusion

Fractures in the elderly are a major problem that has huge economic and health consequences.[40] Men and women who fracture a hip have a 20% risk of dying within the first year and 50% never have the quality of life they once had. It is estimated that 50% of hip fractures is due to falling. Vitamin D deficiency causes proximal muscle weakness, making it more difficult to stand from a sitting position and increases risk of sway. Therefore vitamin D deficiency not only precipitates and exacerbates osteopenia and osteoporosis but increases risk of fracture due to decreased muscle strength and imbalance.

Now that vitamin D deficiency has been linked with so many chronic illnesses a major effort is needed to re-educate both health care professionals and the public about the beneficial effects of sunlight and vitamin D supplementation for health.[2,41] Based on the most recent literature 1000 IU of vitamin D a day will not maintain blood levels of 25(OH)D >30 ng/mL in healthy adults who are not exposed to vitamin D producing sunlight.[36] Thus adults need at least 1500-2000 IU of vitamin D a day. Children require at least 400 IU of vitamin D a day and preferably 1000 IU of vitamin D a day to improve their overall health and well-being, including muscle strength.

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References

1. Bischoff-Ferrari HA, Willet WC, Wong JB, et al: Prevention of nonvertabral fractures with oral vitamin D and dose dependency. *Arch Int Med* 2009;169(6):551-561.
2. Holick MF: Vitamin D deficiency. *N Engl J Med* 2007;357:266-281.
3. Dusso AS, Brown AJ, Slatopolsky E: Vitamin D. *Am J Physiol Renal Physiol* 2005;289:F8-F28.
4. Jones G: Expanding role for vitamin D in chronic kidney disease: Importance of blood 25-OH-D levels and extra-renal 1α-hydroxylase in the classical and nonclassical actions of 1α,25-dihydroxyvitamin D_3. *Semin Dial* 2007;20(4): 316-324.
5. Khosla S: The OPG/RANKL/RANK system. *Endocrinology* 2001;142(12): 5050-5055.

6. Boland R, Norman A, Ritz E, Hausselbach W: Presence of 1,25-dihydroxyvitamin D3 receptor in chick skeletal muscle myoblasts. *Biochem Biophys Res Comm* 1985;128:305–311.
7. Bischoff-Ferrari HA, Dawson-Hughes B, Staehelin HB, et al: Fall prevention with supplemental and active forms of vitamin D: A meta-analysis of randomized controlled trials. *BMJ* 2009;339:843-851.
8. Bischoff HA, Stähelin HB, Dick W, et al: Effects of vitamin D and calcium supplementation on falls: A randomized controlled trial. *J Bone Min Res* 2003;18:343.
9. Endo I, Inoue D, Mitsui T, et al: Deletion of vitamin D receptor gene in mice results in abnormal skeletal muscle development with deregulated expression of myoregulatory transcription factors. *Endocrinology* 2003;144(12):5138-5144.
10. Kato S, Takeyama K, Kitanaka S, et al: In vivo function of VDR in gene expression-VDR knock-out mice. *J Steroid Biochem Mol Biol* 1999;69:247-251.
11. Geusens P, Vandevyver C, Vanhoof J, et al: Quadriceps and grip strength are related to vitamin D receptor genotype in elderly nonobese women. *J Bone Miner Res* 1997;12(12):2082-2088.
12. El-Hajj Fuleihan G, Nabulsi M, Tamim H, et al: Effect of vitamin D replacement on musculoskeletal parameters in school children: A randomized controlled trial. *J Clin Endocrinol Metab* 2006;91:405–412.
13. Ward KA, Das G, Berry JL, et al: Vitamin D status and muscle function in post-menarchal adolescent girls. *J Clin Endocrinol Metab* 2009;94(2):559-563.
14. Wischerts IS, van Schoor NM, Boeke AJP, et al: Vitamin D status predicts physical performance and its decline in older persons. *J Clin Endocrinol Metab* 2007;92(6):2058-2065.
15. Stewart JW, Alekel DL, Ritland LM, et al: Serum 25-hydroxyvitamin D is related to indicators of overall physical fitness in healthy postmenopausal women. *Menopause* 2009;16(6):1093-1101.
16. Bischoff-Ferrari HA, Dietrich T, Orav EJ, et al: Higher 25-hydroxyvitamin D concentrations are associated with better lower-extremity function in both active and inactive persons aged≥60 y. *Am J Clin Nutr* 2004;80(3):752-758.
17. Broe KE, Chen TC, Weinberg J, et al: A higher dose of vitamin D reduces the risk of falls in nursing home residents: A randomized, multiple-dose study. *J Am Geriatr Soc* 2007;55(2):234-239.
18. Visser M, Deeg DJH, Lips P: Low vitamin D and high parathyroid hormone levels as determinants of loss of muscle strength and muscle mass (sarcopenia): The Longitudinal Aging Study Amsterdam. *J Clin Endocrinol Metab* 2003;88(12):5766-5772.
19. Reis JP, von Mühlen D, Miller ER: Vitamin D status and cardiometabolic risk factors in the United States adolescent population. *Pediatrics* August 3, 2009 [Epub ahead of print].
20. Melamed ML, Muntner P, Michos ED, et al: Serum 25-hydroxyvitamin D levels and the prevalence of peripheral arerial disease: Results from NHANES 2001 to 2004. *Arterioscler Thromb Vasc Biol* 2008;28(6):1179-1185.

21. Gorham ED, Garland CF, Garland FC,et al: Optimal vitamin D status for colorectal cancer prevention: A quantitative meta analysis. *Am J Prev Med* 2007;32(3):210-216.
22. Giovannucci E, Liu Y, Rimm EB, et al: Prospective study of predictors of vitamin D status and cancer incidence and mortality in men. *J Natl Cancer Inst* 2006;98(7):451-9.
23. Grant WB: Lower vitamin-D production from solar ultraviolet-B Irradiance may explain some differences in cancer survival rates. *J Nat Med Assoc* 2006;98(3):357-364.
24. Garland CF, Comstock GW, Garland FC, et al: Serum 25-hydroxyvitamin D and colon cancer: Eight-year prospective study. *Lancet* 1989;2:1176-1178.
25. Zittermann A: Vitamin D and disease prevention with special reference to cardiovascular disease. *Prog Biophy Mol Biol* 2006;92:39-48.
26. Scragg R, Holdaway I, Jackson R, Lim T: Plasma 25-hydroxyvitamin D3 and its relation to physical activity and other heart disease risk factors in the general population. *Ann Epidemiol* 1992;2(5):697-703.
27. Rostand SG: Ultraviolet light may contribute to geographic and racial blood pressure differences. *Hypertension* 1997;30(2 pt 1):150-156.
28. Wang TJ, Pencina MJ, Booth SL, et al: Vitamin D deficiency and risk of cardiovascular disease. *Circulation* 2008;117(4):503-511.
29. Dobnig H, Pilz S, Scharnagl H, et al: Independent association of low serum 25-hydroxyvitamin D and 1,25-dihyroxyvitamin D levels with all-cause and cardiovascular mortality. *Arch Intern Med* 2008;168(12):1340-1349.
30. Munger KL, Levin LI, Hollis BW, et al: Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. *JAMA* 2006;296:2832-2838.
31. Merlino LA, Curtis J, Mikuls TR, et al, Iowa Women's Health Study: Vitamin D intake is inversely associated with rheumatoid arthritis. *Arthritis Rheum* 2004;50(1):72-77.
32. Cantorna MT, Zhu Y, Froicu M, Wittke A: Vitamin D status, 1,25-dihydroxyvitamin D3, and the immune system. *Am J Clin Nutr* 2004;80(suppl):1717S-1720S.
33. Hyppönen E, Läärä E, Reunanen A, et al: Intake of vitamin D and risk of type 1 diabetes: A birth-cohort study. *Lancet* 2001;358:1500-1503.
34. Pittas AG, Lau J, Hu FB, Dawson-Hughes B: The role of vitamin D and calcium in type 2 diabetes: A systematic review and meta-analysis. *J Clin Endocrinol Metab* 2007;92:2017-2029.
35. Heaney RP, Davies KM, Chen TC, et al: Human serum 25-hydroxycholecalciferol response to extended oral dosing with cholecalciferol. *Am J Clin Nutr* 2003;77:204-210.
36. Holick MF, Biancuzzo RM, Chen TC, et al: Vitamin D2 is as effective as vitamin D3 in maintaining circulating concentrations of 25-hydroxyvitamin D. *J Clin Endocrinol Metab* 2008;93(3):677-681.
37. Gordon CM, Williams AL, Feldman HA, et al: Treatment of hypovitaminosis D in infants and toddlers. *J Clin Endocrinol Metab* 2008;93(7):2716-2721.

38. Malabanan A, Veronikis IE, Holick MF: Redefining vitamin D insufficiency. *Lancet* 1998;351:805-806.

39. Pietras SM, Obayan BK, Cai MH, Holick MF: Vitamin D2 treatment for vitamin D deficiency and insufficiency for up to 6 years. *Arch Intern Med* 2009;169:1806-1808.

40. Rosen CJ: Postmenopausal osteoporosis. *N Eng J Med* 2005;353:595-603.

41. Holick MF, Chen TC, Sauter ER: Vitamin D and skin physiology: A D-lightful story. *J Bone Miner Res* 2007;22(S2):V28-V33.

Q & A

Q: Dr Holick, can you comment on the vitamin D receptor? Is there resistance in the receptor that can determine the effectiveness of the supplementation of vitamin D?

Dr Holick: The most dominant factor that may be related to vitamin D status probably is the polymorphism for the vitamin D binding protein. You probably are aware that the vitamin D binding protein is an alpha globulin, and it is a major component of the protein makeup of the blood. It looks as if the polymorphism for it helps determine vitamin D status much more so than almost anything else. Vitamin D receptor polymorphism studies have been interesting but often have not had enough subjects to show what is going on. Thus, there have been a lot of studies positive and negative for the various polymorphisms. So we do not yet have good enough data to be able to say that vitamin D receptor polymorphism is related to vitamin D status. Vitamin D binding protein polymorphism certainly does.

Q: We talk about vitamin D deficiency in muscle weakness, but given that all cells have the receptor, do we know whether this is a cell-autonomous event or something on the bone cell that is somehow cross-talking to the muscle? Also, if the receptor is in the muscle, is it regulating mitochondrial genes or other genes that prevent that weakness?

Dr Holick: The vitamin D receptor knockout mouse shows significant structural abnormalities in the skeletal muscle, implying that vitamin D is playing some role in skeletal muscle itself. We do not know at a molecular level how vitamin D is playing a role in muscle function or in muscle strength. It has been suggested that hypocalcemia or, more importantly, hypophosphatemia in a person with vitamin D deficiency might be the major cause of muscle dysfunction. However, studies in rodents show that if you correct calcium and phosphorus metabolism by putting the animals on a high-calcium and high-phosphorus diet, that you still can see some of these defects.

Q: But research with a knock out still would not address what is happening in the actual cell, correct? It could happen in another cell and you could just be missing another factor. So has anyone done even simple experiments on cultured cells to show that if you deplete this receptor and then give the cell vitamin D, you do lose something?

Dr Holick: Research from Argentina, mainly in muscle cells from chicks, has suggested that if you culture them and you do not have 1,25-dihydroxyvitamin D they do not grow and function as well.

Q: That is strength, myofibrillar content, or what?

Dr Holick: It is thymidine incorporation and protein synthesis.

Q: That could be proliferation. Was that done on differentiated muscle or flat myoblasts?

Dr Holick: Myoblasts.

Q: We have sick people in the hospital who stay there and do not get outside. Sometimes they stay in the ICU for 6 or 8 weeks. We try to include vitamin D in some of the formulas, but to be honest, patients are on a lot different things. Do you know what vitamin D levels are likely in hospitalized patients? Is there some relationship between vitamin D levels and, say, falls or in-hospital mortality?

Dr Holick: The half-life of 25-hydroxyvitamin D in the circulation is about 2 weeks. So you could pretty much predict levels. One research group studied inpatients and found that most of them were vitamin D deficient. The problem is that most of the population is vitamin D deficient. We do know that people who are vitamin D deficient have increased risk of mortality, and I suspect that in an ICU, vitamin D deficiency probably plays a significant role in weakness, infirmity, and general health outcomes. It is unfortunate that people have not realized that giving them vitamin D is a simple fix.

Q: Would you suggest that anybody who goes into the ICU or even just the hospital should get 50,000 units of vitamin D automatically? We measure vitamin D on everybody coming out of our ICU. I think I have seen a level of 20 once, and that is the highest I have seen. If the ICU people would get the message, we could at least fix these patients as they come into the hospital.

Dr Holick: Absolutely. Even more frustrating for us is that the orthopedic surgeons will send these patients out after repairing a hip fracture and never think about their vitamin D status. We and others have shown that they are all vitamin D deficient coming in, and they are all vitamin D deficient going out.

Nutrition, Muscle Mass, and Muscular Performance in Middle Age and Beyond

Catherine Johnson, PhD, RD, LD

Aging is associated with many changes in body composition, including reduction of lean body mass with a concomitant increase in fat mass.[1] These changes often have a negative impact on overall health and functional capacity.

Sarcopenia is the degenerative loss of skeletal muscle and strength, beginning as early as age 30, and accelerating with advancing age. Advancing sarcopenia is associated with increased risk of fall and fractures, decreased ability to complete activities of daily living, and increase in fatigue, which all lead to dependency and disability.[2]

A lifestyle behavior that positively affects muscle mass is consumption of dietary protein. Longitudinal studies have shown that older people who consume higher amounts of protein lose less lean muscle mass over 3 years than those who eat lower amounts (eg, 91 g/day vs 57 g/day).[3] Protein quality, quantity, and timing of consumption throughout the day and in conjunction with physical activity are all important to maintenance of muscle mass. Protein sources of high biologic value, namely those from animal sources, will provide the highest concentration of branched-chain amino acids such as leucine, which stimulate muscle protein synthesis.[4,5]

Milk proteins, whey and casein, are shown to stimulate muscle protein synthesis. Both are high-quality proteins and should be consumed daily. However, they produce a different response in young people than they do in older people. Whey, for instance, is digested faster than casein and produces a relatively better response on protein balance in older people. Casein has the opposite effect and has a better response in younger people.[6,7]

The goal of protein consumption and lean body mass gains is to optimize muscle protein synthesis. The quantity of essential amino acids (EAAs) is critical to elicit muscle protein synthesis. Elderly people may require 20–30 g of high-quality protein containing at least 8 g of EAAs, including leucine, three or four times a day.[4,8] Another strategy to maximize muscle protein synthesis is consumption of protein-rich meals more frequently, every 2 or 3 hours.[9]

Leucine is the primary amino acid regulator that "turns on" protein synthesis in the cells, signaling that quality protein is available for protein synthesis. Research shows that adding leucine to a meal that combines carbohydrate and protein is not necessary to get a response in protein synthesis in younger people, but it is necessary to get the same response in older people.[10] These results suggest that the protein-synthesis response is blunted in older people when a meal combines carbohydrate with protein. Reducing simple carbohydrates may be an advantageous strategy to maximize protein synthesis, because this also is shown to reduce loss of lean tissue for people with a negative caloric intake.[9-12]

A minor metabolite of L-leucine, β-hydroxy-β-methylbutyrate (HMB), is a precursor of cholesterol synthesis in skeletal muscle and plays a role in the control of protein homeostasis (Fig 1).[13]

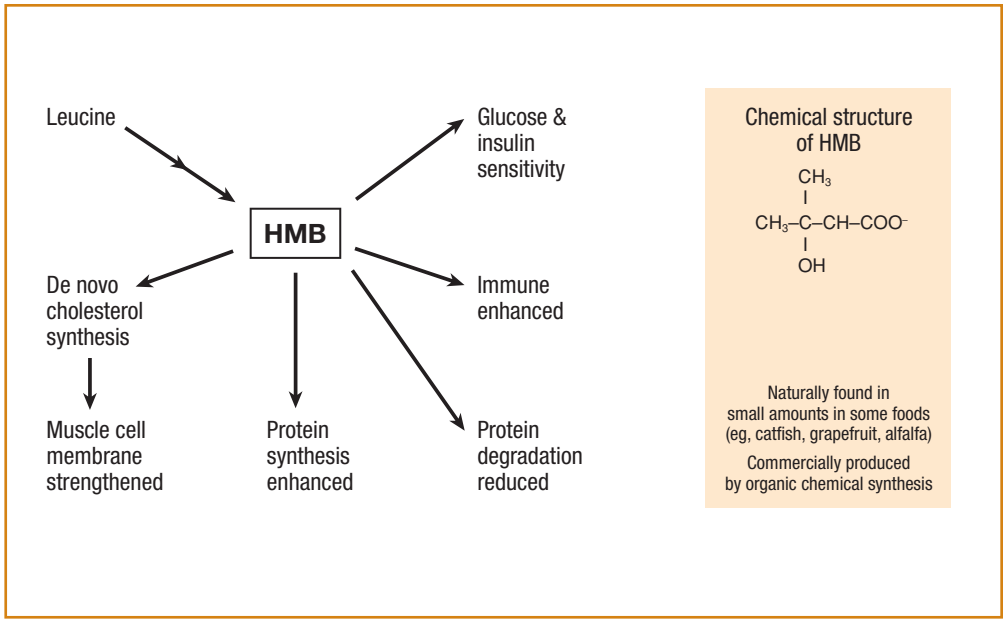


Fig 1. Sources and functions of HMB.[13] HMB=β-hydroxy-β-methylbutyrate

HMB is shown to decrease protein degradation by downregulation of the ubiquitin-proteasome system (Fig 2).[14] It also is shown to stimulate protein synthesis by activation of mammalian target of rapamycin (mTOR), a serine/threonine protein kinase.[15,16]

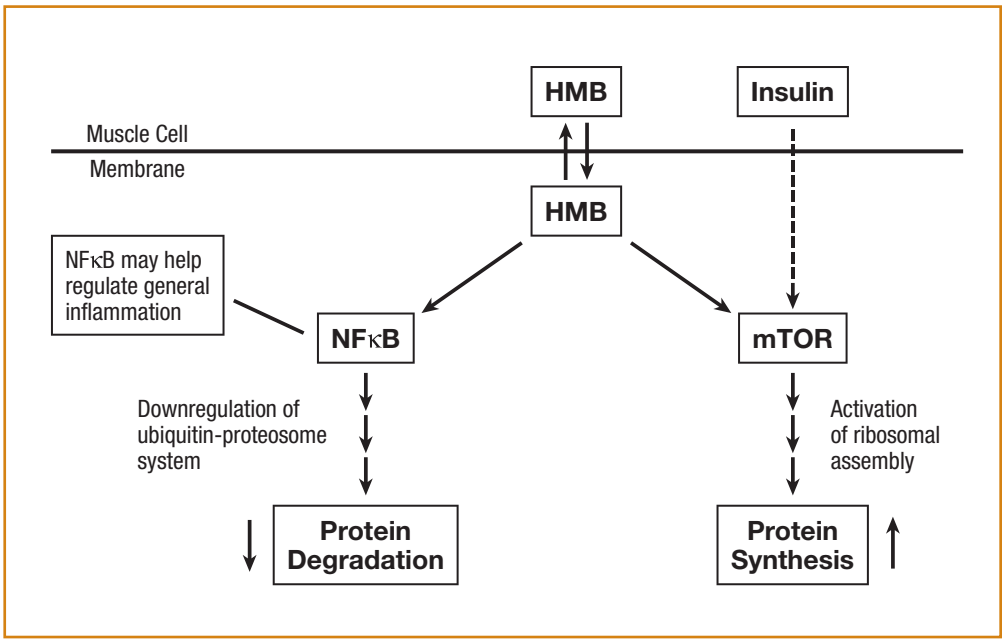


Fig 2. Role of HMB in protein synthesis and degradation. NF-κB=nuclear factor kappa B, HMB = β-hydroxy-β-methylbutyrate, mTOR=mammalian target of rapamycin

Oral administration of HMB is strongly associated with increased strength and lean body mass (LBM) and with decreased fat mass in young- to middle-aged people when combined with resistance exercise. HMB has also been shown to have clinical benefit in a number of muscle wasting/cachectic conditions and in limb immobilization.[14,17,18] These mechanisms appear to be relevant to older people, and clinical studies have demonstrated decreases in body fat percentage, gains in lower- and upper-body strength, increases in limb circumference, leg and handgrip strength, and increases in "get-up-and-go" performance with HMB.[13,19,20] The get-up-and-go functionality assessment involves measuring the time it takes a person to rise from a chair, walk a specified distance, and then return to the chair.

Fig 3 shows the improvements in functionality that resulted when 50 elderly women were supplemented with 2 g HMB, along with arginine and lysine, daily for 12 weeks.[19]

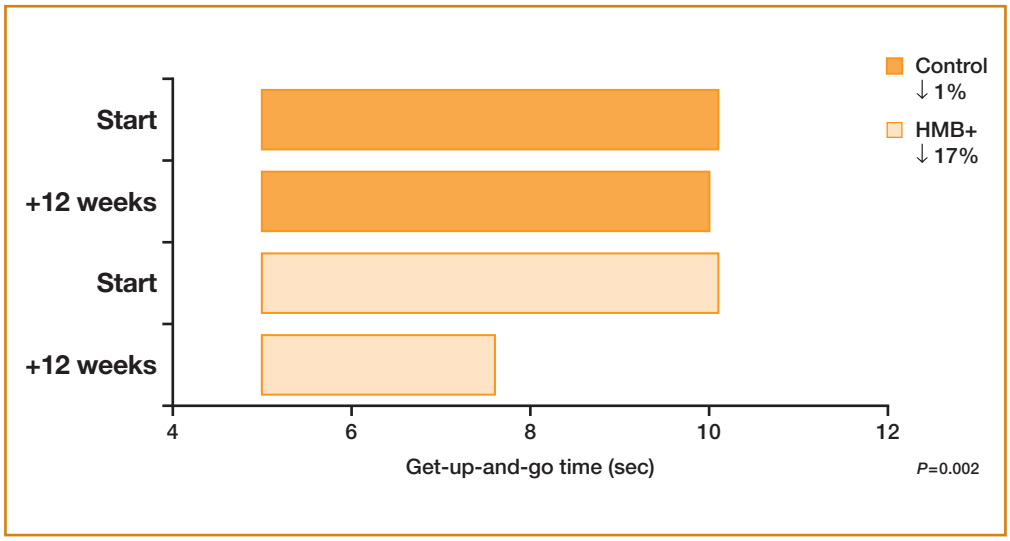


Fig 3. Changes in functionality after 12 weeks of supplementation with HMB+ (HMB, arginine, and lysine) compared to controls.[19] sec=seconds, HMB=β-hydroxy-β-methylbutyrate

After 12 weeks of supplementation, the "get-up-and-go" functionality test results improved by 17% in the experimental group (2.3 +/- 0.5 seconds), but did not change in the placebo group (P=0.002). Improved functionality also was reflected in increased limb circumference, leg strength, and handgrip strength (each P<0.05).

The current recommended dosage for HMB is 3 g/day.[13] This recommendation is based on the finding that increasing HMB from 1.5 g to 3 g increases strength and LBM, while doses greater than 3 g/day do not have an additional effect.[13]

Exercise also is known to increase muscle mass. Muscle mass gains can be maximized by combining the consumption of protein, amino acids, or HMB in close proximity to a session of resistance exercise. An increase in muscle protein synthesis can last up to 36 to 48 hours after a bout of intense exercise. Providing amino acids immediately before or after exercise can increase muscle protein synthesis approximately 2.5 times greater than the effect from exercise alone.[21,22] In the elderly, consumption of protein has to occur immediately after exercise to realize the benefits.[21-23]

In summary, several lifestyle strategies are recommended to promote optimal muscle protein synthesis, including consumption of the following:

- Adequate amounts of high-quality protein, essential amino acids/branched-chain amino acids/leucine, and supplemental HMB
- Protein-rich meals and snacks every 2–3 hours to maximize muscle protein balance
- Moderate amounts of carbohydrate for energy (insulin secretion) and to spare protein from being used for energy
- Foods containing a mixture of protein and carbohydrates 40–120 minutes before exercise and immediately after exercise to increase strength and hypertrophy

References

1. Kyle UG, Genton L, Hans D, et al: Age-related differences in fat-free mass, skeletal muscle, body cell mass and fat mass between 18 and 94 years. *Eur J Clin Nutr* 2001;55:663-672.
2. Hunter GR, McCarthy JP, Bamman MM: Effects of resistance training on older adults. *Sports Med* 2004;34:329-348.
3. Houston DK, Nicklas BJ, Ding J, et al: Dietary protein intake is associated with lean mass change in older, community-dwelling adults: The Health, Aging, and Body Composition (Health ABC) Study. *Am J Clin Nutr* 2008;87:150-155.
4. Symons TB, Schutzler SE, Cocke TL, et al: Aging does not impair the anabolic response to a protein-rich meal. *Am J Clin Nutr* 2007;86:451-456.
5. Paddon-Jones D, Rasmussen BB: Dietary protein recommendations and the prevention of sarcopenia. *Curr Opin Clin Nutr Metab Care* 2009;12:86-90.
6. Dangin M, Boirie Y, Guillet C, Beaufrère B: Influence of the protein digestion rate on protein turnover in young and elderly subjects. *J Nutr* 2002; 132:3228S-3233S.
7. Dangin M, Guillet C, Garcia-Rodenas C, et al: The rate of protein digestion affects protein gain differently during aging in humans. *J Physiol* 2003; 549:635-644.
8. Paddon-Jones D, Sheffield-Moore M, Urban RJ, et al: Essential amino acid and carbohydrate supplementation ameliorates muscle protein loss in humans during 28 days bedrest. *J Clin Endocrinol Metab* 2004;89:4351-4358.
9. Solerte SB, Gazzaruso C, Bonacasa R, et al: Nutritional supplements with oral amino acid mixtures increases whole-body lean mass and insulin sensitivity in elderly subjects with sarcopenia. *Am J Cardiol* 2008;101:69E-77E.
10. Koopman R, Verdijk L, Manders RJ, et al: Co-ingestion of protein and leucine stimulates muscle protein synthesis rates to the same extent in young and elderly lean men. *Am J Clin Nutr* 2006;84:623-632.

11. Koopman R, Wagenmakers AJ, Manders RJ, et al: Combined ingestion of protein and free leucine with carbohydrate increases postexercise muscle protein synthesis in vivo in male subjects. *Am J Physiol Endocrinol Metab* 2005;288:E645-E653.

12. Layman DK: The role of leucine in weight loss diets and glucose homeostasis. *J Nutr* 2003;133:261S-267S.

13. Wilson GJ, Wilson JM, Manninen AH: Effects of β-hydroxy-β-methylbutyrate (HMB) on exercise performance and body composition across varying levels of age, sex, and training experience: A review. *Nutr Metab* (Lond) 2008;5:1.

14. Smith HJ, Mukerji P, Tisdale MJ: Attenuation of proteasome-induced proteolysis in skeletal muscle by β-hydroxy-β-methylbutyrate in cancer-induced muscle loss. *Cancer Res* 2005;65:277-283.

15. Kornasio R, Riederer I, Butler-Browne G, et al: β-hydroxy-β-methylbutyrate (HMB) stimulates myogenic cell proliferation, differentiation and survival via the MAPK/ERK and PI3K/Akt pathways. *Biochim Biophys Acta* 2009;1793:755-763.

16. Eley HL, Russell ST, Tisdale MJ: Attenuation of depression of muscle protein synthesis induced by lipolysaccharide, tumor necrosis factor, and angiotensin II by β-hydroxy-β-methylbutyrate. *Am J Physiol Endocrinol Metab* 2008;295:E1409-E1416.

17. Hsieh LC, Chien SL, Huang MS, et al: Anti-inflammatory and anticatabolic effects of short-term β-hydroxy-β-methylbutyrate supplementation in chronic obstructive pulmonary disease patients in intensive care unit. *Asia Pac J Clin Nutr* 2006;15:544-550.

18. May PE, Barber A, D'Olimpio JT, et al: Reversal of cancer-related wasting using oral supplementation with a combination of β-hydroxy-β-methylbutyrate, arginine, and glutamine. *Am J Surg* 2002;183:471-479.

19. Flakoll P, Sharp R, Baier S, et al: Effect of β-hydroxy-β-methylbutyrate, arginine, and lysine supplementation on strength, functionality, body composition, and protein metabolism in elderly women. *Nutrition* 2004;20: 445-451.

20. Vukovich MD, Stubbs NB, Bohlken RM, et al: Body composition in 70-year old adults responds to dietary β-hydroxy-β-methylbutyrate (HMB) similarly to that of young adults. *J Nutr* 2001;131:2049-1935.

21. Biolo G, Maggi SP, Williams BD, et al: Increased rates of muscle protein turnover and amino acid transport after resistance exercise in humans. *Am J Physiol* 1995;268:E514-E520.

22. Biolo G, Tipton KD, Klein S, Wolfe RR: An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. *Am J Physiol* 1997;273:E122-E129.

23. Esmarck B, Andersen JL, Olsen S, et al: Timing of postexercise protein intake is important for muscle hypertrophy with resistance training in elderly humans. *J Physiol* 2001;535:301-311.

Discussion

Leader: Christine Steele, PhD, Abbott Nutrition, Columbus, OH

Dr Wheeler: Dr Phillips, a lot of the information you shared about the benefits of resistance training and nutrient timing and so forth seems to be relevant for young and middle-aged fit people. What kind of exercises can the elderly do? Can they do enough to see the same benefits? If, in fact, they can benefit, how do we begin to educate people about it?

Dr Phillips: We are sure that with exercise older people can make gains, sustain function, and do very well, but older people have to consider the combination of exercise and nutrition. Older people start off at a lower baseline than younger people. They also may have some underlying pathologies such as insulin resistance that would be barriers to maximizing the full potential of their nutrition. One understudied population that we know can make gains is older women. We have a hard time discerning how postmenopausal women gain any lean mass at all. It is difficult. We have underestimated the ability of estrogen and progesterone, which older women lose, to act as anabolic stimuli. But older men retain some of that, and circulating testosterone.

However, they can all make gains. I think that the timing of the nutrition is important, probably more important than in younger people. I also think we have opportunities to develop exercise strategies that may be more broadly applicable. If you can stand up and lower yourself to about 90° with a bent-knee squat and then stand up, that is about 30% of single repetition maximum (1RM). Try doing that to failure. Everybody in this room could do that, and you would find that their knees kind of burned afterward. Why not ask elderly people to do that? They do not need to go to the gym to do it.

Dr Suetta: I disagree with Dr Phillips about the intensity of resistance training for elderly people. Robust data—solid evidence—exists that shows we should advise elderly people to aim for at least 80% of 1RM, because that level increases muscle mass and achieves muscular gains most effectively. There is a statement about this from the American College of Sports Medicine (ACSM). Some studies have shown this even in frail elderly and very elderly people. So we have enough evidence to say that 30% of 1RM might increase muscle mass a little, but not as much as fairly heavy resistance training intensities.

Discussion

Dr Phillips, you showed that the muscle protein synthesis rate increased with both 90% and 30% of 1RM, and you stated that neither of those intensities increased muscle mass much.

Dr Phillips: Oh, but they did.

Dr Suetta: Not as much as training with around 70% to 80% of 1RM. Could you speculate on what the synthesis rate would have been if you had tested at 80% of 1RM?

Dr Phllips: We tested at 90% of 1RM, which is only 10% higher, so you would expect that the response at 90% would mirror that at 80%. There is nothing magic about 80%. In fact, studies using lower intensities have never taken the intensity to fatigue. That is the whole point of 30% of 1RM to fatigue. If you consider the size principle, 30% of 1RM to fatigue begins to recruit type II fibers in an orderly manner, much the same way that 90% does. So the ACSM position aside, which I think is based on poorly reviewed evidence, no one has compared studies using lower intensities performed to fatigue. However, a large body of literature exists describing research that used intensities of around 20% of 1RM and used, for example, vascular occlusion to show that they could induce fatigue that achieved substantial hypertrophy. In fact, some colleagues of yours did a study in which they exercised young men at 16% of 1RM and got a 2.5% increase in hypertrophy.

Dr Suetta: I am aware of that study, and it is interesting. But I would always advise elderly and frail people, who have only a certain amount of energy to train, to do the optimal thing. I would never advise them to train at an intensity of 30% of 1RM. Later, I will present data showing that one of the side effects of resistance training is gains in muscle function and power, but not with an intensity of 30% of 1RM. There are good reasons to go to the gym and not just do stand-ups.

Dr Phillips: You do not gain at 30% of 1RM if you only do 10 reps, but our subjects are failing after 23 reps. So the onus is on me to prove to you that 30% to failure is enough to increase strength.

Dr Suetta: Do you think they increase muscle power?

Dr Phillips: Not power. Even exercising at 80% of 1RM will not increase power. You have to perform fast and train powerfully to increase power.

Dr Suetta: I disagree. A lot of evidence has demonstrated that older individuals gain power and explosive muscle strength (rate of force development) by training at intensitities of 60%-80% 1RM, including several studies from our own lab [Caserotti

P et al: *Scand J Med Sci Sports* 2008;18:773-782; Suetta C et al: *J Appl Physiol* 2004;97:1954-1961; de Vos NJ et al: *J Gerontol A Biol Sci Med Sci* 2005;60:638-647]. An interesting study by de Vos et al compared different training intensities in old individuals and clearly showed that training at around 80% of 1RM increases muscle power much more effectively than any other intensity [*J Gerontol A Biol Sci Med Sci* 2005;60:638-647].

Dr Phillips: If they trained at 30% of 1RM but did it fast, then I would argue that they would increase power. That is the definition of power—force developed rapidly. So if someone trains at 80% doing slow repetitions up and down, they do not increase power as much as if they trained specifically for power at lower percentages of 1RM so they could move the weight rapidly (ie, developing maximal power). The proliferation of mitochondria might actually be greater when exercising at 30% of 1RM rather than at 80%, and thus the aerobic stimulus is greater at 30%. So exercise at 30% 1RM produces two benefits—increased strength and muscle mass, and increased mitochondrial content.

Dr Suetta: Again, I disagree with you. I think this point is very crucial, because there are many people who seem to misunderstand how you get improvements in muscle power and rate of force development. As I just mentioned, several studies have demonstrated that heavy-resistance training improves muscle power and explosive muscle strength (rate of force development [RFD]).

Notably, Macaluso and De Vito reported a load intensity of 60% maximal isometric voluntary contraction as the optimal load to produce the largest lower limbs' muscle power in older women [*Eur J Appl Physiol* 2003;90:458-463]. When optimal load for power development was assessed according to the 1RM method, one study reported that the greatest lower limbs' muscle power was achieved using a load intensity of 70% 1RM in elderly males [Izquierdo M et al: *Acta Physiol Scand* 1999;167:57-68]. Another advantage with this type of training is that you gain about three times more muscle mass and muscle strength compared to training at low intensities at about 30% of 1RM.

Dr Phillips: What if I told you we have the fractional synthetic rate of both mitochondria and myofibrillar?

Dr Suetta: I think you might get a small increase in muscle mass and an even smaller increase in muscle function.

Dr Phillips: No one has shown that, so I guess the onus is still on me to prove it to you. If you look at studies in which exercise at 30% of 1RM been done to failure or

is done for a long time under tension, up and down, they show that people gain strength and gain mass. It is essentially the same mechanism as exercising at a higher intensity. It simply drives the protein synthetic process to be active in a type II fiber that normally would not be activated at that level. This will not happen if you do only 10 reps at 30%, but try doing 24 reps.

Dr Suzette Pereira [Abbott Nutrition]: Dr Holick, what is your take on providing vitamin D supplements to patients with end-stage renal disease? The kidney obviously is not functioning, and that is a main organ. You did mention that there are other tissues that can activate it.

Dr Holick: The clinical practice guidelines of the National Kidney Foundation recommend to all nephrologists that blood levels of 25-hydroxyvitamin D at all stages of chronic kidney disease need to be above 30 ng/mL, apart from those who need to get an active vitamin D analogue later in their disease. So we urge all nephrologists and primary care physicians to be alert to this and to confirm that their patients have at least 30 ng/mL, even if they have no kidney function. We believe that the parathyroid glands have 1-hydroxylase activity, and we will suppress some of the parathyroid hypertrophy by increasing 25-hydroxyvitamin D levels.

Dr Baracos: I have a question for Dr Phillips and Dr Volek. I am impressed by the elegance of the nutritional, metabolic, and physiological approaches you have taken, adjusting the composition of protein and other elements of the diet with the aim of increasing muscle mass and muscle function. You do that in people I see as having no medical need—recreationally active males and some categories of athletes. Is there a reason why you do not level your sights on the hosts of people who have consequential muscle wasting and loss of function? Is this because of resources or interests? I may be incorrect, but I perceive that if you look at the scope of literature you talked about this morning, that 95% of it would have been done in normal healthy young men, and 5% would have been done in other people.

Dr Volek: I think that is a great question, and a multifactorial one. Both resources and interests play a role. I am not in a medical school, so I have access to college students, staff, and faculty—generally a healthy population. I guess this access has dominated our work with resistance training and nutritional interventions. Having said that, we also have studied bone and muscle in adolescents, looking at milk consumption and vitamin D supplementation, focusing, probably mistakenly, on calcium. And we have done resistance training studies in elderly people, as well.

Dr Phillips: I concur with Dr Volek. A lot of these protocols take 7 or 8 hours to do, and young college men have the time to do them, and they are willing to do six or

seven biopsies. We are doing some work in middle-aged women, and we have done some studies in elderly men and women employing the same principles as those used with younger subjects. We can establish proof of principle concepts in young male populations in which we can be fairly invasive, and then use those principles in other populations.

Dr Volek: There also is a prevention aspect that does not get as much play as it should. Our medical system really focuses on treatment, and I guess my personal interests lie more in prevention, in this case, prevention of muscle atrophy and muscle wasting diseases. Certainly resistance training and nutrition in relatively healthy adults can help prevent and attenuate the decline in muscle mass as people age.

Dr Reid: Dr Volek, if your group has not studied creatine in patient populations, have others? If so, to what degree has creatine been beneficial for sick people?

Dr Volek: Creatine research took off in 1992, so there is nearly 2 decades of literature. During the first 10 years or so, the research was almost exclusively in the realm of sports nutrition in athletes and increasing performance. But because of its mechanism of action, creatine was attractive for researchers studying muscular and neurodegenerative disorders. So in the last 5 or 10 years, interest has grown in muscular dystrophies and a variety of other disorders that seem to have a dysfunction in creatine metabolism associated with them. The National Institutes of Health has funded several studies of creatine in Parkinson's disease, and there have been some recent animal studies on memory and brain function. Some work has been done on muscle function and strength in elderly people that shows a pretty positive effect. So literature on clinical therapeutic applications of creatine in patient populations has grown.

Dr Morley: Potentially, I think the best data on sick and older people will come from studies being done by Evans and Wolfe in Arkansas. They are studying bed rest, which, as you know, causes older people to lose muscle mass rapidly. They have some data showing that a balanced amino acid supplement can attenuate this to a large degree. As Dr Holick will tell you, a problem with these studies is that nobody ever measures vitamin D first. And if we just gave these older people vitamin D, maybe we would not have any of these problems. Still, the reality probably is that both play a role. Hospital meta-analysis data clearly show that giving protein supplements and/or caloric supplements can improve mortality and hospital length of stay in sick older people. I think these supplements are grossly underused because physicians have nearly no interest or training in nutrition. We have failed to fix this. Nutrition is still a minor part of any medical school curriculum, and I think this has to be changed.

Challenges of Defining Sarcopenia: Status Report of the EUGMS Working Group on Sarcopenia

Tommy Cederholm, MD, PhD

Breakdown of muscle, bone, and fat are characteristic features of cachexia, sarcopenia, frailty, and starvation. These are overlapping conditions with unclear distinctions. The progressive decline of lean body mass leads to decreased mobility, impaired functionality, metabolic disturbances, and ultimately to death. In order to foster the understanding of sarcopenia and to develop treatment options, an operational definition of sarcopenia for both research and clinical practice is urgently needed.

For this purpose, the European Geriatric Medicine Society (EUGMS) has gathered a group of international experts in the field of geriatrics and nutrition (ie, the European Sarcopenia Working Group [ESWG]). Other European scientific organizations, such as the European Society of Clinical Nutrition and Metabolism (ESPEN), the International Academy of Nutrition and Aging (IANA), and the International Association of Gerontology and Geriatrics—European Region (IAGG-ER), have nominated representatives to the group.

The ESWG addresses the following questions:

- What is sarcopenia?
- Which items will define sarcopenia?
- What are the planned measurements of these items?
- How does sarcopenia relate to other diseases/conditions?

Definition of Sarcopenia

In 1989, Irwin Rosenberg proposed the term sarcopenia (Greek sarx or flesh + penia or loss) to describe the age-related decrease of muscle mass.[1] Sarcopenia has become a strong concept, but it still needs dissemination into public awareness, as well as into medical practice. The ESWG defines sarcopenia as a "syndrome characterized by progressive loss of muscle mass and strength with a risk of adverse outcomes." It is moreover defined as a geriatric syndrome (ie, a condition that is common, complex, and a costly state of impaired health in older individuals).[2] Geriatric syndromes result from incompletely understood interactions of disease

and age on multiple systems, producing a constellation of signs and symptoms. Delirium, falls, and incontinence are other examples of geriatric syndromes.

Discussion is ongoing about whether to restrict the definition of sarcopenia to mere age-related muscle wasting, and thus to distinguish sarcopenia from the muscle wasting that occurs with disease (cachexia), physical inactivity, bed rest, and starvation.[3,4] Alternatively, sarcopenia could serve as an umbrella concept including all forms of muscle wasting (Fig 1). So far, studies that have reported on prevalence of sarcopenia use muscle mass alone as the determinant. None distinguish the etiology of muscle wasting, mainly because of the probable fact that it is impossible to clearly separate the individual etiologic contributions to muscle wasting. This would argue for the use of sarcopenia as an umbrella concept. Moreover, if sarcopenia is viewed as an age-related phenomenon alone, the concept will mainly become an issue for gerontologists with relevance primarily for public health measures. Sarcopenia as an umbrella concept for all muscle wasting would enable sarcopenia to serve as a tool for physicians to use in clinical care.

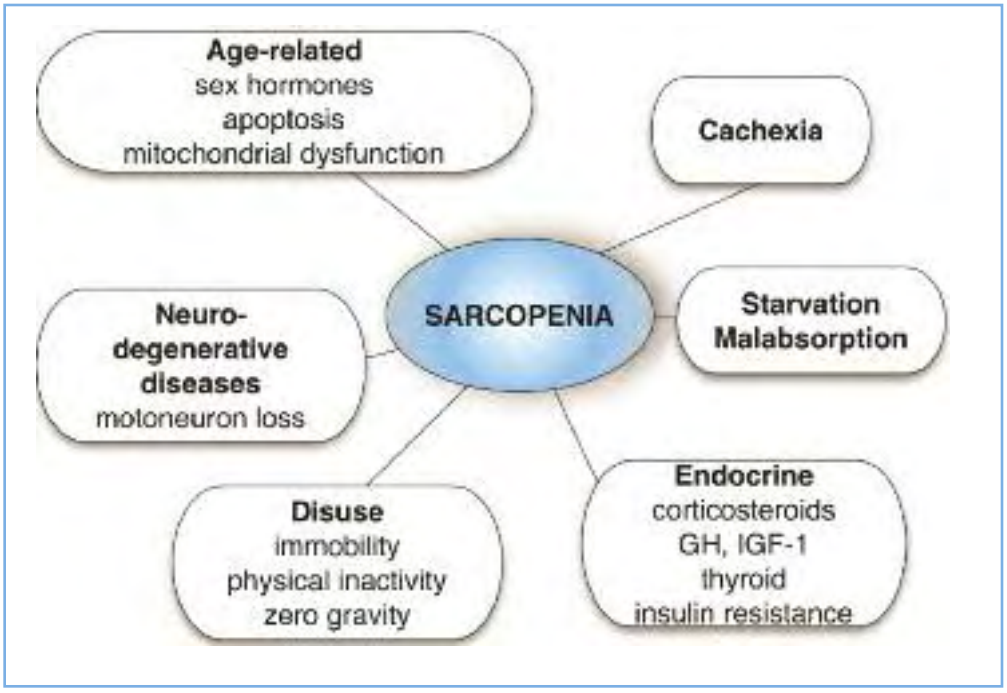


Fig 1. Sarcopenia as an umbrella concept with multiple etiologies.[5] GH=growth hormone, IGH-1=insulin-like growth factor 1

Items That Define Sarcopenia

Current definitions rest on muscle mass determinations. Mainly T-score definitions are used (eg, absolute muscle mass less than two standard deviations [SD] below the mean for healthy young men and women are used as cutoff).[6] The skeletal muscle mass index (SMI) (ie, skeletal muscle mass/body mass x 100) is suggested, where class I sarcopenia is defined as SMI within 1 to 2 SD of young adults, and class II sarcopenia as SMI <2 SD of young adults.[7] Schutz et al suggested the fat-free mass index (ie, fat-free mass/height2 <median of a reference population).[8]

The ESWG recommends an extension from muscle mass alone as the basis for the diagnosis. The alternative is to combine low muscle mass plus low muscle function (strength or performance) for the diagnosis. A suggestion is to use a diagnosis based on documentation of any two of the three criteria—low muscle mass, low muscle strength, or low physical performance. The Special Interest Group of Nutrition in Geriatrics of ESPEN recently adopted the combination of reduced muscle mass ≥2 SD below mean of percentage of muscle mass in young adults (in National Health and Nutrition Examination Survey [NHANES]) plus impaired muscle function as evidenced by 4-meter (m) walking speed of <0.8 m/second.

Measurement of Sarcopenia

Muscle mass is measured by computed tomography (CT) scan, magnetic resonance imaging (MRI), dual energy X-ray absorptiometry (DEXA), and bioimpedance analysis. For measurement of muscle strength, lower limbs are perhaps more relevant than upper limbs for gait and physical function. However, handgrip strength is widely used and is well correlated with most relevant outcomes. A wide range of tests of physical performance are available. The short physical performance battery (SPPB) evaluates balance, gait, strength, and endurance.[9] The SPPB recently was recommended by an international working group for use in clinical trials in frail older persons.[10] Usual gait speed may serve as an easier measurement and give enough information,[11] at least in the clinical setting. Further alternatives for potential measurement options, including the Timed-Up-and-Go Test, are described in the Table below.

Table. Measurements of Sarcopenia

Dimension	Research	Clinical Practice
Muscle mass	Anthropometry CT MRI Potassium DEXA BIA	Anthropometry Bioelectrical impedance analysis (BIA) DEXA
Muscle strength	Handgrip strength Knee flexion/extension (1RM) Peak expiratory flow	Handgrip strength Peak expiratory flow
Physical performance	SPPB Gait speed 6-minute walk Stair climbing Timed Up and Go	SPPB Gait speed Timed Up and Go

CT=computed tomography, MRI=magnetic resonance imaging, DEXA=dual energy X-ray absorptiometry, SPPB=short physical performance battery

Sarcopenia Subcategories and Staging

The ESWG has discussed possible subclassification and staging of sarcopenia. Because a single cause of sarcopenia is sometimes identified, whereas otherwise no evident cause is isolated, the categories of primary and secondary sarcopenia sometimes are useful. Sarcopenia is considered primary (or age-related) when no other cause is evident but aging itself, while sarcopenia is considered secondary when one or more causes are readily identified, such as disuse-related sarcopenia, disease-related sarcopenia, or starvation-related sarcopenia.

Sarcopenia staging (ie, determining the severity of the condition) also is discussed. A suggestion is to use the stages mild, moderate, and severe, or an alternative terminology of latent, preclinical, and clinical sarcopenia. These are issues that the coming work of the ESWG will solve and present in a consensus report.

Research Areas

The ESWG has indicated several areas of research for the development of the sarcopenia concept. Examples are definition of reference populations for mass, strength, and performance; longitudinal and cross-sectional studies of risk factors and associated conditions; new target populations such as post acute, nursing home, and sarcopenic obese individuals; and new validated diagnostic tools and standardization of some instruments.

As main outcome measures in intervention trials, physical performance (SPPB, gait speed) sometimes is advocated.[10] Secondary outcome measures include strength (handgrip, knee flexion), mass (DXA, CT, MRI), activities of daily living (basic, instrumental), quality of life, and mortality.

Suggested Screening Strategy

The ESWG may suggest gait speed measurement as the easiest and most reliable way to begin sarcopenia screening in practice (Fig 2). A cutoff of less than 0.8 m/second would identify risk for sarcopenia.[10,12] Coming work will settle these questions.

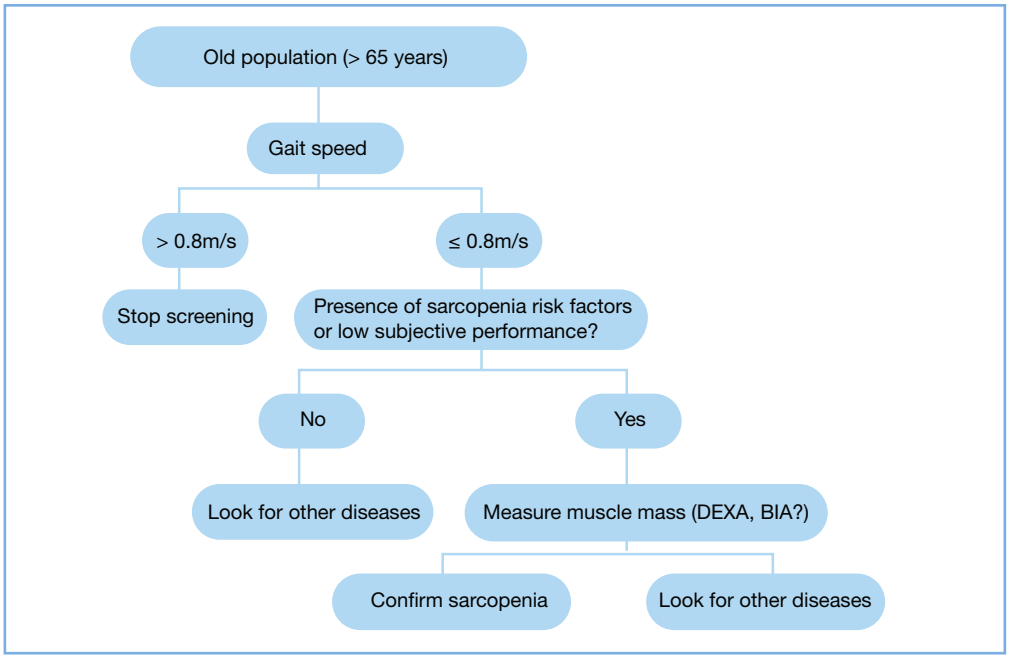


Fig 2. ESWG algorithm for sarcopenia screening in older individuals.

ESWG=European Sarcopenia Working Group, DEXA=dual energy X-ray absorptiometry, BIA= bioelectrical impedance analysis

Conclusion

The reported suggestion is the result of two 2-day seminars during the spring of 2009. The schedule is to have one more seminar and finalize the conclusions by early fall 2009, draft a consensus paper, and have it endorsed by the supporting scientific organizations before submission of the manuscript prior to the end of 2009.

References

1. Rosenberg IH: Sarcopenia: Origins and clinical relevance. *J Nutr* 1997;127 (suppl 5):990S-991S.
2. Inouye SK, Studenski S, Tinetti ME, Kuchel GA: Geriatric syndromes: Clinical, research, and policy implications of a core geriatric concept. *J Am Geriatr Soc* 2007;55:780-791.
3. Thomas DR: Loss of skeletal muscle mass in aging: Examining the relationship of starvation, sarcopenia and cachexia. *Clin Nutr* 2007;26:389-399.
4. Jensen GL: Inflammation: Roles in aging and sarcopenia. *JPEN* 2008;32: 656-659.
5. Muscaritoli M, Anker SD, Argilés J, et al. Consensus definition of sarcopenia, cachexia and pre-cachexia: joint document elaborated by Special Interest Groups (SIG) “cachexia-anorexia in chronic wasting diseases” and “nutrition in geriatrics.” *Clin Nutr* [Epub ahead of print.]
6. Baumgartner RN, Koehler KM, Gallagher D, et al: Epidemiology of sarcopenia among the elderly in New Mexico. *Am J Epidemiol* 1998;147:755-763.
7. Janssen I, Heymsfield SB, Ross R: Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability. *J Am Geriatr Soc* 2002;50:889-896.
8. Schutz Y, Kyle UU, Pichard C: Fat-free mass index and fat mass index percentiles in Caucasians aged 18-98 y. *Int J Obes Relat Metab Disord* 2002;26:953-960.
9. Guralnik JM, Simonsick EM, Ferrucci L, et al: A short physical performance battery assessing lower extremity function: Association with self-reported disability and prediction of mortality and nursing home admission. *J Gerontol* 1994;49:M85-M94.
10. Working Group on Functional Outcome Measures for Clinical Trials: Functional outcomes for clinical trials in frail older persons: Time to be moving. *J Gerontol A Biol Sci Med Sci* 2008;63:160-164.
11. Guralnik JM, Ferrucci L, Pieper CF, et al: Lower extremity function and subsequent disability: Consistency across studies, predictive models, and value of gait speed alone compared with the short physical performance battery. *J Gerontol A Biol Sci Med Sci* 2000;55:M221-M231.
12. Abellan van Kan G, Rolland Y, Andrieu S, et al: Gait speed at usual pace as a predictor of adverse outcomes in community-dwelling older people—an Internal Academy on Nutrition and Aging (IANA) Taskt Force. *J Nutr Health Aging* 2009;13:881-889.

Q & A

Q: I like the framework. The problem is the term “sarcopenia.” Sarcopenia has been used in multiple studies to look at muscle mass. It is hard to take a term that is in everybody’s mind as one thing and change it to another thing without confusing people.

Perhaps you should consider using another term. The newer term “dynapenia” may better fit what you are defining, because what you are defining is a change in strength and power. And you are close to defining frailty. In fact, I could take your definition and call it frailty, and very few people would disagree. So you are no longer defining sarcopenia, because you are now taking executive function into account. Once you take everybody with dementia into account, you are going to have major problems.

In my opinion, you need to stick to muscle, because it is very important. It should extend way beyond just old age, and particularly ICU and hospitalized patients.

You have to step back and say, would we be better off leaving sarcopenia as loss of muscle mass? There is good literature for that. It is a step, but it is not what we are interested in. We are interested in strength and power. Use things that would define power. I would use stair climb, for instance. If you use gait speed, it has got to be 1 m/second and not <0.8 m/second, because I think all of the recent literature has said the cutoff is at 1 m at this stage.

It is easier to come up with a new term than to change what everybody believes sarcopenia is. Whether you use “dynapenia” or you come up with a great European term, I think that would make a huge difference in the acceptability, certainly in the United States and most probably throughout the world.

Dr Cederholm: You can compare that to other processes of finding a feasible or acceptable definition. Discussion on improvement of osteoporosis has taken place, not only to go for bone mass, but to define osteoporosis.

Most of us acknowledge that this is actually a moving target and a process. I really do not share your thought in this way. Of course we have heard “dynapenia” suggested. “Myopenia” has been suggested. Sarcopenia is still a strong concept, and I think it would be acceptable to find a somewhat changed definition.

Q: You are misusing the etymology of the word, though, because sarcopenia is loss of tissue or loss of muscle mass. It has nothing to do with the other things, and it is really important to differentiate those.

We have heard many talks about how basically loss of muscle mass does not always equal loss of strength, does not always equal power. Tendons come in when you are starting to look at power, and I think you have got to be very careful not to lump these together because, as I say, once you lump them together, I think you have gone to frailty. You really need something to define the problems with muscle. I think you are going to struggle with "sarcopenia" as an acceptable term. It may happen, but it is going to cause confusion, and confusion is never good for a field.

Lean Body Mass Loss With Age

Douglas Paddon-Jones, PhD

Sarcopenia is an age-related, multifactorial process characterized by the progressive loss of lean tissue mass. The onset of sarcopenia is insidious, but its progression may be accelerated by physical inactivity and poor nutrition. Research continues to focus on the mechanisms contributing to sarcopenia, including changes in protein metabolism and cell signaling, voluntary or imposed reductions in physical activity, malnutrition, and reduced anabolic efficiency to protein ingestion.

Elderly individuals are at increased risk of becoming physically incapacitated or placed on bed rest for an extended period. The loss of lean body mass is dramatically increased during inactivity and is driven by a chronic imbalance between muscle protein synthesis and breakdown and facilitated by decreased activation of nutrient signaling pathway.[1-3] In recent studies examining changes in protein synthesis and muscle mass in healthy adults subjected to bed rest, older subjects experienced an approximate three-fold greater loss of lean leg muscle mass compared to a cohort of younger individuals confined to bed for 28 days (Figs 1 and 2).[2,4]

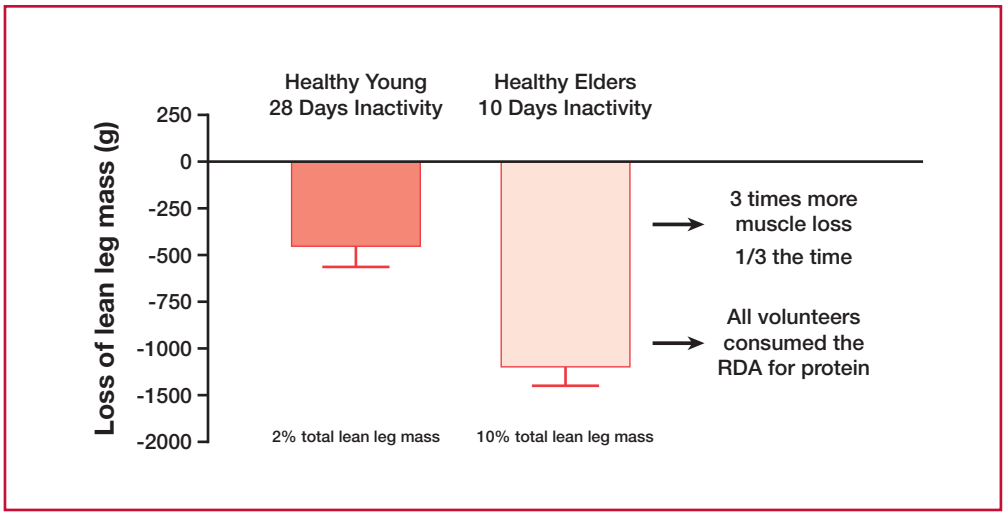


Fig 1. Inactivity and aging muscle. After 10 days of inactivity, older healthy subjects experienced an approximately three-fold greater loss of lean leg muscle mass than a cohort of younger individuals confined to bed for 28 days.[2,4] (1000 g=2.2 lb muscle loss)

Lean Body Mass Loss With Age

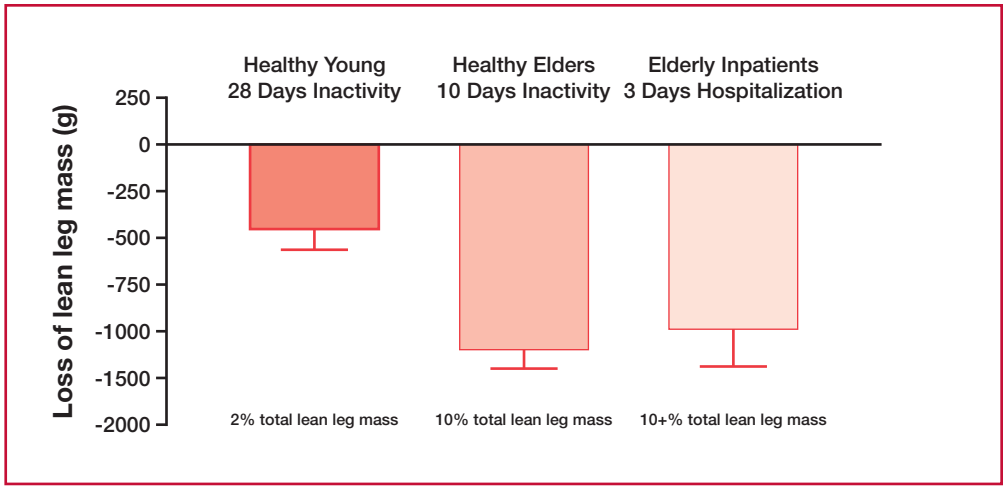


Fig 2. Muscle loss in hospitalized elders. After 3 days of hospitalization, elderly inpatients lost approximately the same amount of lean leg muscle mass as healthy older subjects experienced in 10 days of inactivity—approximately three-fold greater loss of lean leg muscle mass than a younger cohort confined to bed for 28 days.[2,4]

General consensus exists that a moderate-to-large serving of protein or amino acids increases muscle protein synthesis similarly in both young and elderly.[4-12] Unlike earlier *proof of concept* studies using free-form amino acid supplements, several recent studies have adopted a more practical approach and sought to examine the ability of protein-rich foods (eg, milk and beef) to stimulate protein anabolism. These studies are important as they more closely reflect responses to actual dietary practices and provide information on how meal choices may influence accrual of muscle mass and ultimately functional capacity. In one study directly comparing young and elderly, Symons et al[13] reported that a moderate 113 g (≈4 oz) serving of an intact protein (ie, lean beef) contains sufficient essential amino acids (EAAs) (30 g total; ≈12 g EAAs) to increase mixed-muscle protein synthesis by 50% in both young and elderly men and women (Fig 3).

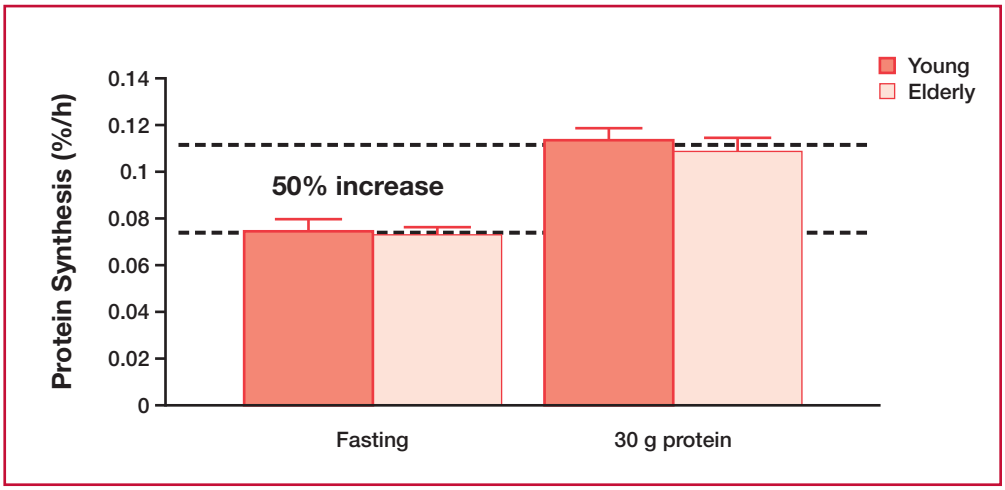


Fig 3. Aging does not impair the ability to increase muscle protein synthesis following ingestion of 113 g of lean beef (30 g protein). h=hour

The adequacy of the recommended dietary allowance (RDA) for protein has recently been the subject of renewed debate.[14-19] The current recommendation for protein intake for adults is 0.8 g/kg^{-1}/day^{-1}. While a modest increase in protein intake beyond 0.8 g/kg^{-1}/day^{-1} is likely to be beneficial for many elders, there is a greater need to specifically examine the dose and distribution of protein across each meal. For a 75-kg individual, the RDA represents 60 g protein/day, or if distributed evenly across three meals, 20 g protein/meal. A 20-g serving of most protein contains 5–8 g of EAAs, which are primarily responsible for stimulating muscle protein synthesis.[10] This is important because aging appears to be associated with an inability of skeletal muscle to respond to low doses of protein (<20 g) or EAAs (<8 g), whereas higher doses (protein >25 g; EAAs 10–15 g) are capable of stimulating muscle protein synthesis in older adults to a similar extent as in the young (Fig 4).[7,20]

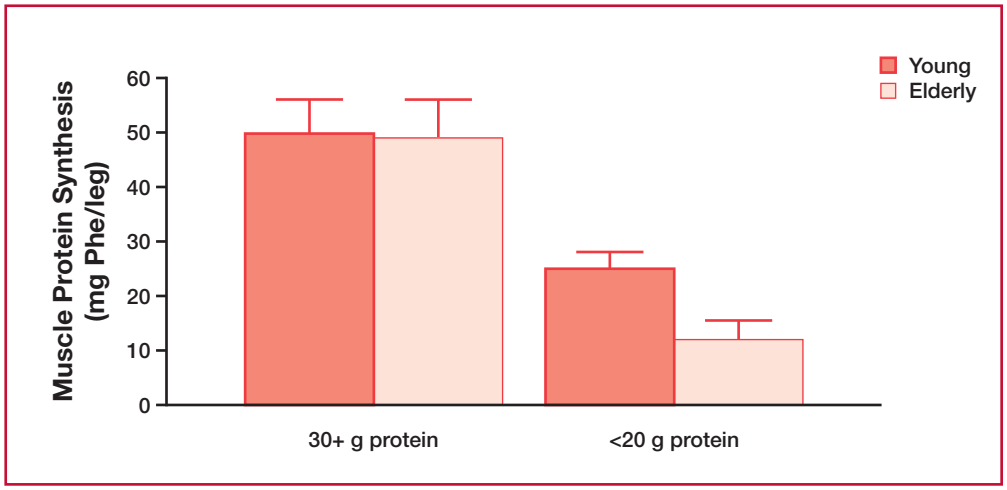


Fig 4. Older adults exhibit a blunted anabolic response to a lower "subthreshold" dose of amino acids or protein measured by the uptake of phenylalanine (mg Phe) per leg (adapted from Katsanos et al[20]).

To examine the effect of protein dose on muscle protein synthesis using a high-quality, protein-rich food, we demonstrated that a large single 340-g (≈12 oz) serving of lean beef (90 g protein) does not elicit a greater anabolic response in healthy young and elderly people than a serving one third the size.[21] This suggests that, despite the additional protein and energy content, ingestion of more than 30 g of protein in a single meal may be an energetically inefficient means of stimulating muscle protein synthesis. If we accept that 25–30 g of high-quality protein (≈10 g EAAs) are necessary to maximally stimulate skeletal muscle protein synthesis, then it seems reasonable to suggest that ingestion of this amount of high-quality protein at each meal could be a useful strategy to maintain muscle mass in the elderly (Fig 5).

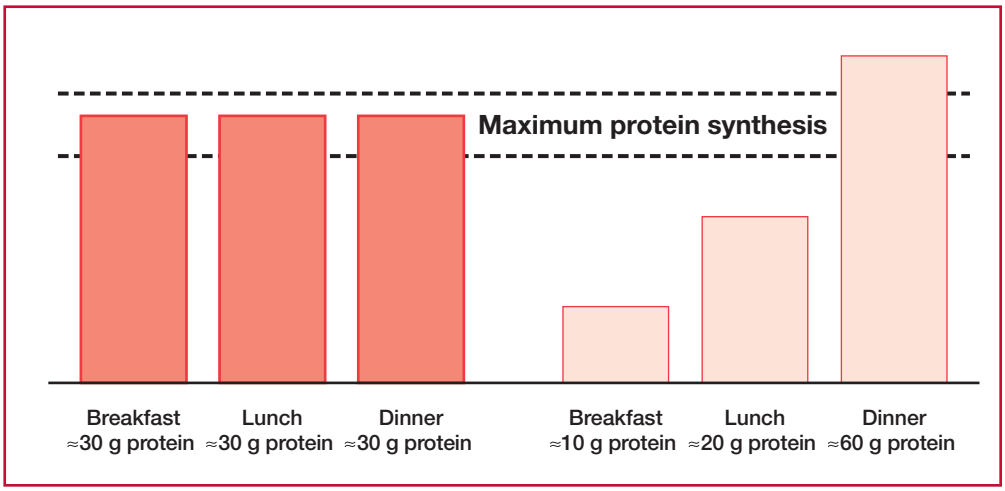


Fig 5. Ingestion of 90 g of protein, distributed evenly over three meals is more likely to provide a greater 24-hour protein anabolic response than an unequal protein distribution.

Thus, research indicates that ingestion of protein, consumed in adequate amounts over the course of a day, can ameliorate the effects of sarcopenia in older adults.

References

1. Dreyer HC, Glynn EL, Lujan HL, et al: Chronic paraplegia-induced muscle atrophy downregulates the mTOR/S6K1 signaling pathway. *J Appl Physiol* 2008;104:27-33.
2. Kortebein P, Ferrando A, Lombeida J, et al: Effect of 10 days of bed rest on skeletal muscle in healthy older adults. *JAMA* 2007;297:1772-1774.
3. Paddon-Jones D, Sheffield-Moore M, Cree MG, et al: Atrophy and impaired muscle protein synthesis during prolonged inactivity and stress. *J Clin Endocrinol Metab* 2006;91:4836-4841.
4. Paddon-Jones D, Sheffield-Moore M, Urban RJ, et al: Essential amino acid and carbohydrate supplementation ameliorates muscle protein loss in humans during 28 days bedrest. *J Clin Endocrinol Metab* 2004;89:4351-4358.
5. Paddon-Jones D, Sheffield-Moore M, Aarsland A, et al: Exogenous amino acids stimulate human muscle anabolism without interfering with the response to mixed meal ingestion. *Am J Physiol Endocrinol Metab* 2005;288:E761-E767.
6. Paddon-Jones D, Sheffield-Moore M, Katsanos CS, et al: Differential stimulation of muscle protein synthesis in elderly humans following isocaloric ingestion of amino acids or whey protein. *Exp Gerontol* 2006;41:215-219.
7. Paddon-Jones D, Sheffield-Moore M, Zhang XJ, et al: Amino acid ingestion improves muscle protein synthesis in the young and elderly. *Am J Physiol Endocrinol Metab* 2004;286:E321-E328.

8. Paddon-Jones D, Wolfe RR, Ferrando AA: Amino acid supplementation for reversing bed rest and steroid myopathies. *J Nutr* 2005;135:1809S-1812S.
9. Volpi E, Ferrando AA, Yeckel CW, et al: Exogenous amino acids stimulate net muscle protein synthesis in the elderly. *J Clin Invest* 1998;101:2000-2007.
10. Volpi E, Kobayashi H, Sheffield-Moore M, et al: Essential amino acids are primarily responsible for the amino acid stimulation of muscle protein anabolism in healthy elderly adults. *Am J Clin Nutr* 2003;78:250-258.
11. Volpi E, Lucidi P, Cruciani G, et al: Contribution of amino acids and insulin to protein anabolism during meal absorption. *Diabetes* 1996;45:1245-1252.
12. Volpi E, Mittendorfer B, Wolf SE, Wolfe RR: Oral amino acids stimulate muscle protein anabolism in the elderly despite higher first-pass splanchnic extraction. *Am J Physiol Endocrinol* 1999;277:E513-E520.
13. Symons TB, Schutzler SE, Cocke TL, et al: Aging does not impair the anabolic response to a protein-rich meal. *Am J Clin Nutr* 2007;86:451-456.
14. Campbell WW, Trappe TA, Wolfe RR, Evans WJ: The recommended dietary allowance for protein may not be adequate for older people to maintain skeletal muscle. *J Gerontol A Biol Sci Med Sci* 2001;56:M373-M380.
15. Wolfe RR: The underappreciated role of muscle in health and disease. *Am J Clin Nutr* 2006;84:475-482.
16. Wolfe RR, Miller SL: The recommended dietary allowance of protein: A misunderstood concept. *JAMA* 2008;299:2891-2893.
17. Leidy HJ, Carnell NS, Mattes RD, Campbell WW: Higher protein intake preserves lean mass and satiety with weight loss in pre-obese and obese women. *Obesity* (Silver Spring) 2007;15:421-429.
18. Thalacker-Mercer AE, Fleet JC, Craig BA, et al: Inadequate protein intake affects skeletal muscle transcript profiles in older humans. *Am J Clin Nutr* 2007;85:1344-1352.
19. Millward DJ, Layman DK, Tomé D, Schaafsma G: Protein quality assessment: Impact of expanding understanding of protein and amino acid needs for optimal health. *Am J Clin Nutr* 2008;87:1576S-1581S.
20. Katsanos CS, Kobayashi H, Sheffield-Moore M, et al: Aging is associated with diminished accretion of muscle proteins after the ingestion of a small bolus of essential amino acids. *Am J Clin Nutr* 2005;82:1065-1073.
21. Symons TB, Sheffield-Moore M, Wolfe RR, Paddon-Jones D: A moderate serving of high-quality protein maximally stimulates skeletal muscle protein synthesis in young and elderly subjects. *J Am Diet* Assoc 2009;109:1582-1586.

Effects of Unloading in Old Versus Young Humans

Leader: Charlotte Suetta, MD, PhD

The loss of muscle mass with aging (ie, sarcopenia and the concomitant decline in muscle strength) is associated with increased disability and mortality.[1,2] In addition, elderly individuals are more prone to periods of bed rest because of a higher degree of comorbidity and hospitalization,[2] which per se result in a rapid and accelerated loss of skeletal muscle mass.[3,4]

Despite this, very little is known about the physiological consequences of unloading on muscle mass and neuromuscular function in the elderly, while even less is known about the regenerative capacity of skeletal muscle in the elderly human being. Furthermore, although it is evident that aging leads to a multitude of changes in the neuromuscular system that are similar to those evoked by unloading,[5] the lack of research into the effect of unloading in elderly humans makes it difficult to ascertain what effects are attributed to a decreased physical activity per se and which to the aging process.

In the present study, concurrent data were obtained on the change in muscle contractile function, muscle size, central activation, and muscle architecture induced by unloading and retraining in old vs young human individuals, respectively.[6] It was the purpose to address the effect of aging on the magnitude of acute muscle disuse atrophy and the adaptive plasticity of subsequent exercise rehabilitation. By assessing these changes, we also aimed to study the potential interaction between changes in muscle contractile properties, specific force, and muscle mass characteristics after immobilization, and further to examine the regenerative capacity of old men (OM) compared to young men (YM).[6]

Immobilization was accomplished by 2 weeks of randomized unilateral whole-leg casting, using a light-weight fiber cast applied from just above the malleolus to just below the groin. Normal range of motion was obtainable at the knee joint. The retraining protocol was accomplished by 4 weeks of surveyed and supervised unilateral strength training on the immobilized leg, with three sessions each week. To induce a sufficient response in the thigh musculature, the training intensity was three to four sets x 12 reps in week 1 (15 repetition maximum [RM]), 5 sets x 10 reps in weeks 2 and 3 (12 RM), and 4 x 10 reps in week 4 (12 RM). Training load was adjusted on a weekly basis by the use of 5-RM tests.

Effects of Unloading in Old Versus Young Humans

In brief, both young and old subjects experienced decreases in maximal muscle strength, resting twitch peak torque, and twitch rate of force development, quadriceps muscle volume, muscle fiber pennation angle, and specific force after 2 weeks of unilateral lower limb immobilization (*P*=<0.05).[6] The decline in quadriceps volume (OM: -5.3%, YM: -8.9%) and muscle fiber pennation angle (OM: -6.5%, YM: -9.3%) was smaller in old compared to young (*P*=<0.05). In contrast, only OM experienced a decrease in quadriceps activation (OM: -9.9%, *P*=<0.05; YM: -1.0%, ns [ns=not significant]).[6] In addition, retraining induced smaller gains in quadriceps volume in old compared to young (OM: +3.8%, YM: +8.2 %, *P*=<0.05), and muscle fiber pennation angle increased in young only (YM: +12.0%, *P*=<0.05).[6]

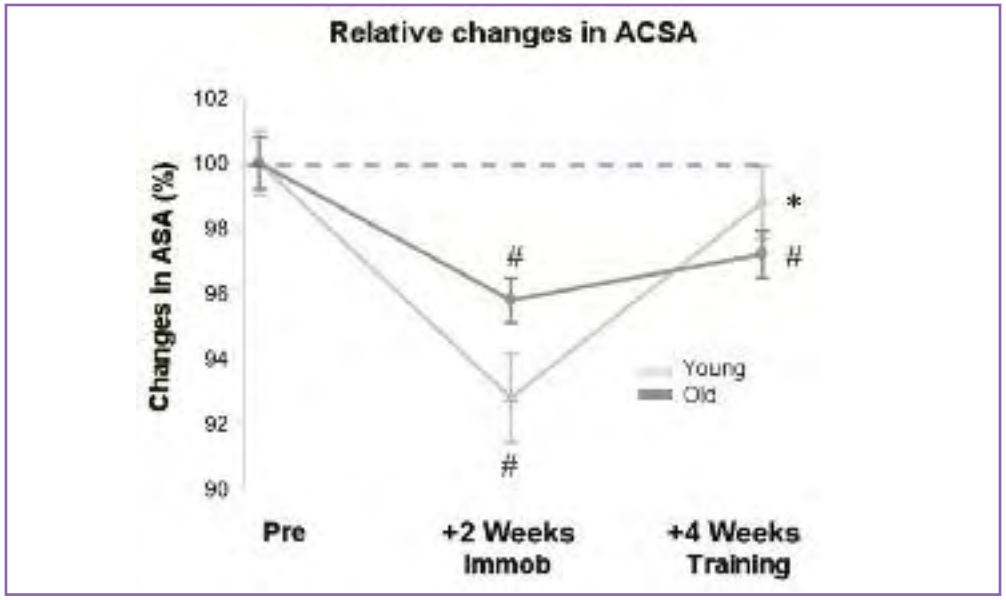


Fig. Changes in anatomical cross-sectional area prior to immobization (pre), after 2 weeks of immobilization, and after 4 weeks of retraining.[6]

Suetta C et al: Effects of ageing on human skeletal muscle after immobilization and retraining. *J Appl Physiol* 2009;107:1172. © 2009 American Physiological Society. Reprinted with permission.

In conclusion, the main and novel findings were that young subjects showed a greater magnitude of muscle atrophy and more marked changes in muscle fiber pennation angle after immobilization compared to old subjects, old subjects demonstrated a diminished capacity to restore muscle size and muscle architecture during subsequent retraining, and immobilization led to reduced muscle activation in old but not young subjects. Thus, the present data suggest that the adaptive

plasticity in skeletal muscle mass and central nervous system function associated with unloading and subsequent remobilization, respectively, may differ substantially between old and young individuals.

The present data thus indicate that aging affects the response to short-term immobilization and the regenerative capacity of human skeletal muscle. In the present study, young subjects demonstrated a greater reduction and subsequent increase in muscle mass because of immobilization and retraining, respectively, compared to old subjects. Conversely, it seemed neural function was more affected in old subjects, who showed a larger decline in neuromuscular activation than young subjects. Together these findings put forward that old individuals apparently are more affected with respect to neural function, whereas young individuals are more affected in terms of muscle size. Furthermore, the present data indicate that aging is accompanied by an impaired ability to recover from disuse muscle atrophy. Consequently, old individuals may need longer time to recover from periods of disuse compared to young individuals.

References

1. Janssen I, Heymsfield SB, Ross R: Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability. *J Am Geriatr Soc* 2002;50:889-896.
2. Manton KG, Corder LS, Stallard E: Estimates of change in chronic disability and institutional incidence and prevalence rates in the U.S. elderly population from the 1982, 1984, and 1989 National Long Term Care Survey. *J Gerontol* 1993;48:S153-S166.
3. Hill AA, Plank LD, Finn PJ, et al: Massive nitrogen loss in critical surgical illness: Effect on cardiac mass and function. *Ann Surg* 1997;226:191-197.
4. Okeefe SJ, Sender PM, James WP: Catabolic loss of body nitrogen in response to surgery. *Lancet* 1974;2:1035-1038.
5. Vandervoort AA: Aging of the human neuromuscular system. *Muscle Nerve* 2002;25:17-25.
6. Suetta C, Hvid LG, Justesen L, et al: Effects of ageing on human skeletal muscle after immobilization and retraining. *J Appl Physiol* 2009;107:1172-1180.

Q & A

Q: Thank you for the interesting study. Were the elderly and the young controlled for diet?

Dr Suetta: No, we did not control for diet.

Q: Did you measure any inflammatory cytokines or inflammatory markers and the effect of immobilization on them?

Dr Suetta: We have blood samples, and we have taken muscle biopsies as well. However, we do not have the data yet on cytokines or inflammatory response, but it is definitely something we will look into. That possibly explains some of the differences between our findings, that maybe bed rest increases inflammatory response to a greater degree than just unloading one limb.

Q: I think the issue about recovery in these older folks is really important. I was intrigued by the primary data that you showed from the explosive force production protocol. One of the things that was obviously different between the young and the old is that the original tracings from the old individual had a periodicity to them; so, there was an oscillatory characteristic to it that looked like the first inflection, which was most reproducible at about 80 or 100 milliseconds if I read it correctly, suggesting maybe a spinal reflex inhibition.

Do you see inhibitory effects on the EMGs [electromyograms]? Is this a neural component to the difference between the two age groups?

Dr Suetta: Yes, it seems like this is the case.

Q: I thought the satellite cell data was very intriguing. The thing that makes me wonder, and I do not know if anybody has ever done this, if you take satellite cells from these older individuals and grow them in culture, do they have a different replicative capacity? Can you see that they either develop less rapidly when you switch serum or that they replicate poorly, or that they do not form myofibrils as well? This is a very interesting finding.

Dr Suetta: I was waiting for that question. Irina Conboy's group from UC Berkely has done many of those studies in mice [Conboy IM et al: *Nature* 2005;433:760-764], and I was fortunate enough to be at Berkeley University last spring, where we came to talk and decided to see if the satellite cells are reacting the same way in

humans. We took some biopsies from old and young, and cultured them just as they previously did in mice.

It seems like if you culture the satellite cells from old humans in their old serum, they do not grow very well. However, if they are cultured in young sera, they grow very well [Carlson et al: *EMBO Mole Med* 2009;1:381-391], which means that there is definitely something in the systemic environment that activates them.

Q: Well, that is even more fantastic. Are you in the process of trying to identify that substance?

Dr Suetta: Irina Conboy group has shown that it seems like the Notch and TGF-β control each other [Carlson ME et al: *Nature* 2008;454:528-532]. What they find in mice is that Notch is lowered and TGF-β is increased. What we recently published together is that the same pattern is seen in human muscle tissue [Carlson et al: *EMBO Mole Med* 2009;1:381-391]. What is even more interesting is that this difference actually is bonded with the resistance training. So, resistance training is very potent in many ways.

Q: The satellite cells, as I understand it, are really two types of satellite cells now with some of the newer staining techniques, one of which seems to be a little more potent than the other. How did you stain for your satellite cells? And which of the two types? The one sort of looks like a true muscle, as I understand it, and the other one is sort of a questionable muscle? I am not very good at it, but clearly they are becoming different.

Dr Suetta: It is kind of new for me, too. In Copenhagen, we stain with NCAM or Pax7. Right now we are trying to compare the two stainings, but with these methods, we are not able to distinguish between the satellite cells.

Q: If you are suggesting that there is a neural component that is going awry because of immobilization, then do you really believe that giving them protein, like Dr Paddon-Jones has suggested, over this period of time would actually help correct for loss of strength or loss of power in the muscle? Or is there something else we need to be looking at?

Dr Suetta: That is a very good question. My opinion is that we need to provide patients with a protein supplement to counteract a decrease in muscle mass, because the size of a given muscle mass is strongly correlated to the force it can produce. However, despite similar decreases in maximal muscle strength in young

and old individuals after the immobilization period, the old individuals experienced a larger decrease in qualitative muscle strength (rate of force development), which indicates that a neural component definitely exists and is very important for muscle function. To my point of view, the neural component is not affected by dietary supplementation, but needs to be maintained with exercise.

Q: Is anyone exploring that right now to see if there are things that could be done to help?

Dr Suetta: It is quite simple. Several studies have shown, including some of our own [Suetta C et al: *J Appl Physiol* 2004;97:1954-1961] that resistance training during a hospitalization can counteract a decrease in both muscle mass and muscle function.

Q: I am intrigued as to why sarcopenia occurs, and I know very little about it. If you take a cross section of a muscle and measure the number of muscle fibers between a young and an old person, are they the same number but thinner in the older person? Or do you see a reduced number of muscle fibers per unit area?

Dr Suetta: Very classical studies from Sweden that are done on cadavers very clearly show that you lose both muscle size and a huge amount of muscle fibers with age. You do not do that with disuse. It is very different pathways that govern it.

Discussion

Leader: Tracy Smith, PhD, RD, LD, Abbott Nutrition, Columbus, OH

Conference attendee: Dr Suetta, when you measure specific strength in humans, you could understand how specific strength would decrease because of neural factors and maybe even an increase in the noncontractile tissue in the muscle, if you did not account for that with your physiological cross-sectional area. The production of force depends on adequate neural drive to the muscle. If neural drive is lower, the apparent specific tension may be lower for reasons other than the amount of contractile tissue. But with the isolated fibers, I guess that is ruled out, because you have essentially removed the nervous system. What is it about the contractile proteins themselves (they are something that is intrinsic to the muscle) in the absence of a nervous system that decreases the force per cross-sectional area?

Dr Suetta: Interestingly, the loss of muscle fibers, as observed with aging, predominantly occurs in type 2 fibers. After immobilization, we also see decrease in specific tension, predominantly in type 2 fibers, and they seem to be more insensitive to calcium after immobilization, and the type 2 fibers of old individuals get more affected than those of the young. Then we speculated that it could be because of the difference observed in the crossbridge circles between type 1 and type 2 fibers. However, it also could be because of a smaller myosin content in old myofibers, as nicely demonstrated by Bottinelli's group [D'Antona G et al: *J Physiol* 2003;552(Pt 2):499-511, Fig 7].

Conference attendee: If you were to dunk these fibers in a calcium bath to cause them to generate tension, would those differences in specific strength go away? I assume they were electrically stimulated.

Dr Suetta: We do not stimulate the fibers electrically. They are chemically skinned and then put in different concentrations of calcium, where we can measure isometric-specific force at different fiber levels. After immobilization, specific force decreased in both young and old, but still the type 2 fibers of the old individuals were affected the most. We are going to look more into the possible reasons for that.

Dr Reid: We have been interested in these same sorts of myofilament changes with a variety of insults. We do not look at aging, but colleagues down the hall have been looking at rodent muscle. What they find in rodent diaphragm, at least, is that the

mitochondrial volume density is higher. So for a given cross section of the fiber you may have more mitochondria and fewer myofilaments.

That would not account for the change in calcium sensitivity, but it would contribute to the decline in stress. I do not know if that applies to your fibers. Have you looked at mitochondrial content?

Dr Suetta: Unfortunately, no.

Dr Reid: One group of researchers has shown, at least in rodents, that there is a preferential dropout in thin filaments. They have done cross sections with electron microscopy, and the thin filaments drop out and the thick filaments persist, suggesting a different mechanism for this loss in specific force.

Dr Supinski: You can have oxidative stress that will specifically knock down specific force, and caspase and calpain also can specifically knock it down. A multitude of things could account for Dr Suetta's findings. She is going to have to do much work to sort it all out, but I am sure it will be interesting when she is finished.

Dr Schols: Dr Paddon-Jones, you show nicely the importance of high-protein intake spread out over the day. Obviously you were referring to the elderly. One of the problems in cachexia, which we will discuss later, also can be a decreased appetite, and obviously you want the patients to eat as much as possible. What about potential effects of the protein on satiety of these patients and ultimately then on their overall dietary intake?

Dr Paddon-Jones: That is one of the primary practical obstacles we face every day. In elderly patients, we have issues when we give any sort of supplement. In our ACE (acute care for the elderly) unit, we cannot get them to take in enough food, and I think that is amplified with women who have advanced cervical cancer. It is hard to get them to eat any sort of food when we add supplements, and it often leads to nausea. Finding a strategy to get them some nutrition almost requires one-on-one negotiation.

Dr Schols: What is your point of view then? We already discussed nutrition and exercise combination. Both require effort from the elderly, which is good I think, because you consider long-term strategies.

What do you do if you know that is difficult for the elderly to maintain a high-protein intake, which seems to be needed according to all the available studies? What do

you think about combined nutritional and pharmacological approaches to maintain or enhance the muscle mass?

Dr Paddon-Jones: Some of our colleagues are using combined nutrition and testosterone in hypogonadal men, and as we discussed earlier, it does seem to work. If we have a severely compromised population, we need to look at a combination of therapies to try to bring them close to normal. We tend to focus on using nutrition first, as more of a catchall strategy that needs to be in place before we can hope anything like testosterone will work.

Dr Schols: Nutrition first or exercise first?

Dr Paddon-Jones: Nutrition first, because of the types of patient populations we get. I showed you the step activity count on some of these inpatients. They are in the hospital because they cannot exercise, so we tend to focus on nutrition first. Then anything else is almost a bonus. We know we will have a positive synergistic effect if we can add exercise and if we can add hormonal therapy.

Dr Schols: With creative exercise, you can try to stimulate muscle. But what about electrical stimulation? Do you see that as an alternative for those patients?

Dr Paddon-Jones: Yes. We are going to trial that. We know that we cannot get many of our patients to do anything. They are bed bound for maybe a few days, so we are going to try some electrical stimulation while they are in bed to provide a minimal amount of muscle contraction.

For some patients, we left the bed boards on the bottom of the bed. The patients rested their feet against them and just tapped—a minimal amount of activity, but it preserved much of the functional characteristics in isolated single fibers.

In terms of function, it takes a minimal amount of exercise to offset some of the decrements that we saw. Electrical stimulation and minimal amount of exercise are better than nothing.

Dr Morley: Our group looked at supplements, protein, carbohydrate, and fat separately. We did not get an anorectic effect in not-very-healthy older people with between-meal protein, but it has to be given about 2 hours before the meal. When protein is given closer to the meal than that, we got an anorectic effect. So if you space out the protein, it works, and you will not see the anorectic effect.

Exercise is a big problem because we deal with exercise theoretically in the hospital. It is called physical therapy. We deal with it theoretically in nursing homes,

and the amount of exercise they do does not, in my mind, reach the minimal level of exercise.

There seems to be no concept among physical therapists of how much exercise we really need, and we know minimal is better than none. I am totally convinced we could get people out of a nursing home 10 to 15 days earlier if we actually tried to exercise them, because we do not. It is about timing and how much time the physical therapist has.

The question around now is, what about vibration exercise such as that used by the National Aeronautics and Space Administration? Research in older people shows some improvement by just putting them on a vibration stair, which is doable for almost anyone because they are strapped in. Some people have done the same thing with stroke patients, and 3 to 4 years post-stroke these patients suddenly improve dramatically.

I think there is no question that we grossly underexercise. We should be doing basic research on innovative ways to get patients to exercise. Otherwise nutrition is the only choice we have.

Dr Suetta: I have a comment on electrical stimulation. We performed a study some years ago in which we compared electrical stimulation with normal physiotherapy and resistance training in postoperative hip replacement patients. We saw that with conventional physical therapy, patients had a further drop in muscle mass 5 weeks after the surgery, while the electrical stimulation could maintain their muscle mass, although they had a diminished level compared to the healthy leg. With resistance training, muscle mass actually increased. My point is that resistance training in most conditions is the optimal way of training, but if for some reason it is not possible, it seems to be a good idea to apply electrical stimulation.

Dr Schols: That is an important message from a hospitalization point of view. We are glad if we can maintain muscle mass in hospitalized patients. We do not think of improving it.

Dr Supinski: There is no doubt that we should try to move the majority of patients around and exercise them. But some research has shown that in those with infections, exercise can break down the subsarcolemmal support structure, the spectrin in muscle. Then when it contracts, it breaks. You get holes in muscle.

These same researchers found that they could reduce the amount of diaphragmatic injury during an infection by putting the patients on a ventilator for 24 hours. So

there may be some circumstances in which a patient is so sick, with activated caspases and calpains throughout the body, that exercise actually might be harmful and break muscle down for the first day or two right after some event such as pneumonia.

Dr Schols: I can imagine if you want someone to walk or cycle or whatever, that is not possible, but if you perform resistance type of exercise, you are not challenging the diaphragm that much.

Dr Supinski: I am not saying this is always the case. In fact, I may be the only one here who sees these patients, but in the first day or two after they come in, the spectrin is unstable. We have seen this in the leg, as well as the diaphragm. We want to do a study to see what exercise actually does in these circumstances, because we hypothesize that if we exercise animals too soon after they are infected, their condition will get worse.

Dr Schols: Obviously then, you are talking about a different issue, because then you are interfering, and maybe should not interfere, with the normal acute phase response that occurs during acute disease.

Dr Supinski: Perhaps, but I think there is a period during which exercise might be dangerous, say the first 24 or 48 hours.

The same thing may be true with some muscular dystrophy patients. When they get acutely ill, their muscle breaks down, and then they have impaired repair responses. So they go through a period of cycles where they get sicker and sicker. During those acute phases it might be best to prevent infection in them or have some other approach to treatment. But exercising muscular dystrophy patients when they are acutely ill might be bad for them.

Dr Tisdale: Do you have any thoughts about the world of reactive oxygen species? As we heard from Dr Reid earlier, reactive oxygen species are thought to be important in muscle protein loss. Some experiments in animals have knocked out copper-zinc superoxide dismutase, resulting in changes in muscle reminiscent of those in older animals. The animals lose muscle mass and force contraction.

It is probably not a coincidence that type 2 fibers are more sensitive to oxidative stress than type 1 fibers. It may explain the serum effect from older people—why the satellite cells are not propagating properly. I wonder whether anybody has looked at this in humans or just in animal studies of experimental scientists.

Discussion

Dr Reid: I think the lab that is doing the best work on oxidative stress in aged muscle is a group near Liverpool. They have shown that oxidative stress contributes to both the loss of muscle mass and the loss in specific force seen at least in aged rodents. They have not done nearly as much work in humans as they have in rodents, but the work that they have done in rodents has been instructive.

Dr Tisdale: I agree about the rodent studies. The question is, is anybody going to look at this in humans to see whether they can alter the situation that we see in older adults? If you do experiments in humans, do not use vitamin E, tocopherol, as an antioxidant, because that can act as a pro-oxidant. The reason we see these anomalous results using so-called antioxidants is because they are not true antioxidants. They actually can propagate a chain reaction.

Dr Supinski: Conley et al has written about this [*Curr Opin Clin Nutr Metab Care* 2007;10:688-692]. He has postulated that reactive oxygen species may play a role in producing mitochondrial dysfunction in his older patients.

Dr Tisdale: As we talk about various forms of treatment, maybe that should be factored in as well.

Dr Reid: The paper to which Dr Tisdale refers has to do with adaptation to exercise, and the subjects were sort of normal healthy people who were given antioxidants. It was found that the antioxidants inhibited the adaptation to training.

The response in sick people or the response in aged people may be qualitatively different in the sense that you are giving antioxidants in an environment in which oxidative stress is tonic. So there can be phasic oxidative stimuli to which we adapt, and then there are baseline shifts in redox status in tissue. People with chronic oxidative stress such as the elderly may benefit from antioxidant therapy, whereas healthy young people might not. I think it is a worthwhile experiment to do.

Dr Wheeler: If we have muscle mass and show retention of muscle mass, but they do not convert into functionality, what have we gained for these patients? We know that muscle mass alone is not going to necessarily translate into functionality, unless the patients use the muscle and develop the strength and power. If we go back to research that shows things like gait speed being related to mortality (ie, the lower the gait speed, the slower the individual and the higher the rate of mortality or risk for mortality), then muscle without functionality conversion becomes what?

I pose this as a general question: Are we really saying that the muscle mass is the thing we are going for? Or is it muscle mass plus functionality? Are both not critical to the patient populations we are speaking of?

Conference attendee: A related question is, is there any evidence that doing things to decrease protein breakdown preserves lean mass such as essential amino acids? The essential amino acids stimulate protein synthesis, but by decreasing protein breakdown do we end up with muscle mass that contains proteins that may be modified in certain ways that inhibit their function? Doing that, we are not accomplishing anything functional; we are just increasing mass that does not function the way it should.

Dr Hegazi: Back to the point about whether patients like the protein. We know that they need a protein supplement or even basic nutrition. That is a great point for clinical practice. All the time, we see patients and provide them with nutrition support, for instance, by prescribing a nutritional supplement. However, we find that the patients have not touched the supplements. I think we should be aggressive in these cases. We could do this by counting calories, and if intake did not meet 50% of caloric needs, we would place a nasogastric tube and start feeding the patients.

My question to the whole panel is, is there a correlation between protein synthesis, skeletal protein synthesis, and visceral protein synthesis?

Dr Paddon-Jones: Yes.

Dr Hegazi: Question answered. My point is that if we are talking about patient undernutrition in the setting of stressed semi-starvation, coupled with immobilization for a couple of weeks, is there concomitant visceral or intestinal barrier protein depletion? In other words, is there visceral protein depletion in general and then organ dysfunction in association with skeletal muscle protein depletion?

Dr Supinski: Some literature argues that the gut epithelium requires glutamine for adequate function. One question is whether we need different amino acid supplements to make sure that we are maintaining protein synthesis and the integrity of the gut, and whether this differs from what is required for skeletal muscle? This may not be important for the average person walking around, but it is critically important for somebody who is very sick, because if the gut epithelium is not maintained, the patient will get bacterial translocation, and he or she can die from that.

Dr Morley: Some but not all literature suggests that glutamine does not improve outcomes, particularly in patients who are critically ill. Some people believe it does, but we concluded that while a little glutamine is good, a lot of glutamine, as has been used in ICU settings, does not seem to work.

Dr Supinski: There are some experimental data in animals, though.

Dr Morley: Animals are not humans.

Dr Supinski: No, I understand, but some people argue that if you are not sure, what is the harm of giving somebody a little bit of glutamine? Is what we see in skeletal muscle the same as in every other organ, or do some organs require a different nutrient mix?

Dr Schols: I want to follow up on Dr Wheeler's question regarding whether it is muscle mass or muscle function that is important. Obviously, when your outcome is physical functioning, quality of life, and being able to perform activities of daily living, then the translation of maintenance or improvement of muscle mass to muscle function is a logical one. We know that muscle mass is an independent predictor of survival in some chronic diseases and also in the elderly. The question is, why is this so?

In patients with COPD, it has been shown that quality of life is strongly determined by their exercise capacity and less by muscle mass per se. However, some studies have shown that low muscle mass or weight loss is an independent predictor of hospitalization or acute exacerbations. We cannot yet say that, in disease, if an improvement in muscle mass does not immediately translate into one of the markers that we use to assess muscle strength or muscle function, that maintaining or increasing muscle mass is not important.

We can try to find the proof by performing longer-term intervention studies with survival as the end point, or in chronic diseases with exacerbations or hospitalization as the end point. The problem is that such studies may require a follow-up of 3 years. These are expensive, so we are searching for biomarkers. There are markers for sarcopenia, for instance, that are relatively simple to measure but that are not very sensitive, such as a handgrip strength. That is a bit of a challenge for all of us.

Dr Rebecca Biga [Abbott Nutrition]: Does anyone here have any thoughts on why the elderly, compared to young people, respond as they do and the benefits of the way they respond?

Dr Suetta: My answer will be speculative, because my findings are different from those of Dr Paddon-Jones. I think the data show that when we just looked at young people, we saw strong correlations between initial muscle mass and how much they lost. There seems to be a generic potential—myogenic potential—both for how

much you lose and how much you regain after a period of disuse, which might make some sense. If you do not have a lot of muscle mass, you are not going to lose it all after a period of disuse, indicating that there are some mechanisms that retain muscle mass.

However, knowing this, I think counteracting muscle loss during hospitalization is even more important. A certain amount of muscle mass is needed to base the exercise on. We know that muscle strength is strongly correlated to muscle mass and maybe more important muscle function and disability.

Dr Pereira: Dr Paddon-Jones, you showed that aged muscles did not respond well to anabolic stimulus, say an amino acid, at a certain level. Michael Rennie thinks this has something to do with a dysfunctional S6 kinase [Cuthbertson D et al: *FASEB J* 10.1096/fj.04-2540fje. December 13, 2004]. Have you been looking at that mechanism?

Dr Paddon-Jones: In terms of the difference between young and older people, if we give the elderly adequate nutrition and exercise, it is hard to detect the difference in their responses compared to young people. It is only when something goes wrong that we put them in bed or we give them suboptimal amounts of protein. When we give them a less than adequate dose of protein, which may be 7 g of essential amino acids or the equivalent, we do see a blunted, diminished response in the elderly.

We just did an encouraging pilot study in which we gave a small amount of leucine, about 3 g/meal, to elderly patients and were able to restore basal and postabsorptive protein synthesis to what we think were youthful levels. In those cases, it did not take a large intervention to restore elderly back to a youthful response.

Dr Bosaeus: I think we have been rightly focusing on muscle here, but at the same time I must expose my great ignorance in not knowing what factors contribute in an environment of systemic inflammation. The same thing that produces protein breakdown in muscle tends to increase or at least preserve protein synthesis in the liver and the inter-organ transport here also. There was a debate about this a number of years ago because the amino acid composition of the liver acute-phase protein synthesis and the muscle breakdown do not match. There is a mismatch about 2.1 to 2.6, if I remember correctly.

As you said, muscle mass in itself without function may not be a beneficial thing, but it could be a good proxy marker for how much metabolism is disturbed and at what pace it goes. But this is pure speculation.

Dr Reid: A simple reason we might want to maintain muscle mass is purely for metabolism. It is a glucose sink. In many conditions, not only is muscle contractile function compromised, but glucose regulation can be compromised. That is one reason that I would like to have more meat around patients.

Data from Galveston the last few years and the data that Dr Paddon-Jones showed about amino acid supplementation suggest that if the primary response is preservation of muscle mass, we might still have a deficit in specific force. Our observation in rodents is that antioxidants were pretty good at protecting specific force, but they did not do much for muscle mass. Perhaps we can devise a strategy by two different parallel mechanisms and preserve both components of the muscle, both force and mass.

Dr Volek: I think metabolic health is an important point with insulin sensitivity. I would add that muscle also is the main contributor to metabolic weight. We mainly are talking about muscle wasting here, but obesity is also a big problem. Maintaining metabolic rate is crucial for long-term success. Lean body mass accounts for about 80% of metabolic rate, another reason that muscle is important beyond its functional capacity.

One thing we have not discussed is the issue of variability among people. I think personalized or customized medicine and nutrition will be important advancements. Any of us who have done research and looked at the data sets know that nobody responds like the mean. Since we treat people, we must understand some of the factors that contribute to that variability.

We do not have many tools to do that now, but there is some precedent in the pharmaceutical world. For example, it is now possible to identify people who may have an adverse response to certain drugs, or to even titrate drugs such as warfarin based on the presence of specific single nucleotide polymorphisms in their genetic code. This is an exciting area. If we really want to be ahead of the curve, we will try to understand the variability rather than frowning upon it because we do not like it when we do our statistics.

Dr Morley: What I am about to say always gets me into trouble. It is feasible that muscle mass could be bad for you when you are really sick or malnourished. Studies of starving African children, the only relatively clean model, show that when

they start to eat, they put down fat. Why do they put down fat before they put down muscle? Because of resting metabolic rate. Muscle uses up energy. If we are putting down muscle and do not have a store of energy to feed our brain, our heart, and other organs, we can run into trouble. We have to be careful that we have adequate fat. People die when they have lost all their fat. It is important to have a store of energy that does not cost you anything.

I do not think muscle size alone is useful. The Osterman study showed that if we put back functional muscle in patients with cancer, we improve power. I think we have to have functional muscle, but we have to be careful about this muscle; if it is just there soaking up energy in a sick person, it may not be useful.

The other point was about why older people look different. First, despite everything we have heard, maybe they are different, but maybe they are not. Maybe at baseline they are not. The best weight lifters in the world by the time they are 80 years of age are 60% to 70% worse than at age 30. Clearly, muscle mass goes down dramatically with aging, and its function goes down as well. The question is why.

There is a rate of living theory that has many problems, but basically it says that the more energy we use up, the more rapidly we will die. We have only so much potential energy in our life, so we might as well moderate it. We need a lot when we are young because we have to go out to hunt and gather, at least our ancestors did. As we get older, we can sit and think, and we do not need as much energy.

Look at the decreases in hormonal levels, particularly those that occur at menopause and beyond—and men have their quasi-menopause around the same age. All these changes are trying to slow us down as we get older. Slowing down may not always be bad as long as we can function.

If I am really into muscle building, I would think that is an anathema, and I would not accept either of my two comments. However, the reality is somewhere in between. We never should believe that muscle does not go down dramatically with aging, because the real life experiment, the muscle builders and weight lifters, clearly shows that we have incredible loss of strength no matter how hard we train.

We have to be careful in the very sick. I do not think that we should let them lose muscle, but I do not think that we should be adding muscle in ICU patients.

Structured Panel Discussion

Leader: Neile Edens, PhD, Abbott Nutrition, Columbus, OH

Dr Edens: During the last 1½ days, we have had some great presentations and discussions, and I for one feel replete with data. Now I would like to integrate some of those data. Because we have both basic and clinical scientists at this conference, I would like to hear everybody's unique perspective on the following question: Can we reduce or how can we reduce the severity or the risk of sarcopenia?

Dr Morley: Clearly, we should build muscle mass when we are young. It is like bone. If you build bone when you are young, you do better when you are old. I am convinced that if we did not build muscle when we were a teenager, it will be much harder when we are old. We see patterns within muscle in older people. People who used to exercise seem to do better, at least in my patient population, when I try to re-exercise them.

I think we need adequate protein throughout life. And we have to be careful about the common belief that as we get older and have some renal failure that we should cut back on protein. The data I know indicate that protein may hasten the time to dialysis in people with severe renal failure, but it does not necessarily kill.

There are endocrine interventions, but the amount of data on them is insufficient to recommend anything other than trials. Of these interventions, the one that appears the best is the testosterone anabolic hormones.

I think that we have to "fix" people before they are born, through childhood, and through middle age. It is never too late to do exercise. It is never too late to eat relatively well. However, it is much harder when you are 85 years old, if you did not exercise and eat well when you were 40 years old. It is a little late to fix people at age 85 and tell them it is now time to exercise.

Dr Supinski: I am in favor of nutrition, exercise, and endocrine interventions, but not to the level of intervention in some sports stars.

Exercise is great, but it is hard. In one Woody Allen movie he was in a future time when we would take a pill and not need to exercise. That maybe is not a bad idea. I think we need to learn more about all these pathways, and that if we are really smart and clever, we might come up with drugs to manipulate these things, because there

always will be people who will not exercise, no matter how hard we urge them to do it, and there always will be people who will not eat adequately. So there must be, in the long run, pharmacological therapies.

We age. Data show that weight lifters do not lift as much when they get older. I think subtle alterations in the gene programming of these satellite cells and other cells occur, and that if we understood that process, we could develop a pharmacological means of blocking it. Maybe some of the pharmacological interventions will be biopharmaceuticals, but I have more hope for the future. I think in the long run, we will come up with treatments for these things that will block the advance of sarcopenia and weakness, even if we cannot exercise all of our patients.

Dr Suetta: Because we do not have that pill yet, I think the quick answer is exercise. It is not a pill we have to take only once when we are young or middle-aged. We have to do it through all ages. In contrast to what Dr Morley says, I believe the important take-home message is that the muscle function of older weight lifters is much better than that of their nonweight-lifting counterparts.

It has been shown that specific force in aged individuals who have been performing resistance exercise for a long period is not different from that of young, in contrast to endurance-type-trained elderly individuals or sedentary individuals [Klitgaard H et al: *Acta Physiol Scand* 1990;140:41-54]. By doing resistance exercise, we can counteract many of these decreases that occur with age. Of course, we cannot counteract everything. We cannot counteract loss of type 2 fibers and some of the neuronal components in sarcopenia. If we train throughout life, however, we can maintain an independent level of life, and that is important.

Dr Volek: In the spirit of succinctness, consume adequate protein, plenty of leucine, plenty of good fats, primarily monounsaturated fat, and carbs in an amount you can tolerate. I see little downside to adding creatine and β-hydroxy-β-methylbutyrate (HMB) for muscle mass. Of course, there are thousands of caveats to those general recommendations.

Dr Schols: Start as young as possible with a healthy lifestyle that includes exercise and nutrition, focusing on nutrition, not only to maintain muscle mass but also to modulate cardiovascular risk. Do not start smoking, or stop if you are smoking.

Generally, children are fit, but we also want to reduce risk for various chronic diseases. As people age, depending on their genetic profile and their lifestyle, they will sooner or later develop functional impairments. I wonder whether the exercise

focus should shift from endurance to resistance type of exercise as people age. I would not advocate that for young people.

Because of aging and variability in the population, I advocate for tailored intervention strategies, including nutrition, exercise, and, if needed, endocrine interventions.

Dr Reid: I agree. It is hard to argue with physical activity. I have learned much at this session about the importance of protein. I am eating my protein. Vitamin D is an interesting nutritional approach that I have not given much thought to, and it looks very interesting. I agree with Dr Tisdale that it is worth exploring antioxidants as a possible application.

I am not a sportsman and was not an athletic kid, but I am becoming more interested in physical activity as I get older, because I see my body changing. I think there is the potential to get people up and out, and get them active, even as they start to move into their older years. We need to inform them about the importance of this activity.

Dr Bosaeus: I would advocate nutrition and physical activity instead of exercise, more for the purpose of upholding a normal energy metabolism. Throughout life, in the absence of specific disease, this will create an endocrine environment that probably will link in with what we consume. There may be different windows in life when these things are more important than others—metabolic programming, for instance, more in young childhood than in utero. I know very little about reprogramming of metabolic functions in old age, but perhaps the relation of diet with the changing endocrine environment is worth looking into.

As to high-peak muscle mass achievement, I think it is a bit more complicated than just saying that the high-peak muscle mass is such and such. Women tend to live longer than men with lower muscle mass to start with, and males with a high muscle mass tend to lose it faster. What is the balance of that? I do not know if there is a specific gender effect or if it is a functional muscle mass per se.

Dr Hegazi: I would pay more attention to nutrition in the hospital. For elderly patients in the hospital, I would not depend on nutritional assessment and a given recommendation. I would be more aggressive. If the patient is not taking oral nutritional supplements, they could best be helped by initiating tube feeding to meet nutritional needs and selecting nutritionals that could help break the catabolic cycle. Perhaps we can develop nutrients in pill form, not just a nutritional supplement that the patient either takes or not.

Dr Johnson: My grandmothers were physically active and did their own work way into their 80s and 90s. I always saw them eating well, and right now, I am counseling my own mother to drink some milk before she takes a walk and again when she comes back. This is an inexpensive intervention.

We know that if people with a very-low body weight have snacks or oral nutrition supplements in between meals, it does not affect the quantity of food they eat at their meals. So I would probably recommend that people consume more frequent, smaller meals throughout the day, trying to do as much of their own house and yard work as possible, as well as walking some every day.

Dr Cederholm: For prevention, I think it is crucial to increase public awareness of the role of exercise, for weight control, but more for maintaining muscle mass. We know that "fat and fit" lives longer than "lean and lazy." When it comes to treatment later in life, it is never too late to start exercising. Muscle is a plastic tissue, much more plastic than bone.

To repeat what the others have said, the data on leucine and HMB are very interesting. We can hope to develop our knowledge about certain nutritional compounds such as amino acids and combinations with eicosapentaenoic acid (EPA).

It also is crucial to increase awareness among professionals, because what medical professionals in the hospital find to be important will diffuse into society. When professionals acknowledge the importance of muscle mass and muscle function, I think we will have an effect with the public as well.

Dr Tisdale: This is important for me, because I am in the age group that gets sarcopenia.

Unfortunately, I know very little about it, but I would say until we understand the molecular mechanisms that are responsible for sarcopenia, we cannot answer the question that Dr Edens asked. We might be biased one way or another, but we cannot truthfully answer the question without knowing the molecular mechanisms.

I would, however, disagree with Dr Morley about the importance of muscle protein. He says we die when we run out of fat, but we get cold when we run out of firewood and that is basically because we do not burn the house down to keep warm. It is the same way with the body. We die when we run out of fat, because once we start destroying the "house," we lose everything and we die. So muscle protein is important.

Dr Baracos: I would like to introduce the topic of synergy. I think there is an evident and important synergy between anabolic stimuli to muscle of whatever nature and having the building blocks necessary and essential to build it. I am also struck by the negative synergy between, for example, immobilization or bed rest and old age and other insults. I posit that a woman who is admitted to a hospital for repair of an abdominal aortic aneurysm, develops an infectious complication, and stays in hospital for 2 weeks, will experience the vast proportion of total lifetime muscle loss during the course of that event. If I had a drug or a food product, I would aim it more pointedly in the direction of those people at risk for synergistic catabolic catastrophes rather than in some vague way toward people living in the community who might be having a muscle loss of a very different nature.

Dr Guttridge: I have learned much in these last few days because nutrition is not my expertise, and I have really come to appreciate it. However, I can draw from some of my background in cancers. The number one killer in cancer is lung cancer. For many years, people have been trying to understand the mechanisms of lung cancer and trying to find the perfect pill to treat lung cancer. Yet the correlation between smoking and lung cancer has been evident for many years. What is making a dent in that disease is the aggressive anti-smoking campaign that children are exposed to at a young age. My third grader at school is being told not to smoke and not to take drugs. This is very different than when I grew up. That generation probably will grow up not smoking, and we will really see the fatalities drop. It was not a pill; it was just good common sense.

I am a huge supporter of exercise, but increasing it is going to take the same kind of aggressive campaign. We need to reach the population before they are age 60, to encourage exercise in combination with eating right, creatine, HMB, and other helpful measures to supplement that exercise.

Dr Paddon-Jones: A more global approach to exercise is to find something that we like to do and can do regularly. Then, if we want nuanced improvements, we can consider creatine, those sorts of value-added supplements. The second prong is more acute. When we face a catabolic crisis, we should react aggressively and use a combination of strategies. That could be amino acids, antioxidants, and maybe physical therapy, if and when appropriate.

Dr Wheeler: I agree with everything that has been said here. One thing that still bothers me is that from a clinical perspective, we continue to overuse body mass index (BMI). I would like to see us get away from that as a gold standard of health, and start using body composition and educating people about body composition. Publications including those from the American Heart Association always focus on

BMI. It is our fault, because we are not teaching people about what the distribution of muscle and fat means relative to overall health.

I also think that it is important to emphasize resistance weight training. My mother, who is 86 years old, had an abdominal aortic aneurysm and is extremely frail. I have seen the effects of her not doing positive things such as specific exercises at the age of 50, 60, and 70 years. What would she be like today and how would she have survived that horrific surgery that saved her life, but left her frail, if she had taken better care of her body? We need to begin to educate and push more toward, not just exercise, but specifically whole-body resistance exercise.

Dr Rosemary Riley [Abbott Nutrition]: I have a couple of concerns. First of all, I am concerned that our young generation is going to be hitting the sarcopenic categories earlier than our parents and ourselves, because of their lifestyle—the way they eat and their general sedentary behavior. Our recommended diet that includes a moderate or good dose of protein three to four times a day is not how they eat, and they are giving up milk at a very early age. So we are challenged to help young people change their diet and lifestyle, and of course, we need to treat those people who need treatment right now.

Cachexia Associated With COPD

Annemie Schols, PhD

Research during the past 2 decades consistently has shown that chronic obstructive pulmonary disease (COPD) is not only a chronic inflammatory lung disease but also a metabolic disorder affecting multiorgan systems. Weight loss, skeletal muscle wasting, and a decreased muscle oxidative phenotype are well documented in advanced COPD and a target for multimodal intervention strategies. Existing strategies have obtained promising results from exercise and nutritional supplementation with or without anabolic agents. Furthermore, experimental research rapidly advances an understanding of the molecular mechanisms of altered muscle plasticity in COPD progression, providing new leads for nutritional intervention.

Whole Body and Cellular Energy Metabolism

In comparison with other chronic wasting conditions, part of the weight-losing COPD patients are characterized by elevated activity-induced and daily energy expenditure.[1-3] Without a corresponding increase in caloric intake, patients inevitably lose weight.[1] A recent randomized controlled trial (RCT) reported the effect of dietary counselling and food fortification in COPD outpatients. While the treated group gained weight during the nutritional intervention period of 6 months and maintained weight during the 6 months follow-up, the control group progressively lost weight.[4]

It is postulated that pulmonary pathology increases the work of breathing and thus daily energy expenditure.[5-8] In addition, impaired cellular and whole-body energy metabolism could result from intrinsic muscle abnormalities. Disturbed levels of energy-rich phosphates, such as adenosine triphosphate (ATP) and creatine phosphate, are reported in rest, as well in response to an exercise bout, indicative of impaired oxidative energy metabolism.[9-12] Consequently, the affected muscles rely more on anaerobic energy metabolism to produce ATP, which is far less efficient.[13]

The most prominent intrinsic muscular abnormality in COPD that is likely to cause the previously mentioned impairment in cellular energy metabolism is the loss of muscle oxidative phenotype because of a fiber type I→II shift in lower limb skeletal muscle and in parallel, reduced activities of enzymes involved in muscle oxidative metabolism.[14-17] It seems that these alterations are more pronounced in

emphysematous patients who, strikingly, also are more prone to weight loss.[16] Accordingly, loss of muscle oxidative phenotype also is reflected by decreased mitochondrial mass and mitochondrial function, which was more pronounced in patients with low body mass index.[18-21]

These data suggest that the regulation of muscle oxidative phenotype and specifically mitochondrial biogenesis is altered in cachectic COPD patients. The transcription factors peroxisome proliferators-activated receptor (PPAR) α and β/δ and in particular their coactivator PPAR coactivator-1α (PGC-1α) are considered as key regulators of muscle oxidative phenotype.

Recently decreased expression levels of these regulators were reported in muscle biopsies of patients with COPD and were more pronounced in the cachectic patients, again suggesting that loss of muscle oxidative phenotype and muscle mass are somehow interrelated.[22] Enhanced daily energy expenditure could originate from the loss of muscle oxidative phenotype because of less efficient energy metabolism. Alternatively, elevated muscle oxidative stress, which is consistently demonstrated for COPD, has likely involvement in muscle protein breakdown. Oxidative stress occurs when the production of oxidants exceeds the capacity of the antioxidant defense system.

Evidence shows that the antioxidant system is impaired in COPD; basal muscular levels of the antioxidant glutathione were reduced in emphysema patients, who also adapted less well to exercise training because their muscular antioxidant defense system did not improve as it did in healthy controls.[23,24] On the other side of the balance, enhanced muscular reactive oxygen species (ROS) production was reported for COPD as well, and it is likely that the impaired muscle oxidative capacity itself is the source of enhanced ROS production.[25-27] Moreover, it was shown that muscle-derived mitochondria from COPD patients produce more ROS as compared to healthy controls.[20]

Muscle Maintenance and Protein Balance

Atrophy of skeletal muscles in COPD appears to selectively affect glycolytic type IIA/IIX fibers.[28] Whether fiber type II atrophy is causally linked to the I→II fiber type shift still is undetermined.

Insulin-like growth factor-I (IGF-I) is an important mediator of anabolic pathways in skeletal muscle cells. While improvements in molecular signatures of muscle anabolism are described after exercise or pharmacological modulation in nonwasted COPD patients,[29-31] no studies are available on the muscle protein

anabolic response in skeletal muscle of cachectic COPD patients. However, several studies consistently showed that protein/carbohydrate-rich supplements as an integrated part of a pulmonary rehabilitation program are effective in inducing (muscle) weight gain and improving physical performance.[32,33] Optimizing protein intake and essential amino acid intake may not only stimulate protein synthesis per se, but also enhance efficacy of anabolic pharmacological agents. However, no clinical data are available regarding the response to anabolics in cachexia during normal and high-protein intake (0.8-1.0 g/kg vs >1.5 g/kg body weight, respectively).

In cachectic patients with COPD, consistently reduced plasma levels of branched chain amino acids (BCAAs) are reported.[34-36] Some indications show that low-plasma BCAAs in COPD patients are because of specific alterations in leucine metabolism, an amino acid that has received much attention recently for its potential to modulate muscle anabolic signalling.[37] However, only limited studies have compared muscle anabolic effects of selective proteins or specific amino acids in patients at risk or suffering from cachexia. In a short-term tracer experiment, supplementation of BCAA to a soy-protein meal resulted in a significant acute increase in whole-body protein synthesis in weight-stable COPD patients with borderline muscle mass but not in age-matched healthy controls.[38]

While short-term RCTs consistently showed the anabolic potential of multimodal interventions, muscle rapidly wasted away when this stimulus stopped.[39] This is not surprising because experimental research shows that maintenance of muscle mass by insulin/IGF-I signalling also involves suppression of protein degradation. Doucet et al[40] recently reported increased expression of atrogin-1 and MuRF1 in skeletal muscle biopsies of COPD patients with muscle atrophy. Inflammation is the most explored trigger for impaired muscle maintenance in cachexia. However, despite abundant and elegant experimental evidence for a role for tumor necrosis factor (TNF)-α in muscle wasting, much controversy still exists regarding its role in COPD.

Nevertheless, two recent RCTs investigated the effect of nutritional anti-inflammatory modulation on muscle maintenance in COPD. Matsuyama et al[41] investigated the effects of a dietary supplement containing n-3 polyunsaturated fatty acid (PUFA) during 2 years and reported a significant decrease in leukotriene B4 levels in serum and sputum and in TNF-α and interleukin-8 (IL-8) in sputum, while no effect was observed in the control group receiving a nutritional supplement enriched with n-6 PUFA.

Broekhuizen et al[42] investigated the effect of PUFAs as adjunct to exercise training in COPD patients eligible for pulmonary rehabilitation. No effects were (yet) seen on

systemic inflammatory profile, but PUFAs significantly enhanced improvement in cycle endurance time and peak workload. Experimental research indicates that inflammation may not only modulate muscle mass but could also affect muscle oxidative phenotype by downregulation of the PPARs. A bidirectional antagonism, described between the PPAR and NF-κB signalling pathway, is a possible explanation for the effects of anti-inflammatory modulation on exercise capacity.[43] Indeed, increased NF-κB activation and decreased muscle messenger ribonucleic acid (mRNA) PPARα expression are demonstrated in underweight COPD patients, and are inversely associated with systemic inflammation.[22]

Conclusion

Recent approaches to translation research clearly have advanced our understanding of the pathophysiology of cachexia in COPD. A cure for this complex clinical syndrome may perhaps not yet exist, but would certainly benefit from a multidimensional intervention approach. In addition to further mechanistic insight into the interaction between oxidative phenotype and muscle maintenance regulation, longitudinal COPD studies are needed to unravel genetic and environmental factors underlying cachexia susceptibility, what initiates the cachexia process, and what the sequential molecular steps are.

References

1. Schols AM, Soeters PB, Mostert R, et al: Energy balance in chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1991;143:1248-1252.
2. Baarends EM, Schols AM, Westerterp KR, Wouters EF: Total daily energy expenditure relative to resting energy expenditure in clinically stable patients with COPD. *Thorax* 1997;52:780-785.
3. Slinde F, Ellegard L, Gronberg AM, et al: Total energy expenditure in underweight patients with severe chronic obstructive pulmonary disease living at home. *Clin Nutr* 2003;22:159-165.
4. Weekes CE, Emery PW, Elia M: Dietary counselling and food fortification in stable COPD: A randomised trial. *Thorax* 2009;64:326-331.
5. Baarends EM, Schols AM, Akkermans MA, Wouters EF: Decreased mechanical efficiency in clinically stable patients with COPD. *Thorax* 1997;52:981-986.
6. Vaughan P, Oey IF, Steiner MC, et al: A prospective analysis of the inter-relationship between lung volume reduction surgery and body mass index. *Eur J Cardiothorac Surg* 2007;32:839-842.
7. Takayama T, Shindoh C, Kurokawa Y, et al: Effects of lung volume reduction surgery for emphysema on oxygen cost of breathing. *Chest* 2003;123:1847-1852.

8. Nezu K, Yoshikawa M, Yoneda T, et al: The change in body composition after bilateral lung volume reduction surgery for underweight patients with severe emphysema. *Lung* 2000;178:381-389.
9. Green HJ, Burnett ME, D'Arsigny CL, et al: Altered metabolic and transporter characteristics of vastus lateralis in chronic obstructive pulmonary disease. *J Appl Physiol* 2008;105:879-886.
10. Pouw EM, Schols AM, van der Vusse GJ, Wouters EF: Elevated inosine monophosphate levels in resting muscle of patients with stable COPD. *Am J Respir Crit Care Med* 1998;157:453-457.
11. Serres I, Hayot M, Préfaut C, Mercier J: Skeletal muscle abnormalities in patients with COPD: Contribution to exercise intolerance. *Med Sci Sports Exerc* 1998;30:1019-1027.
12. Maltais F, Simard AA, Simard C, et al: Oxidative capacity of the skeletal muscle and lactic acid kinetics during exercise in normal subjects and in patients with COPD. *Am J Respir Crit Care Med* 1996;153:288-293.
13. Steiner MC, Evans R, Deacon SJ, et al: Adenine nucleotide loss in the skeletal muscles during exercise in chronic obstructive pulmonary disease. *Thorax* 2005;60:932-936.
14. Gosker HR, Zeegers MP, Wouters EF, Schols AM: Muscle fibre type shifting in the vastus lateralis of patients with COPD is associated with disease severity: A systematic review and meta-analysis. *Thorax* 2007;62:944-949.
15. Green HJ, Bombardier E, Burnett M, et al: Organization of metabolic pathways in vastus lateralis of patients with chronic obstructive pulmonary disease. *Am J Physiol Regul Integr Comp Physiol* 2008;295:R935-R941.
16. Gosker HR, van Mameren H, van Dijk PJ, et al: Skeletal muscle fibre-type shifting and metabolic profile in patients with COPD. *Eur Respir J* 2002;19:617-625.
17. Maltais F, Sullivan MJ, LeBlanc P, et al: Altered expression of myosin heavy chain in the vastus lateralis muscle in patients with COPD. *Eur Respir J* 1999;13:850-854.
18. Gosker HR, Hesselink MK, Duimel H, et al: Reduced mitochondrial density in the vastus lateralis muscle of patients with COPD. *Eur Respir J* 2007;30:73-79.
19. Picard M, Godin R, Sinnreich M, et al: The mitochondrial phenotype of peripheral muscle in chronic obstructive pulmonary disease: Disuse or dysfunction? *Am J Respir Crit Care Med* 2008;178:1040-1047.
20. Puente-Maestu L, Pérez-Parra J, Godoy R, et al: Abnormal mitochondrial function in locomotor and respiratory muscles of COPD patients. *Eur Respir J* 2009;33:1045-1052.
21. Rabinovich RA, Bastos R, Ardite E, et al: Mitochondrial dysfunction in COPD patients with low body mass index. *Eur Respir J* 2007;29:643-650.
22. Remels AH, Schrauwen P, Broekhuizen R, et al: Peroxisome proliferator-activated receptor expression is reduced in skeletal muscle in COPD. *Eur Respir J* 2007;30:245-252.

23. Engelen MP, Schols AM, Does JD, et al: Altered glutamate metabolism is associated with reduced muscle glutathione levels in patients with emphysema. *Am J Respir Crit Care Med* 2000;161:98-103.

24. Rabinovich RA, Ardite E, Troosters T, et al: Reduced muscle redox capacity after endurance training in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2001;164:1114-1118.

25. Barreiro E, Rabinovich R, Marin-Corral J, et al: Chronic endurance exercise induces quadriceps nitrosative stress in patients with severe COPD. *Thorax* 2009;64:13-19.

26. Couillard A, Maltais F, Saey D, et al: Exercise-induced quadriceps oxidative stress and peripheral muscle dysfunction in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2003;167:1664-1669.

27. Delample D, Durand F, Severac A, et al: Implication of xanthine oxidase in muscle oxidative stress in COPD patients. *Free Radic Res* 2008;42:807-814.

28. Gosker HR, Engelen MP, van Mameren H, et al: Muscle fiber type IIX atrophy is involved in the loss of fat-free mass in chronic obstructive pulmonary disease. *Am J Clin Nutr* 2002;76:113-119.

29. Vogiatzis I, Stratakos G, Simoes DC, et al: Effects of rehabilitative exercise on peripheral muscle TNFalpha, IL-6, IGF-I and MyoD expression in patients with COPD. *Thorax* 2007;62:950-956.

30. Casaburi R, Bhasin S, Cosentino L, et al: Effects of testosterone and resistance training in men with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2004;170:870-878.

31. Lewis MI, Fournier M, Storer TW, et al: Skeletal muscle adaptations to testosterone and resistance training in men with COPD. *J Appl Physiol* 2007;103:1299-1310.

32. Creutzberg EC, Wouters EF, Mostert R, et al: Efficacy of nutritional supplementation therapy in depleted patients with chronic obstructive pulmonary disease. *Nutrition* 2003;19:120-127.

33. Steiner MC, Barton RL, Singh SJ, Morgan MD: Nutritional enhancement of exercise performance in chronic obstructive pulmonary disease: A randomised controlled trial. *Thorax* 2003;58:745-751.

34. Kutsuzawa T, Shioya S, Kurita D, Haida M: Plasma branched-chain amino acid levels and muscle energy metabolism in patients with chronic obstructive pulmonary disease. *Clin Nutr* 2009;28:203-208.

35. Engelen MP, Wouters EF, Deutz NE, et al: Factors contributing to alterations in skeletal muscle and plasma amino acid profiles in patients with chronic obstructive pulmonary disease. *Am J Clin Nutr* 2000;72:1480-1487.

36. Hofford JM, Milakofsky L, Vogel WH, et al: The nutritional status in advanced emphysema associated with chronic bronchitis: A study of amino acid and catecholamine levels. *Am Rev Respir Dis* 1990;141(4 pt 1):902-908.

37. Kimball SR, Jefferson LS: Molecular mechanisms through which amino acids mediate signaling through the mammalian target of rapamycin. *Curr Opin Clin Nutr Metab Care* 2004;7:39-44.

38. Engelen MP, Rutten EP, De Castro CL, et al: Supplementation of soy protein with branched-chain amino acids alters protein metabolism in healthy elderly and even more in patients with chronic obstructive pulmonary disease. *Am J Clin Nutr* 2007;85:431-439.

39. Bolton CE, Broekhuizen R, Ionescu AA, et al: Cellular protein breakdown and systemic inflammation are unaffected by pulmonary rehabilitation in COPD. *Thorax* 2007;62:109-114.

40. Doucet M, Russell AP, Léger B, et al: Muscle atrophy and hypertrophy signaling in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2007;176:261-269.

41. Matsuyama W, Mitsuyama H, Watanabe M, et al: Effects of omega-3 polyunsaturated fatty acids on inflammatory markers in COPD. *Chest* 2005;128:3817-3827.

42. Broekhuizen R, Wouters EF, Creutzberg EC, et al: Polyunsaturated fatty acids improve exercise capacity in chronic obstructive pulmonary disease. *Thorax* 2005;60:376-382.

43. Pascual G, Fong AL, Ogawa S, et al: A SUMOylation-dependent pathway mediates transrepression of inflammatory response genes by PPAR-gamma. *Nature* 2005;437:759-763.

Q & A

Q: In the study with the nutritional intervention, what were the supplements and how many calories did they have?

Dr Schols: Supplements were given three times daily, 125 mL. That is because in previous studies, we showed that we can improve the efficiency of nutritional intervention in these patients by providing them with smaller portions spread out during the day. It was 30% protein and 60% carbohydrate, and in total they were given nearly 600 kcal daily.

Q: Did you assess tolerance?

Dr Schols: Yes, we did assess it, and I also have all the data of the mean intake of supplements throughout the maintenance phase, but I thought that was too much detail for this presentation.

Q: I admire the integration of very basic science with elegant clinical studies. One observation that you mentioned briefly in passing is that the TNF link to muscle structure function in patients was based on the TNF expression in the muscles of the patients, suggesting that TNF plays an autocrine-paracrine role. Do you have any idea what the stimulus is for TNF upregulation in muscles of COPD patients? Do you think it is a TNF-induced TNF expression, or is there some other circulating mediator that you think is responsible?

Dr Schols: That is an important question that I cannot answer, but one we are trying to solve. It also still is not clear whether or not there is spillover from the pulmonary compartment to systemic inflammation. We try to address that in experimental models.

Quite some controversy exists in the literature regarding whether or not there is elevated TNF expression in the muscle, but I think that this is partly related to very small-size studies with different patient populations. I think what was nice in our study is that we included a large group of patients.

This analysis shows us that we cannot generalize for all COPD patients, because apparently some of the patients may have increased inflammation in the muscles; others do not. We now are analyzing whether or not this is related to systemic inflammation. That could be easy, because then we would have an easy biomarker, but I cannot give you the answer yet.

Q: I wonder about the omega-3 PUFA studies that you did. Do you think that the baseline levels of the patients have any effect or any influence on the effect? I think that Dutch people usually eat a lot of fish. So, would you expect the effects of omega-3 PUFA supplementation to be better in a population in which people do not eat as much fish?

Dr Schols: Well, they do not eat much fish in Holland. So, that is a misconception. In Holland, we are not the type of people who believe everything; not many people take supplements or capsules. We verified this, and it was not an interfering factor in our study, which was the case in previous cancer cachexia trials.

I think it is also interesting to ask whether genetic TNF polymorphisms are predictive for the response to the intervention. Japanese researchers performed a nutritional intervention trial including omega-3 fatty acids. As in our study, they did not see an enhancing effect of the omega-3 fatty acids on muscle mass and on body weight. This was provided as part of a nutritional supplement. They did show some effects on induced sputum on markers of inflammation. What I like of the concept of the PUFAs is that maybe you have an intervention that not only targets the muscle, but also could benefit the lungs, because we still are dealing with a disease and with a primary impairment.

Q: I know the tissue macrophages respond to hypoxia to produce cytokines. Did you look at oxygenation in the patients? Did you see a pattern showing that the more wasted individuals have hypoxia?

Dr Schols: This is a topic of interest within our group. We certainly are working on this, both in experimental models as well as in human tissue. I cannot give you an answer. Several research groups have shown evidence that tissue hypoxia or hypoxia possibly is related to mitochondrial dysfunction.

It certainly seems logical that this contributes to the abnormalities in muscle metabolism, as well as maybe enhancing the effect of the inflammation. However, we do not have a clear-cut answer yet. It also is very difficult to measure tissue hypoxia in humans.

Measurement of Lean Body Mass Using CT Scans

Vickie Baracos, PhD

Body Mass Index (BMI) and Weight Loss: Conventional Elements of How Cachexia and Nutritional Status Have Been Defined in Cancer Patients

Our understanding of cancer cachexia in the past has focused on losses of body weight. Since ancient times, the relationship between loss of body weight over time and poor cancer outcomes has been known. Even though measures such as loss of lean body mass may be better predictors of patient outcomes, weight loss remains firmly entrenched as a criterion for identification of malnutrition and cachexia. Body weight loss, for instance, still is used as a major criterion of inclusion and also as a principal end point in randomized clinical trials for various forms of cachexia treatment, including nutritional support. In a review of 55 clinical trials looking at the effect of appetite stimulants on cancer cachexia, Yavuzsen et al[1] reported that 91% of studies examined used overall body weight change as an outcome.

Progressive loss of weight is conventionally viewed as culminating in a cachectic (ie, emaciated) state. Current demographics of cancer patients, however, are progressively affected by increasing rates of obesity, as well as by an increasing prevalence of cancer in obese people. As a result, patients with advanced disease are reported to have a high prevalence of obesity in spite of ongoing weight losses.[2]

Despite considerable weight loss in some of these patients, the classic image of cachexia (ie, emaciation) is becoming less common. We conducted population-based profiling of BMI and weight loss history in a cohort (n=2695) of patients with gastrointestinal cancer. Patients were newly referred to medical oncology clinics in a regional cancer center. A computerized database of all cancer cases in the province (Alberta Cancer Registry) was used to capture disease site and morphology, along with biological, clinical, and demographic information. Analysis of BMI reveals an average BMI clinical and demographic information. Analysis of BMI reveals an average BMI >26 kg/m^2 and a preponderance of overweight and obese patients, with only 5% presenting with a BMI <18.5 kg/m^2. A history of weight loss was

common, with an average loss of 8.6±8.9%. The population quartiles of 6 month weight loss were -19.3%, -10.6%, -4.5%, and +2.4%. In the following Table, the data are stratified by time to death.

Table. BMI Distribution (%) and 6-Month Weight Loss at Different Times From Death in Patients With Advanced Solid Tumors (n=2695)

	Days to Death				
	<90 (n=509)	90 to 180 (n=312)	181 to 360 (n=346)	361 to 540 (n=396)	>540 (n=1132)
BMI <20.0 (%)	23	22	15	12	8
BMI 20.0 to 24.9 (%)	39	44	37	38	38
BMI 25.0 to 29.9 (%)	28	25	36	35	36
BMI ≥30.0 (%)	10	9	12	15	18
BMI (Mean±SD)	23.9±5.3	23.7±4.6	24.9±5.0	25.5±5.4	26.0±5.0
6 month weight loss (%, Mean±SD)	11.7±9.0	9.3±8.8	7.8±8.8	7.0±7.5	5.5±7.9

Despite a mean weight loss of about 12%, the mean BMI of patients within 90 days of death was high at 23.9 kg/m^2. Owing to high body weights, many patients remained obese or overweight in spite of considerable weight loss. These data appear to reflect the generally heavier body weights in Westernized countries.

A Reconceptualization of Cancer Cachexia

Skeletal muscle wasting can hide within the bulk of body weight and body weight change, and recognition of sarcopenia (age-related muscle wasting) as a clinically important phenomenon is emerging. The term sarcopenia denotes a reduced quantity of skeletal muscle (ie, >2 standard deviations below that typical of healthy adults).[3] Sarcopenia is associated with loss of physical function, disability,[2-4] risk of fractures and of falls,[3] increased length of hospital stay,[5] nosocomial infections,[6] and decreased survival[7] in nonmalignant diseases. Sarcopenia is not restricted to people who are thin or wasted.[2,8] The aging process is often paralleled by decreases in muscle and increases in fat mass, which may culminate in sarcopenic obesity.[9,10] Recent studies[11,12] point to an increasing prevalence of sarcopenic obesity in elderly people in North American and Europe.

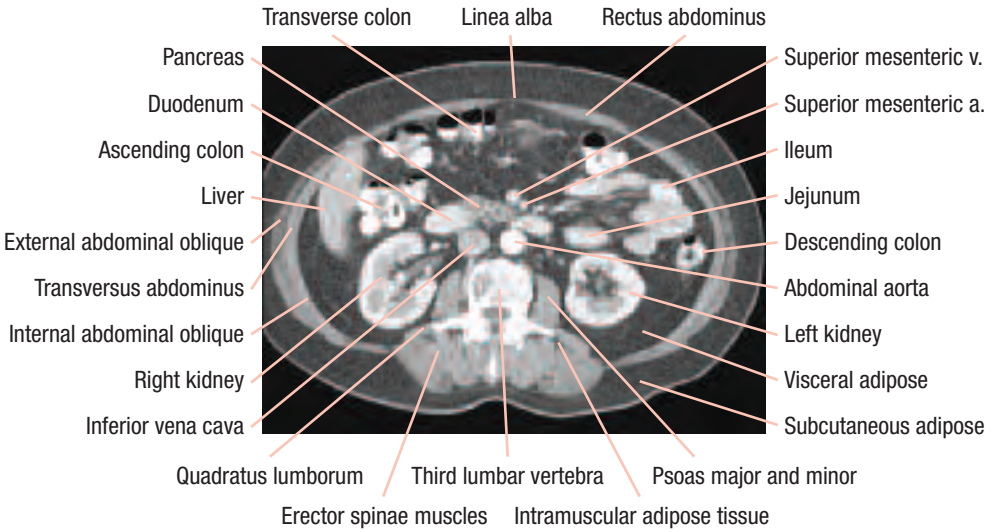
Experts are starting to acknowledge the independent behavior of muscle and adipose tissues on wasting syndromes. A recently convened consensus conference

on the definition of cachexia[13] notably made a distinction between the behavior of skeletal muscle and adipose tissue: “Cachexia, is a complex metabolic syndrome associated with underlying illness and *characterized by loss of muscle with or without loss of fat mass*. The prominent clinical feature of cachexia is weight loss in adults (corrected for fluid retention) or growth failure in children….” This perspective acknowledges the possibility of a persistent muscle loss in the absence of any change in fat mass.

The Use of Diagnostic Images To Assess Body Composition Changes and Sarcopenia in Cancer Patients

A highly differentiated understanding of human body composition has evolved in tandem with image-based technologies such as computed tomography (CT) and magnetic resonance imaging (MRI). These methods enjoy a high degree of specificity for the separate discrimination of many organs and tissues (Fig 1).

Fig 1. Structures present in a computerized tomography image at the 3rd lumbar vertebra.



CT is considered a gold standard method used to assess body composition[14]; however, its use in noncancer populations is limited.

Measurement of Lean Body Mass Using CT Scans

Cancer patients undergo frequent routine scans for diagnosis and to monitor disease progression. Although these patients are routinely evaluated by high-resolution diagnostic imaging, the information content of these images is barely exploited, in part owing to lack of deployment of relevant methods and concepts in a cancer care setting. We have proposed the opportunistic use of these high-quality images, which are readily available in the medical records of these patients, to provide accurate and practical studies of body composition across the cancer trajectory.[15] These images are a considerable resource and are now in many institutions stored and accessible in a digitized format. For example, our large cohort of patients with solid tumors of the lung and gastrointestinal tract typically undergo imaging four times a year during active treatment. An extensive reliance on diagnostic imaging gives specialists in cancer care an unprecedented ability to evaluate their patients' body composition, including repeated measures over time.

The premise of CT scans for body composition research has been described in detail elsewhere.[14,15] It is important to note that these methods are accessible. Image analysis software is commercially available, and the quantification can be done by any qualified person with appropriate knowledge of human anatomy, the nature of the images, and the capacities of the software.

Diagnostic images taken in cancer care do not usually encompass the whole body; this requires that patients be evaluated at a standardized location. Specific skeletal landmarks in the lumbar region appear in a majority of work in nonmalignant disease as well as in cancer.[2,15] As illustrated in Fig 1, this region contains visceral, subcutaneous, and intermuscular adipose tissue, psoas and paraspinal muscles (*erector spinae* and *quadratus lumborum*), as well as *transversus abdominus*, external and internal oblique abdominals, and *rectus abdominus*. Since the cross-sectional areas of tissues in single images in the lumbar area are strong correlates of whole-body adipose tissue, muscle, and lean tissue mass,[15-17] we make use of the 3rd lumbar vertebra (L3) in our characterization of cancer patients. Individuals may be compared directly on this basis; however, these quantities may be translated to approximate whole-body tissue masses using regression equations from earlier work.[15-17] Specific tissues are identified based on their anatomical features (Fig 1) and then demarcated and quantified (Fig 2) based on pre-established thresholds of Hounsfield units (units of radiation attenuation) using commercially available imaging analysis software.

Cross-sectional areas (cm^2) are computed automatically by the program once the desired tissues are demarcated. Demarcated images (Fig 2) illustrate body composition changes in a lung cancer patient over time; this person lost skeletal muscle during progressive disease.

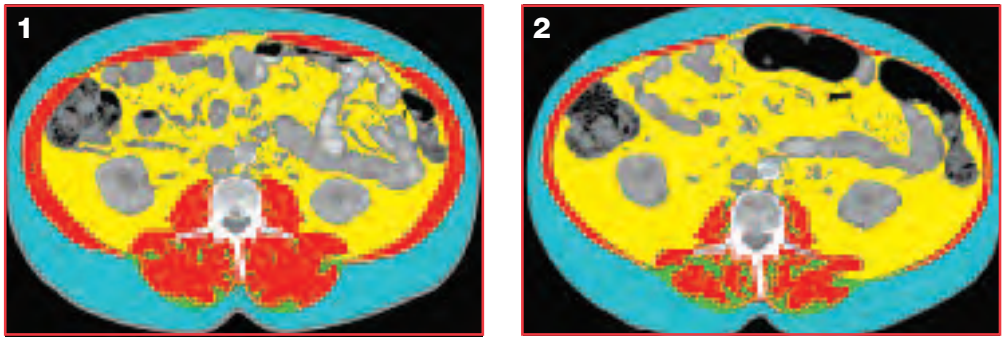


Fig 2. Skeletal muscle loss in a patient with lung cancer. Segmented CT images for a male lung cancer patient at two separate time points. Number 1 was taken 390 days before death, and 2 was taken 58 days before death. Segmented tissues of interest: ■ is skeletal muscle, ■ is visceral adipose tissue, ■ is subcutaneous adipose tissue, and ■ is intramuscular adipose tissue. During this 332-day period, skeletal muscle area decreased from 173 cm^2 to 86.7 cm^2.

Conclusions

Current demographics of weight and body composition suggest a need to reconceptualize cancer cachexia. Substantial depletion of skeletal muscle is a widespread abnormality of body composition in patients with advanced solid tumors, which is present in people at any BMI and strongly related to outcome. Valid and convenient approaches for determining muscularity are required to evaluate this feature in cancer patients, and the secondary analysis of CT images is an accessible means of making this evaluation.

References

1. Yavuzsen T, Davis MP, Walsh D, et al: Systematic review of the treatment of cancer-associated anorexia and weight loss. *J Clin Oncol* 2005;23:8500-8511.
2. Prado CM, Lieffers JR, McCargar LJ, et al: Prevalence and clinical implications of sarcopenic obesity in patients with solid tumours of the respiratory and gastrointestinal tracts: A population-based study. *Lancet Oncol* 2008;9:629-635.
3. Baumgartner RN, Koehler KM, Gallagher D, et al: Epidemiology of sarcopenia among the elderly in New Mexico. *Am J Epidemiol* 1998;147:755-763.
4. Janssen I, Heymsfield SB, Ross R: Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability. *J Am Geriatr Soc* 2002;50:889-896.
5. Pichard C, Kyle UG, Morabia A, et al: Nutritional assessment: Lean body mass depletion at hospital admission is associated with an increased length of stay. *Am J Clin Nutr* 2004;79:613-618.

6. Cosqueric G, Sebag A, Ducolombier C, et al: Sarcopenia is predictive of nosocomial infection in care of the elderly. *Br J Nutr* 2006;96:895-901.
7. Metter EJ, Talbot LA, Schrager M, Conwit R: Skeletal muscle strength as a predictor of all-cause mortality in healthy men. *J Gerontol A Biol Sci Med Sci* 2002;57:B359-B365.
8. Baumgartner RN: Body composition in healthy aging. *Ann N Y Acad Sci* 2000;904:437-448.
9. Zamboni M, Mazzali G, Fantin F, et al: Sarcopenic obesity: A new category of obesity in the elderly. *Nutr Metab Cardiovasc Dis* 2008;18:388-395.
10. Roubenoff R: Sarcopenic obesity: Does muscle loss cause fat gain? Lessons from rheumatoid arthritis and osteoarthritis. *Ann N Y Acad Sci* 2000;904:553-557.
11. Bouchard DR, Dionne IJ, Brochu M: Sarcopenic/obesity and physical capacity in older men and women: Data from the Nutrition as a Determinant of Successful Aging (NuAge)—the Quebec Longitudinal Study. *Obesity* (Silver Spring) 2009;17:2082-2088.
12. Rolland Y, Lauwers-Cances V, Cristini C, et al: Difficulties with physical function associated with obesity, sarcopenia, and sarcopenic-obesity in community-dwelling elderly women: The EPIDOS (EPIDemiologie de l'OSteoporose) Study. *Am J Clin Nutr* 2009;89:1895-1900.
13. Evans WJ, Morley JE, Argilés J, et al: Cachexia: A new definition. *Clin Nutr* 2008;27:793-799.
14. Ross R, Janssen I: Computed tomography and magnetic resonance imaging, in Heymsfield S, Lohman T, Wang Z, Going S (eds): *Human Body Composition*, ed 2. Champaign, Ill: Human Kinetics, 2005, p 523.
15. Mourtzakis M, Prado CM, Lieffers JR, et al: A practical and precise approach to quantification of body composition in cancer patients using computed tomography images acquired during routine care. *Appl Physiol Nutr Metab* 2008;33:997-1006.
16. Shen W, Punyanitya M, Wang Z, et al: Visceral adipose tissue: Relations between single-slice areas and total volume. *Am J Clin Nutr* 2004;80:271-278.
17. Shen W, Punyanitya M, Wang Z, et al: Total body skeletal muscle and adipose tissue volumes: Estimation from a single abdominal cross-sectional image. *J Appl Physiol* 2004;97:2333-2338.

Long-Term Outcomes of the Effect of ICU Stay on Lean Body Mass and Strength

Gerald Supinski, MD

The number of patients populating critical care units in the United States has grown steadily over the past 2 decades. This growth has occurred as the result both of implementation of technological advances that can keep patients alive longer and also in response to the application of ever-more sophisticated therapies (ie, organ transplants and advanced chemotherapy) that often have complications that require intensive care. While there is an overall benefit to the use of increasing complex technologies to keep patients alive, substantial morbidity also is associated with use of these therapies.

One major cause of morbidity in this patient population is the development of significant limb and respiratory skeletal muscle weakness and atrophy. Recent work indicates that these patients develop far more severe weakness than usually is recognized by the clinicians taking care of them, and this weakness lasts far longer after discharge from the hospital than people realize.[1-3]

This phenomenon is best supported by data from a study by Herridge et al, which measured the post-discharge strength and exercise tolerance of patients who were hospitalized for adult respiratory distress syndrome (ARDS).[1] ARDS, characterized by acute lung injury, affects 200,000 Americans each year. Approximately 70,000 of these patients die during hospitalization, but 130,000 survive the syndrome and are eventually discharged.

Herridge et al found that, on average, these patients do not return to work until 8-12 months after hospital discharge, and at 1 year, have a level of exercise capacity that is only 66% of the normal level (Table). Moreover, pulmonary function returns to normal in the majority of these patients at an early point after discharge, and it appears that reductions in long-term exercise capacity are linked to peripheral skeletal muscle dysfunction. Herridge et al recently have updated these data and find that exercise capacity remains substantially lower than normal, even 5 years after hospital discharge. Anecdotally, many of these patients give histories of having the ability to walk only short distances (eg, 40 feet) immediately after discharge, with an impaired ability to perform many of the tasks of daily living.

Table. Walk Distance and Return to Work Status of Survivors of ARDS[1]

Outcome	3 Months	6 Months	12 Months
Distance walked in 6 minutes			
Number evaluated	80	78	81
Median (m)	28	396	422
Interquartile range (m)	55-454	244-500	277-510
Percentage of predicted value	49	64	66
Returned to work Number/total number (%)	13/83 (16)	26/82 (32)	40/82 (49)

m=meter

Adapted from Herridge et al: Canadian Critical Care Trials Group: One-year outcomes in survivors of the acute respiratory distress syndrome. *New Engl J Med* 2003;348:683. © 2003 New England Journal of Medicine.

Top panel presents distance walked in 6 minutes; bottom presents number returned to work. At 1 year after discharge, walk distance was only 66% of predicted, and the number that had returned to work was only 49%.[1]

In addition to this severe limb muscle weakness, some work suggests that many critically ill patients develop significant weakness of the respiratory muscles. This is of special concern, because weakness of this particular muscle group contributes to respiratory failure and can prolong the time these patients require mechanical ventilatory support. Theoretically, this problem could result in a downward spiral, with critical care illnesses engendering respiratory muscle weakness, respiratory muscle weakness necessitating continued ventilatory support, and continued ventilator support leading to complications that sustain or worsen the level of critical care illness.

In support of this concept, two recent studies reported that critically ill patients requiring sustained mechanical ventilation have surprisingly severe reductions in diaphragm strength.[2,3] Both studies used bilateral magnetic stimulation to activate the phrenic nerves in the neck, and both recorded the transdiaphragmatic pressure evoked by supramaximal twitch stimuli to achieve an objective index of respiratory muscle strength. Laghi et al found that the average patient requiring mechanical ventilation had diaphragm twitch transdiaphragmatic pressures that were only 23%

of the level measured in a healthy control population.[2] Watson et al reported similar data, with transdiaphragmatic pressures decreased on average to levels only 36% of controls.[3] Furthermore, we have found some groups of critically ill patients to have even more severe reductions in diaphragm strength, with levels as low as 2%-5% of normal values recorded in individual patients.

Several factors appear to predispose critically ill patients to the development of skeletal muscle weakness and wasting. For one thing, these patients often are heavily sedated and relatively immobile, with disuse atrophy probably a major factor contributing to the development of weakness. Moreover, the respiratory muscles seem especially prone to the development of disuse atrophy, such that use of ventilator modes that result in little or no respiratory muscle contraction (eg, controlled volume-cycled or pressure-limited ventilation in the presence of heavy sedation and/or paralytic agents) may rapidly induce severe diaphragm atrophy and weakness.[4] In addition, patients often fail to receive even minimal levels of nutrition because of procedural delays, and malnutrition may become a major factor contributing to skeletal muscle weakness and atrophy.

A high percentage of ICU patients are infected at some point during their ICU stay with infections, both precipitating the need for ICU admission (eg, pneumonia, urinary tract infection) and complicating ICU stay (eg, line infections, ventilator-associated pneumonia). It is known that infection-induced systemic inflammation has profound effects on muscle function because of the direct and indirect effects produced by cytokines, and even minor infections (eg, colds) can produce enormous reductions (30%-40%) in the functional capacity of respiratory and limb muscles.[5] More serious infections produce even larger reductions in muscle function, with reductions in strength up to 50%-80% observed in both human and animal studies of severe sepsis (Fig 1).[6] Other factors that are present in critically ill patients and are associated with the development of skeletal muscle weakness include hyperglycemia, congestive heart failure, vitamin D deficiency, uremia, hypophosphatemia, and steroid administration.

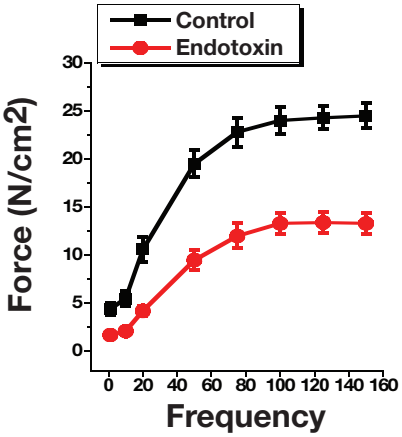


Fig 1. Skeletal muscle force-frequency curves with and without endotoxin treatment. Animals were treated with saline or endotoxin, killed at 24 hours, skeletal muscle excised, and muscle force determined in vitro in response to electrical excitation over a range of stimulation frequencies. Force was markedly reduced at all stimulation frequencies for muscle samples taken from lipopolysaccharide (LPS) (endotoxin)-treated animals.[7]

A substantial amount of recent work has examined the mechanisms by which these various stresses induce muscle weakness. Our own work has focused on the mechanisms by which infections alter muscle function, but many of the cellular pathways involved in sepsis-related muscle weakness also contribute to muscle dysfunction engendered by other pathophysiological processes (eg, disuse, hyperglycemia). Studies suggest that infection-induced muscle dysfunction does not result from activation of a single enzyme pathway and is not attributable to damage of a single subcellular structure, but probably represents the interacting effects of activation of a number of pathophysiological processes, which damage a number of subcellular organelles.

Animal models of infection, for example, are shown to cause a disruption of skeletal muscle sarcolemmal membranes, a depletion of sarcolemmal sodium-potassium ion exchange proteins, alterations in sarcoplasmic reticulum calcium turnover, a marked reduction in the intrinsic force-generating capacity of the contractile proteins, a reduction in mitochondrial adenosine triphosphate (ATP) synthesis rates, a reduction in mitochondrial ATP transport capacity, depletion of critical mitochondrial electron transport constituents, a reduction in creatine kinase levels, and depletion and dysfunction of skeletal muscle phosphofructokinase, a key enzyme required for glycolysis.[8-11] These subcellular alterations reduce the ability of muscle to both generate a single contraction and to sustain repetitive contractions.

Several pathophysiological processes are perhaps responsible for producing these multiple defects in skeletal muscle functional capacity. It is possible to broadly group these into two classes, factors that accelerate protein degradation in muscle and factors that impair protein synthesis. Recent studies demonstrate a marked increase in multiple elements of the proteasomal proteolytic pathway in sepsis, including an increase in ubiquitin, the 20S proteasome component, and several E3 ligases (atrogin and MuRF1 [ie, muscle ring finger 1]).[12,13] In keeping with the importance of the proteasome in sepsis, administration of proteasome inhibitors in an animal model of sepsis is shown to result in a marked reduction in protein degradation, as judged by use of the tyrosine release assay.[14]

In addition, calpain and caspase proteases are both shown to become activated in skeletal muscle in inflammatory states (Fig 2).[15,16] Both proteases can cleave important components of skeletal muscle contractile apparatus and cytoskeleton, including actin, actinin, and spectrin in the case of caspase, and myosin, talin, and spectrin in the case of calpain. It is thought that calpain and caspase activation may result in destabilization of the contractile protein matrix, leading to a disruption of force generation and facilitating release of contractile elements that can be subsequently degraded by the proteasome.[17,18] These proteolytic pathways are regulated by signaling pathways, and recent reports indicate that several upstream kinases, including p38 and protein kinase R (PKR), play essential roles in regulating the activation of proteolytic processes in response to inflammatory stimuli.[19,20]

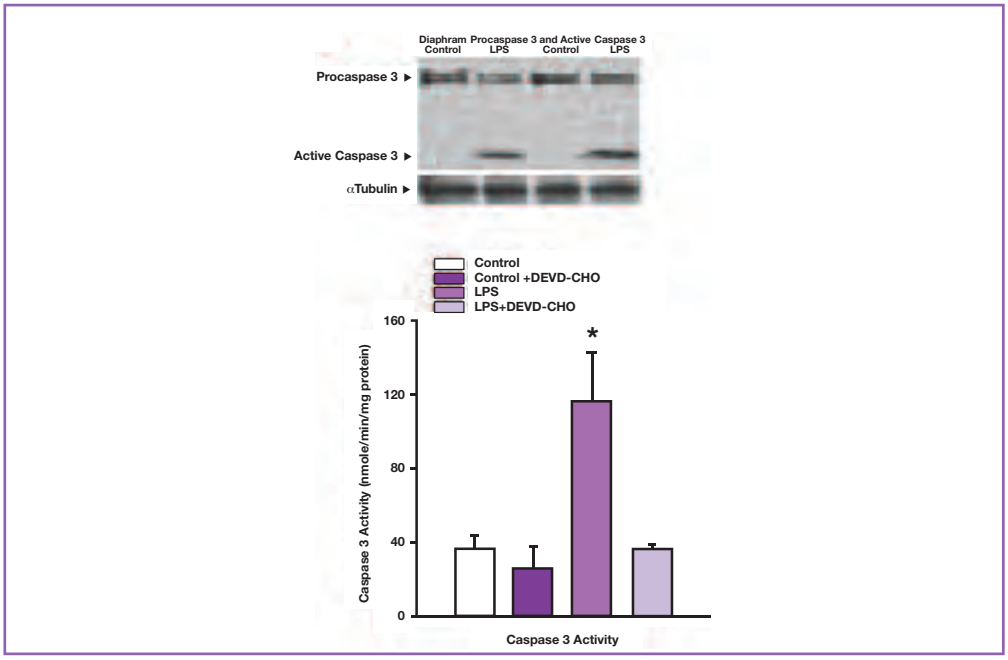


Fig 2. Skeletal muscle caspase activity following endotoxin administration. Top panel presents active caspase protein levels for skeletal muscle from control and endotoxin (LPS)-treated animals. LPS induced a large increase in active caspase protein. Bottom panel presents caspase activity levels assessed for muscle samples from control and LPS-treated animals; as an additional control, samples were run with and without addition of DEVD-CHO, an exogenous caspase inhibitor. LPS administration markedly increased muscle caspase activity.[16]

Supinski et al: Caspase activation contributes to endotoxin-induced diaphragm weakness. *J Appl Physiol* 2006;100:1770. © 2006 American Physiological Society. Reprinted with permission.

The precise mechanisms by which inflammation reduces protein synthesis also has undergone considerable recent study and appears to involve alterations in circulating hormone levels that normally modulate protein synthesis (eg, corticosteroid levels) and, in addition, muscle-specific alterations in the activity of regulators of translation, such as eIF2Bepsilon (eukaryotic initiation factor 2B epsilon) and eIF2α.[21,22] Reductions in protein synthesis by as much as 50% can occur within a few days in response to infection and other inflammatory stimuli, and this process alone may result in a significant reduction in skeletal-muscle protein stores.

Summary

Loss of functional capacity of skeletal muscle is a major cause of morbidity in patients with critical illnesses. Weakness in these patients can manifest as either severe limb muscle weakness and/or respiratory muscle weakness, requiring mechanical ventilatory support. Several factors appear to interact to induce

weakness in these patients, including inactivity, poor nutrition, infection, drugs, and hyperglycemia. These various factors are thought to interact to activate several proteolytic pathways (ie, the proteasome, caspase, and calpain) and to impair protein synthesis. A complete understanding of the cellular pathways by which skeletal-muscle proteolysis is enhanced and protein synthesis is reduced in critically ill patients should lead to the discovery of cellular pathways that can prevent weakness and wasting in this patient population through therapeutic targeting.

References

1. Herridge MS, Cheung AM, Tansey CM; Canadian Critical Care Trials Group: One-year outcomes in survivors of the acute respiratory distress syndrome. *N Engl J Med* 2003;348:683-693.
2. Laghi F, Cattapan SE, Jubran A, et al: Is weaning failure caused by low-frequency fatigue of the diaphragm? *Am J Respir Crit Care Med* 2003;167:120-127.
3. Watson AC, Hughes PD, Louise Harris M, et al: Measurement of twitch transdiaphragmatic, esophageal, and endotracheal tube pressure with bilateral anterolateral magnetic phrenic nerve stimulation in patients in the intensive care unit. *Crit Care Med* 2001;29:1325-1331.
4. Levine S, Nguyen T, Taylor N, et al: Rapid disuse atrophy of diaphragm fibers in mechanically ventilated humans. *N Engl J Med* 2008;358:1327-1335.
5. Poponick JM, Jacobs I, Supinski G, DiMarco AF: Effect of upper respiratory tract infections on respiratory muscle strength in patients with neuromuscular disease. *Am J Respir Crit Care Med* 1997;156(2 Pt 1):659-664.
6. Lanone S, Mebazaa A, Heymes C, et al: Muscular contractile failure in septic patients: Role of the inducible nitric oxide synthase pathway. *Am J Respir Crit Care Med* 2000;162:2308-2315.
7. Supinski G, Nethery D, Nosek TM, et al: Endotoxin administration alters the force vs. pCa relationship of skeletal muscle fibers. *Am J Physiol Regul Integr Comp Physiol* 2000;278:R891-R896.
8. Callahan LA, Nethery D, Stofan D, et al: Free radical-induced contractile protein dysfunction in endotoxin-induced sepsis. *Am J Respir Cell Mol Biol* 2001;24:210-217.
9. Callahan LA, Stofan DA, Szweda LI, et al: Free radicals alter maximal diaphragmatic mitochondrial oxygen consumption in endotoxin-induced sepsis. *Free Radic Biol Med* 2001;30:129-138.
10. Callahan LA, Supinski GS: Downregulation of diaphragm electron transport chain and glycolytic enzyme gene expression in sepsis. *J Appl Physiol* 2005;99:1120-1126.
11. Callahan LA, Supinski GS: Diaphragm and cardiac mitochondrial creatine kinase are impaired in sepsis. *J Appl Physiol* 2007;102:44-53.
12. Roth GA, Moser B, Krenn C, et al: Heightened levels of circulating 20S proteasome in critically ill patients. *Eur J Clin Invest* 2005;35:399-403.

13. Wray CJ, Mammen JM, Hershko DD, Hasselgren PO: Sepsis upregulates the gene expression of multiple ubiquitin ligases in skeletal muscle. *Int J Biochem Cell Biol* 2003;35:698-705.
14. Kadlciková J, Holecek M, Safránek R, et al: Effects of proteasome inhibitors MG132, ZL3VS and AdaAhx3L3VS on protein metabolism in septic rats. *Int J Exp Pathol* 2004;85:365-371.
15. Fareed MU, Evenson AR, Wei W, et al: Treatment of rats with calpain inhibitors prevents sepsis-induced muscle proteolysis independent of atrogin-1/MAFbx and MuRF1 expression. *Am J Physiol Regul Integr Comp Physiol* 2006;290:R1589-R1597.
16. Supinski GS, Callahan LA: Caspase activation contributes to endotoxin-induced diaphragm weakness. *J Appl Physiol* 2006;100:1770-1777.
17. Du J, Hu Z, Mitch WE: Cellular signals activating muscle proteolysis in chronic kidney disease: A two-stage process. *Int J Biochem Cell Biol* 2005;37:2147-2155.
18. Powers SK, Kavazis AN, DeRuisseau KC: Mechanisms of disuse muscle atrophy: Role of oxidative stress. *Am J Physiol Regul Integr Comp Physiol* 2005;288:R337-R344.
19. Supinski GS, Callahan LA, Ji X: p38 MAP kinase modulates endotoxin induced diaphragm caspase activation. *Am J Respir Cell Mol Biol* 2010;43:121-127.
20. Supinski GS, Callahan LA: PKR kinase modulates endotoxin induced diaphragm caspase 8 activation [publication page A4201]. Presented at: American Thoracic Society Meeting; May 19, 2009; San Diego, CA. Available at: http://www.abstracts2view.com/ats09/view.php?nu=ATS09L_4547&terms. Accessed January 23, 2010.
21. Svanberg E, Frost RA, Lang CH, et al: IGF-I/IGFBP-3 binary complex modulates sepsis-induced inhibition of protein synthesis in skeletal muscle. *Am J Physiol Endocrinol Metab* 2000;279:E1145-E1158.
22. Vary TC, Deiter G, Kimball SR: Phosphorylation of eukaryotic initiation factor eIF2Bepsilon in skeletal muscle during sepsis. *Am J Physiol Endocrinol Metab* 2002;283:E1032-E1039.

Acknowledgement

Written in collaboration with Dr Leigh Ann Callahan, associate professor of medicine, Dept of Medicine and the Muscle Biology Center, University of Kentucky, Lexington.

Q & A

Q: The data you show with LPS is very similar or maybe in a slightly different order to what I [Dr Tisdale] showed with the LPS in tissue culture. We also find, by the way, that hyperglycemia induces an activation of PKR, so you could have the same mechanism.

The question I was going to ask was that I [Dr Tisdale] showed Tuesday that HMB [β-hydroxy-β-methylbutyrate] also was effective in attenuating the increase in caspase and PKR and preventing the muscle protein degradation in vitro. Did you consider using this, as well as EPA [eicosapentaenoic acid], in your animal model and maybe patients?

Dr Supinski: That is a great suggestion, and we would like to test a number of therapies in the animal model. That is a great therapy, because I think we could rapidly translate it to human use. We have found some other potential treatments in animals that are theoretically usable in people. Obviously, I cannot give a calpain inhibitor 3 to a patient, but there are other ways to inhibit caspase and calpain. I think that is an excellent suggestion.

Q: My question is with the selectivity of the mediators, such as the caspase and calpain and p38. Are we talking about something at the receptor level?

Dr Supinski: Well, it is one of the things we focused on. We think that the receptors involved in this are probably cytokine receptors, TNF, and interleukin. In sepsis and in critical patients in general, use of receptor antagonists to cytokines has not worked out very well.

Our thesis and contention are that cytokine receptor antagonists block these pathways too far upstream, inhibiting the good as well as the bad effects of cytokines such as TNF [tumor necrosis factor]. You want TNF to activate neutrophils. You want neutrophils to kill bacteria. As a result, we believe that it may be an important therapeutic strategy to try to selectively block some of the far-downstream effects of cytokines, focusing on blocking detrimental effects alone. The processes we are trying to block (ie, caspase, calpain, and PKR) are fairly downstream. We believe this helps in two ways. First, we will not interfere with the normal functions of cytokines, such as TNF, and second because we are blocking later pathophysiological events, we have more time from the onset of illness to administer our treatments.

Q: You mentioned that force dropped as the first thing, and then mitochondrial function.

Dr Supinski: Yes, force drops first, then mitochondrial function declines, and finally we see loss of muscle mass and protein content (ie, muscle wasting).

Q: I was wondering if you could you speculate about what you think causes this drop in muscle function as the first thing?

Dr Supinski: It is profound, and we have seen it over and over again in every one of our infectious models. We think what is happening initially is that the contractile protein lattice is perturbed. I think it almost has to be this way, because you have to break the proteins out of that lattice before you can degrade them. When they first come out of the lattice or the lattice is disrupted, the proteins remain in the cell. As a result, protein content and muscle mass remain normal initially. Subsequently, these proteins become fodder for the proteasome inhibition pathway.

Now, one of the other things we have seen, which I did not present, is if we give a proteasomal inhibitor, it does nothing to these reductions in strength. I think that protein degradation is a two-step process. First, relatively selective proteases, such as caspase and calpain, break apart the lattice. Afterward, the contractile proteins fall out and are available for degradation by the proteasome. Force drops when the lattice is initially disrupted; muscle mass and protein content decline when the removed proteins are degraded. Perhaps the proteasome may be largely degrading proteins that do not work anyway in our sepsis models. This is our interpretation.

Now, the mitochondrial alterations are also interesting. We observe increased free-radical generation from mitochondria very early (and far before we observe reductions in mitochondrial function). Is that part of the same process? Is that part of the triggering pathway for activation of all of these proteolytic pathways? I do not know.

Q: I did not catch how long you supplemented with EPA. Was it was long enough to change the membrane composition? I also wonder if you could speculate on EPA's inhibiting calpain activation, and in the backdrop of oxidative stress, if it is through neuroprostanes and isoprostanes.

Dr Supinski: I think it is a good bet that EPA may affect lipid constituents, including isoprostanes. Our initial hypothesis was that EPA administration to septic animals would not affect force. We thought it was going to affect wasting. We measured all these indices (force, mass, and protein content) just to be complete. We were

shocked when we found that EPA was capable of inhibiting calpain and preventing force loss. We would speculate that EPA may stabilize membranes and in some way prevent calcium release as a potential explanation for its ability to inhibit calpain.

To answer your question, I am not completely sure. I will bet it is something related to isoprostanes or PLA2 (phospholipase A2), or something to do with the membrane and calcium release through membranes. It probably is that mechanism. This is the first time anyone has shown, to my knowledge, that with EPA you can affect calpain activation. We certainly were not looking for it, but this is what we found.

Cachexia in Cancer

Ingvar Bosaeus, MD

Severe, progressive malnutrition and wasting often is seen in advanced cancer, with weight loss long associated with decreased survival.[1] The term *cachexia* refers to a progressive weight loss with depletion of host reserves of skeletal muscle and adipose tissue. It also represents the complex and profound metabolic changes seen in advanced cancer, characterized by breakdown of skeletal muscle and abnormalities in fat and carbohydrate metabolism. Other important features of cachexia are anorexia, early satiety, muscle weakness, and fatigue.[2]

The syndrome, however, is not well defined. A recent consensus definition emphasizes the loss of fat and muscle and the complex metabolic changes.[3] Diagnostic criteria are proposed, based on a weight loss of 5% or more in 12 months or less, plus at least three out of five of the following:

- Decreased muscle strength
- Fatigue
- Anorexia
- Low fat-free mass index
- Abnormal biochemistry (inflammatory markers, anemia, and low albumin)

Another proposed three-factor classification emphasizes that patients with weight loss, reduced food intake, and evidence of systemic inflammation are particularly at risk in terms of adverse functional status and prognosis, and that such patients should receive evaluation for intervention as soon as identified.[4]

The Two Pathways to Wasting

The development of cachexia reflects both a reduced food intake and a catabolic metabolism induced by an inflammatory host response to tumor presence and/or tumor factors.[5] This leads to a negative energy and protein balance, manifesting as weight loss as body stores are progressively depleted. Because low food intake, for whatever reason, is in one pathway only, stand-alone nutritional intervention is unlikely to correct the imbalance, unless the metabolic changes are addressed at the same time.[6]

Energy Balance in Cancer Cachexia

When food intake is lower than requirements, body energy stores are mobilized to meet demands. This is normally a metabolic and behavioral adaptation, leading to decreased energy expenditure. Furthermore, body fat stores are preferentially used for fuel, with a relative sparing of the fat-free body mass. In cancer cachexia with systemic inflammation, activation of protein breakdown in skeletal muscle is seen, and the amino acids thus generated are used to fuel hepatic protein and glucose synthesis.[7] Thus, faster breakdown of skeletal muscle occurs with a preservation of visceral organs. Nutrition support is capable of restoring body fat, but muscle breakdown still is driven by systemic inflammation.

Negative energy balance leading to progressive weight loss is attributed to changes in energy intake, components of energy expenditure, or both, mediated by metabolic alterations. Diminished food intake is a prominent feature in weight-losing cancer patients, with most, but not all, studies reporting a low intake. We found a low intake in 297 patients with advanced cancer, mainly gastrointestinal tumors.[8] Mean dietary intake was below maintenance requirements, but not different in normometabolic and hypermetabolic patients. Weight loss of more than 10% was present in 43% of the patients and elevated resting energy expenditure (REE) in 48%. Weight loss was not accounted for by diminished dietary intake, because energy intake in absolute amounts was not different and intake per kilogram body weight was higher in weight-losing patients compared to weight-stable patients. Increased REE could make a large contribution to negative energy balance, if not compensated for by an increase in energy intake.

Thus, metabolic derangements contribute to cachexia development, and these metabolic changes differ from those induced by decreased energy intake or starvation alone.

Body Composition in Cancer Cachexia

In simple starvation, muscle mass usually is preserved at the expense of body fat depots, which are preferentially used to provide energy. In contrast, relatively more muscle tissue is lost in the development of cancer cachexia, and these changes are not reversed if adequate energy and other nutrients are provided, as seen in “pure” starvation states. The active body cell mass (predominantly skeletal-muscle tissue) and its associated intracellular water decrease, while the extracellular space is maintained or more slowly decreased, with or without clinical signs of edema. Thus, body weight changes may not accurately reflect the amount of muscle and fat lost.[9] To better characterize the depletion of cancer cachexia, expansion is needed of the

commonly used body mass index (BMI) in order to reflect these body composition changes, for instance using height-adjusted indices of fat-free mass index (FFMI), fat mass index (FMI), and skeletal muscle index (SMI). However, this requires defined reference values and standardized body composition measurements.[10]

Nutrition Support

The best way to treat cancer cachexia is obviously to cure the cancer, thus normalizing the metabolic abnormalities induced by the tumor and/or tumor/host interactions. When cure is unachievable, an obvious next option is to increase nutritional intake by dietary counseling and oral nutritional supplements or by artificial nutrition. A number of studies have tried to achieve this. However, no benefits were found in terms of anthropometric measures, response rate to therapy, survival, or quality of life.[11]

Parenteral nutrition is difficult to supply over extended periods of time and is associated with a number of complications. A number of earlier, mostly parenteral, nutrition trials have shown no benefit, but rather a tendency to increase infectious complications.[11] Thus, no evidence exists to show that increased nutritional intake alone is effective in the palliation of cancer cachexia.

Anticatabolic Therapy

The disappointing results of stand-alone conventional nutritional supplementation in cancer patients has led to a focus on the metabolic changes in cancer cachexia and attempts to manipulate the metabolic alterations with a variety of pharmacological agents. Thus, it seems that strategies to counteract the inflammatory response and its metabolic consequences are an option.

Steroids are widely used and are shown to improve appetite. However, steroids will not reverse ongoing weight loss and muscle wasting, their symptomatic benefits are often short-lived, and they are associated with a number of adverse effects. Nonsteroidal anti-inflammatory drugs (NSAIDs) are shown to reduce acute-phase proteins and REE and preserve body fat in patients with advanced cancer.[12] Treatment with indomethacin is shown to stabilize performance status and prolong survival.[13] Therefore, NSAIDs seem to have a role in the palliation of cancer cachexia, although effect size and response rate still are not well known.

Anabolic androgenic steroids stimulate net muscle protein synthesis, with testing in a number of catabolic conditions, but their therapeutic potential in cancer cachexia in largely unknown.[6] Much interest also has focused on the importance of the

growth hormone (GH)/insulin-like growth factor (IGF-1) axis on the anabolic regulation of skeletal muscle mass. However, in cancer patients, the ongoing worry remains that growth factors may stimulate tumor growth, and these concerns may limit trials in this area.

We recently have shown that low-dose insulin treatment (0.11±0.05 units/kg/day) stimulated carbohydrate intake and metabolic efficiency during exercise, and improved survival in cancer cachexia.[14] However, fat-free mass, maximum exercise capacity, and spontaneous physical activity were unaffected.

The role of treatments to reverse the underlying metabolic changes in cancer cachexia is presently unclear.

Multimodal Approach

We have studied the effect of nutritional support in combination with anti-inflammatory treatment (NSAID) and anemia prevention (erythropoietin) in 309 patients with progressive cachexia because of solid tumors.[12] As-treated analysis demonstrated that patients receiving nutritional support had a prolonged survival, accompanied by improved energy balance, body fat, and a greater maximum exercise performance. The results support that nutrition is a limiting factor influencing survival and that treatment targeted toward both diminished nutritional intake and metabolic alterations is perhaps more effective. A recent study of patients on palliative chemo/radiotherapy given oral nutrition support plus parenteral nutrition (30% of requirements) also showed improved 48-week survival, body composition, and quality of life.[15]

Perspectives

The metabolic alterations in advanced cancer have many parallels to a chronic systemic inflammatory response and differ considerably from the metabolic changes in starvation. Nutritional support alone does not appear to affect overall survival in advanced cancer, but in combination with treatment targeted against the inflammatory response and/or metabolic abnormalities, focusing also on energy expenditure is possibly of greater value. Therapeutic strategies aimed at modulating the mediators of the catabolic response, such as cytokines and eicosanoids, or metabolic regulation, such as with anabolic and anticatabolic agents, may thus offer more promise in the future. Early detection and intervention also may prove more effective. An optimal therapeutic approach to cancer cachexia is not yet available. Strategies to counteract both catabolism and reduced dietary intake may have

importance for the survival, function, and quality of life of cancer patients, and researchers should continue to explore this in interventional studies.

References

1. DeWys WD, Begg C, Lavin PT, et al: Prognostic effect of weight loss prior to chemotherapy in cancer patients. *Am J Med* 1980;69:491-497.
2. Baracos VE: Cancer-associated cachexia and underlying biological mechanisms. *Annu Rev Nutr* 2006;26:435-461.
3. Evans WJ, Morley JE, Argilés J, et al: Cachexia: A new definition. *Clin Nutr* 2008;27:793-799.
4. Fearon KC, Voss AC, Hustead DS; Cancer Cachexia Study Group: Definition of cancer cachexia: Effect of weight loss, reduced food intake, and systemic inflammation on functional status and prognosis. *Am J Clin Nutr* 2006;83: 1345-1350.
5. Skipworth RJ, Stewart GD, Dejong CH, et al: Pathophysiology of cancer cachexia: Much more than host-tumour interaction? *Clin Nutr* 2007;26:667-676.
6. Fearon KC: Cancer cachexia: Developing multimodal therapy for a multidimensional problem. *Eur J Cancer* 2008;44:1124-1132.
7. Tisdale MJ: Cachexia in cancer patients. *Nat Rev Cancer* 2002;2:862-871.
8. Bosaeus I, Daneryd P, Svanberg E, Lundholm K: Dietary intake and resting energy expenditure in relation to weight loss in unselected cancer patients. *Int J Cancer* 2001;93:380-383.
9. Fouladiun M, Körner U, Bosaeus I, et al: Body composition and time course changes in regional distribution of fat and lean tissue in unselected cancer patients on palliative care—correlations with food intake, metabolism, exercise capacity, and hormones. *Cancer* 2005;103:2189-2198.
10. Prado CM, Birdsell LA, Baracos VE: The emerging role of computerized tomography in assessing cancer cachexia. *Curr Opin Support Palliat Care* 2009;3:269-275.
11. American College of Physicians: Parenteral nutrition in patients receiving cancer chemotherapy. *Ann Intern Med* 1989;110:734-736.
12. Lundholm K, Daneryd P, Körner U, et al: Evidence that long-term COX-treatment improves energy homeostasis and body composition in cancer patients with progressive cachexia. *Int J Oncol* 2004;24:505-512.

13. Lundholm K, Gelin J, Hyltander A, et al: Anti-inflammatory treatment may prolong survival in undernourished patients with metastatic solid tumors. *Cancer Res* 1994;54:5602-5606.

14. Lundholm K, Körner U, Gunnebo L, et al: Insulin treatment in cancer cachexia: Effects on survival, metabolism, and physical functioning. *Clin Canc Res* 2007;13:2699-2706.

15. Shang E, Weiss C, Post S, Kaehler G: The influence of early supplementation of parenteral nutrition on quality of life and body composition in patients with advanced cancer. *JPEN J Parenter Enteral Nutr* 2006;30:222-230.

Discussion

Leader: Anne Coble Voss, PhD, RD, LD, Abbott Nutrition, Columbus, OH

Dr Tisdale: Dr Bosaeus, you suggested that the drive to synthesize acute-phase proteins by the liver was actually driving the breakdown of skeletal muscle. However, several years ago experiments in mice suggested this was not the case. In that experiment, researchers gave interleukin 6 (IL-6) to mice, and they showed that it indeed induced acute-phase response, but it had no effect whatsoever on skeletal muscle protein or body weight. In contrast, another member of the IL-6 family, ciliary neurotrophic factor, actually produced both. It produced an acute-phase response and a depletion of muscle protein. So although there might be a correlation between the two, I do not think there is a direct linkage between the drive to synthesize acute-phase protein and the loss of skeletal muscle.

Dr Bosaeus: I hope I did not imply a direct link there, because I am quite ignorant of all the details. I was trying to illustrate a shift in interorgan metabolism, in which there is a shift to acute-phase proteins in the liver, driven by IL-6 and other cytokine signals. The fuel for that must come from somewhere, and skeletal muscle is probably the best candidate for that. Somehow there has to be a signal, but it does not have to be the same to produce these things. I am not aware of the role of specific cytokines or other signals in orchestrating this, but this is obviously something of interest.

Dr Tisdale: Acute phase proteins can be produced without any loss of skeletal muscle.

Dr Baracos: Skeletal muscle protein is the most likely precursor and fuel to support the acute-phase response; there is not much in the way of alternative resources to commit to the acute-phase response. Theoretically, we could provide more protein intake to drive the acute-phase response in the liver without muscle mobilization. However, as far as I know, the data so far do not show any great reduction in protein breakdown in skeletal muscle by feeding extra protein.

Dr Bosaeus, I told my good friend and colleague, Neal McDonald, that I had a patient who had lost 5 units of body mass index in the last 6 months, and who was extremely sarcopenic and cachexic. Noting that I would reasonably expect this person to die within 1 to 2 weeks, I asked him what anticachexia therapy he would propose. His response: “Are you nuts? I do not propose any anticachexia therapy for this person. He or she has no reasonable expectation to benefit from it.” If this is indeed a clinically appropriate response, then why is it that in many randomized clinical trials of

anticachexia therapy and nutritional support, so many patients very close to death are included?

It may be in the best interest of research done in this area to find a way to exclude such people from trials or analyses of data, because there is indeed no expectation of a treatment response. Benefit of nutritional support is not expected immediately preceding death, like it might well be in longer-surviving cancer patients or COPD patients who are not imminently dying. Is approximately 12 weeks of treatment required to produce a quantifiable benefit? What do you think about this issue?

Dr Bosaeus: I agree. But the main criterion in the studies I described has been expected survival of more than 6 months in the mind of the attending physician. In practice, this measure ends up a mean of 8 months ±130 days. This is crude, but it is one way to go. Perhaps we could find better features than the clinical judgment of the attending physician for identifying those people at an earlier stage.

Another crude measure we have tried is to be on the alert for small changes in weight. So for instance, when initiating an insulin trial, it was on the premise of when weight loss started. In practice, we were able to clinically detect, with the help of the patient, about a 2% weight loss from the stabilization plateau.

Dr Baracos: Another conundrum that strikes me every day as I go into the cancer center worried about cachexia is that there are more new patients diagnosed with metastatic disease who are so fat that they cannot fit in the magnetic resonance imaging (MRI) machine than there are people who are clinically underweight. This may be a leading-edge North American phenomenon, but I do not have an honest answer to what the nutritional strategy should be for a patient who is sarcopenic obese.

Dr Bosaeus: I do not have that answer either. But if a patient has a large fat reserve, which is energy reserve, we could focus less on energy balance and more on muscle. What that would reflect in practice, I would not speculate on now.

Dr Morley: Dr Bosaeus, I liked your approach to cancer cachexia, but the question that now has to arise with the GTx Ostarine™ study in cancer is whether catabolism actually matters. Those researchers used an anabolic agent with minimal anticatabolic effects and showed improved power in a sick group of cancer patients over 6 months to 1 year. I thought the study would fail, so I was surprised at the outcome. We have to recognize the possibility that if we can actually drive protein into muscle, we will build muscle even in the presence of a catabolic stimulus,

which all of these patients had. This turns everything upside down from the way I have thought of it before.

Dr Bosaeus: Perhaps I should have been clearer, but this imbalance with synthesis down, degradation up, probably varies in a single individual and perhaps also over time. If the main problem is on the synthesis side, yes, anabolic stimuli could be the agents of choice, but these would probably be less effective if at the same time degradation was up. I do not know what clinical markers that could be used to classify a patient in these terms, but if it became possible, I think it would be a help.

Dr Morley: I would have thought in a predominantly lung-cancer group, it would have been catabolic. That is what I believed before I saw the data. Our study in assisted living, coupled with other recent studies, suggest that if we provide some sort of protein supplement along with an anabolic stimulus, we may well make a major difference in this population.

A question we are left with in the ICU population is whether we should first give them vitamin D, then give everything else. Should we give essential amino acids and fish oil as people come into the ICU, along with an anabolic stimulus, or should we wait? The insulin trials in the ICU are difficult to interpret. We have no idea what the right answer is anymore.

Dr Supinksi: I think these patients need to be fed early, and giving EPA (eicosapentaenoic acid) when they first come in would be logical.

A characteristic of the ICU population that makes it different from the cancer patients is the ICU patients get acute respiratory distress syndrome (ARDS). The strange thing about ARDS is that procollagen 3 is a biomarker in the lung fluid that predicts bad outcomes. The higher the procollagen 3, the more fibrosis the patients get, the worse the outcome is.

One concern is that anabolic agents tend not to just be anabolic in legs, but they also may be anabolic in lungs. Testosterone, for example, activates procollagen 3 pathways. One marker in patients indicating a response to testosterone is procollagen 3 levels in their blood. We have had that experience when we tried to give anabolic agents such as oxandrolone to patients in the ICU; lung function actually gets worse in response to the anabolic agent. I am concerned about the really critically ill patient population—people who have fibrosis in their lungs or some sort of ongoing pulmonary inflammation. Is there an anabolic agent that can help muscle without damaging the lung? I think that is a serious unanswered question. One controlled study by some surgeons who randomized everybody in

their ICU to oxandrolone vs a placebo found that length of stay on a ventilator increased and time in the ICU increased in the patients who got the anabolic agent [Bulger EM et al: *Ann Surg* 2004;240:472-478].

Dr Reid: Dr Supinski, I am stimulated by the discussions earlier about the effects of nutrition in cancer and in COPD, and by your repeated comments that in the ICU, patients often get very little nutrition and nutrition is not a high priority. Do you think that protein or nutrition per se would fall in the same category, and you would want to be cautious about providing it? Or if you would be willing to give nutrition, how do you go about changing the culture in the ICU to help people understand the importance of nutrition?

Dr Supinski: I am fairly crazy, but I am not so crazy that I think you should not feed patients. I worry about oxandrolone and androgens in general, but I would feed them. I complained this morning that patients are not being fed. I do not think that clinicians do not want to feed patients, but patients fail to receive nutrition because of procedural issues (eg, holding feedings to perform tests, to perform ventilator weaning trials, etc). Sometimes feeding is stopped for a procedure, and people do not get around to resuming it for 24-48 hours. How do you change that? This is a national problem in taking care of patients in ICUs. ICUs are chaotic, and we need to have better approaches that allow a more determined approach to feeding.

Dr Stephen DeMichelle [Abbott Nutrition]: Dr Supinski, I share with Dr Reid a philosophical question. Do you see antiproteolytic therapy being implemented with the new recommendation for lower tidal volume, lung strategies, and fluid therapy in ICU patients with a complex pathophysiology, or do you see it implemented more often in patients weaned off the ventilator and discharged from the hospital?

I am interested in acquired weakness and frailty once the patients leave the hospital, and in the new data coming 5 years later. Is this proteolytic pathway still turned on, or is the issue that patients are discharged, depressed, and delirious and not getting adequate care and good nutritional support?

Dr Supinksi: As I said, these patients should get some nutrition, and we should not wait to provide it. In fact, I do not think that we can wait until they are ready to wean from the ventilator. The data I presented show that by the time the physician gets around to weaning these patients, they are already weak. We need to give nutrients and anabolic agents such as EPA early. We have to set some better standards to feed these people earlier. Much of the delay is caused by the chaos in the ICU unit, but we have to figure out a way to get around that.

Structured Panel Discussion

Leader: Neile Edens, PhD, Abbott Nutrition, Columbus, OH

Dr Edens: In our last session, I would like to turn our attention to clinical science. We have heard over the course of the conference various priorities in clinical investigation. We have heard a need for larger trials, for longer interventions, and for trials in particularly vulnerable populations.

As a nutrition company, we are going to have to make choices and set priorities. So I would like the participants to talk about nutrition intervention questions to address in sarcopenia and cachexia. In particular, as we move nutrition beyond just provision of basic macronutrients and micronutrients into more experimental ingredients, such as EPA for management of inflammation and HMB for management of protein degradation, how would you address how new ingredients factor into what you would like to see in the next wave of clinical innovation?

Dr Wheeler: First, we need to address what the role of nutrition can be vs drug therapy or vs no nutrition. In some of the trials we are involved with all over the world, especially in emerging markets, we are learning a great deal about patient care. People in some areas strongly believe that nutrition can play a role in health care. In those areas, the specific nutrients we have discussed here can have a role. But in other areas, people refuse to consider such a role for nutrition.

So we have to address people's beliefs about what role nutrition plays in health care and when it plays that role, and then decide how we feed patients who are in these circumstances where nutrition is not valued as a valid therapy. And we have to address what important outcomes nutrition interventions can produce working synergistically with drug interventions.

Dr Paddon-Jones: From our vast clinical experience, we have a ton of data on protein metabolism, specifically in young, healthy populations and older populations, but we really do not know how that translates to clinical groups. Our first charge is to see whether we get similar response to protein supplementation in these clinical populations. Theoretically, from a practical point of view, we could make a huge difference, if these effects carry over to those groups. But in reality, we really need to look at more of a minimal, feasible approach. Can we get some improvement with a low-volume supplement, something these patients will actually take, rather than the traditional, high-volume supplements we give to younger, healthier subjects?

Dr Guttridge: When I come to meetings like this, I always try to leave with some new insight into our cancer studies and whether some common mechanisms exist between cancer and others conditions related to cachexia such as COPD (chronic obstructive pulmonary disease). I do not think we spend enough time trying to understand the commonalities or the differences between these conditions.

If you at Abbott Nutrition do see improvement in a particular cachexia state with one of your products, I hope that you would try to understand what the markers are that are being affected. Then hopefully, if we see improvement in another cachexia condition, we can see whether those same markers are affected and be able to understand where the overlap is or what the distinctive features are from one condition to another.

Dr Baracos: We need to ask whether we can transform somebody who is sarcopenic to someone who is no longer sarcopenic. Dr Morley stated earlier that you would not have believed that it was possible to put muscle back on people with lung cancer.

The fundamental question is, do they have anabolic potential? We do not know the answer to it. If they have no exploitable anabolic potential, which they might not have if they had been treated with sorafenib, they might well have under some conditions. When we can answer that, we will take a group of people and push them back up the scale, so that they are no longer sarcopenic. We need to do trials that have outcomes that are really clinically important. For instance, can the patients tolerate their chemotherapy, or live longer, or be discharged from an interim weaning ventilator unit—something that matters materially to their outcome. You cannot impress people with a mere promise of a few kilograms of lean body mass.

Dr Cederholm: I have been thinking about resistance training in the elderly, because we know that resistance training is probably the strongest anabolic stimuli we can use. So why do we not use that much more, even in the elderly population? We know a great deal about the beneficial effects of nutritional supplementation, and we know a great deal about exercise effects, but I think we need to address the combination of strength training and supplementation. Of course, as has been discussed, we then have to try to find the best combinations of amino acids, EPA, or other nutrients. I think we should stress strength training more in the elderly, because we rarely prescribe it in that population. I would also prescribe it for cancer patients and for ARDS (acute respiratory distress syndrome) patients.

Dr Johnson: I agree with Dr Cederholm that after we stabilize a patient after hospital admission, we need to continue the recovery with nutritionals and a

rehabilitation component, and then look at morbidity, mortality, and 1-year survival. Because inpatients are still in the acute phase of illness, when designing a trial, we would need to identify the particular clinical conditions that would or would not respond to nutrition therapy.

Dr Bosaeus: Two things come to mind. First, selection and classification of subjects is crucial, because with respect to nutritional issues we are not talking about a single mechanism, a single target, a single outcome, but a multitude of them. Some are obviously more important than others. For instance, if we recruit patients for a cachexia trial, we should go for patients in the early phases of the condition rather than trying to restore something that has broken down, and ensure that they are not hypogonadal and that they are comparable in terms of physical activity. You also will need some measures of the inflammatory response.

The second thing is determining what outcomes are of interest, because there could be a multitude. I advocate function and survival as primary outcomes, and to use other outcomes as proxy indicators along the line.

Dr Reid: What are the most important questions to address first? I want to echo what my colleagues have been saying repeatedly—the importance of a functional measurement. That is not to dismiss the importance of muscle mass. I think maintaining or increasing muscle mass is valuable, but mass alone is not enough. Functional measures are more physiologically relevant to the patient's life, and therefore a more valuable outcome.

Which trials will have the most impact? The ones that get positive results. It is tough to do that in a diverse population of people over long periods of time in slow-moving processes.

I like the Dr Paddon-Jones's model of the saw-toothed wave form, showing catabolic crises that occur from which people recover slowly, if at all. Those crises make a great opportunity for intervention, because they happen abruptly. We can predict that they are going to happen. We could find clinical or community settings in which we could predict and be prepared to intervene, and we could see an outcome in a fairly short time in a way that would be feasible. What would have the broadest applicability? This depends on what happens most commonly. Infections offer one opportunity, but I think unloading is perhaps a more available crisis in which to intervene. We might find a targeted, specific condition with which we could get positive results in a big population of people—people who get bed rest for 2 weeks, for example, or older people who break their leg and get a cast.

Dr Schols: I think three issues should be addressed. First, there is much exciting experimental research ongoing on trying to unravel mechanisms involved in muscle maintenance. I think we also should invest in translating these studies to specific groups of patients that are phenotypes with respect to their body composition and functional capacity. I think this is more important than just discussing all the definitions for sarcopenia or cachexia.

As Dr Reid said, when we are interested in seeing whether specific nutrients or novel compounds can modulate these processes, we have to go to more acute models, patient situations such as acute exacerbations in chronic diseases such as COPD, and in cancer, maybe when patients have controlled interventions which we know will affect their muscle maintenance because of upregulation of many processes that may adversely affect the muscle. Then we have this patient situation plus an important process—the recovery from an acute intervention.

Last, I want to stress intervening earlier with sarcopenic patients, particularly when you are dealing with chronic patients.

Abbott Nutrition should not focus only on what supplements should be given, but also on how they are positioned within the total diet, because glucose can be good for muscle maintenance or improvement of function. The general diet of these patients, which provides most of their calories, has a composition that can interfere with ongoing inflammatory processes.

Dr Volek: I think the successful nutrition interventions will be targeted interventions. As I heard today, when the various patient populations with metabolic stress were described, the common thread was inflammation, chronic constitutive inflammation, and acute inflammation. Therefore, nutrition interventions that focus on the inflammatory process, in particular, should be promising. I am a little concerned, however, that much nutrition support pushes high-carbohydrate intake and dextrose, even in total parenteral nutrition solutions. Some evidence shows that such nutrition can exacerbate the inflammatory process.

I like the EPA research for a variety of diseases beyond those described today. However, I would point out the need to protect that EPA, especially with high-dose EPA supplementation, as it gets incorporated into the phospholipid membrane. Combining EPA with antioxidants and tocopherols, in particular γ-tocopherol, which is actually anti-inflammatory itself, could be promising. Trials with EPA will have a good chance of showing some efficacy.

Dr Suetta: I agree with Dr Cederholm about how we should be more specific about how and when we apply exercise in our studies, because it is just as variable as nutrient supplementation. If we do that in trials, we should have no problem seeing an effect, especially if we apply both resistance training and supplementation.

I have a comment on focusing on unloading instead of on sarcopenia. I think two different mechanisms are involved. The response we see after an injury or an unloading type of procedure is much faster than what we normally see. We may have to look at this as two different models, but it is not a problem to rebuild muscle, even in patients from the ICU. We just have to do it right.

Dr Supinski: I look at the cells as a factory, making protein instead of, say, cars. If a car factory is not making enough cars, we need more raw materials. Maybe we do not have enough steel. Maybe we do not have enough glass for windshields. Sometimes one of the machines inside the factory breaks or a worker gets sick. Many of these illnesses we are talking about not only have insufficient factory parts, but they have specific workers who are not doing their job. I think we need to use biopharmaceuticals to fix those workers, get them back to work, and then they will be able to use those supplies that they get more efficiently, and you will get more cars.

I think the answer is a combination of good nutrition, plus for specific diseases for which we know specific targets, biopharmaceuticals that reverse those specific injuries.

Dr Morley: This is a complex question, and you can take it many ways. First, we have to decide what to give. Do we give just a leucine-enriched amino acid supplement a couple of times a day to get our peaks? Would that be enough? Do we need to add creatine? HMB (β-hydroxy-β-methylbutyrate)? Do we add EPA? Finally, should we use an oligopolysaccharide? We have shown that decreases inflammation, at least in the elderly in nursing homes. We have to decide what to study. Abbott Nutrition cannot do 40 studies over a year with 40 different things. Maybe you could, but then your bosses would want to know where the profit went. It would be nice, but we are not going to see it. So, you have to find one product that combines what, in your best guess, works.

When you have that product, there are a couple of things to consider. The one I just want to get out of the way is that somebody should study giving protein to everybody who comes into a hospital. Dr Paddon-Jones' literature shows that we can stop much of this muscle loss just by giving people protein from the start for bed rest. If we were doing that, we would make a huge difference.

Otherwise, I would look at a preventive approach. Because I am a geriatrician, I probably would look at COPD. There are many patient models, but I would look at people who exhibit the Fried criteria for frailty or prefrailty, and I would study those living in the community, not in institutions, similar to the study that Chapman and I did.

I would not intervene at first, then I would consider use of a supplement. The supplement would not be given more than twice a day in small amounts, most probably no more than about 300 kcal maximum. Finally, I would look at use of an anabolic stimulus. We know the best anabolic stimulus is resistance exercise. So, I would look at resistance exercise alone first, then add testosterone as another anabolic stimulus and examine the effects of the combination. We have to show that an addition of a supplement to resistance exercise improves outcomes.

The data Chapman and I got show that hospitalizations decrease and power improves in the population. These are incredible outcomes. I think we would need 1000 to 1500 people to be able to clearly show it, because we need four groups, and at least 250 in a group. If you did that work with an Abbott Nutrition supplement, you would be able to say nobody could give any other supplement but yours.

Of course, I forgot that the supplement has to provide 2000 IUs of vitamin D per day. It is a pain in the neck to give vitamin D. We forget. I am obsessed about vitamin D, and I forget to give it to my patients. I suddenly find that they are now vitamin-D deficient again, because I gave them their 50,000 IUs and then forgot to continue it.

I think you have to put that sort of product together and do one big study instead of multiple little studies. Do a multinational study so you make everybody happy. That would basically give you the product, and it is going to work. Resistance exercise plus a good protein product certainly will work.

Dr Wheeler: Thank all of you for contributing to this conference. We appreciate your taking time out of your busy days, your busy schedules, and your busy jobs to spend this few days with us to bring this important topic to life.

We hope that some of the information we discussed here will stimulate other thoughts and other research programs in this important area for months and years to come. We have learned a great deal and had great discussions about lean body mass. We have talked about how muscle mass is related to overall health and that loss of muscle mass can lead to serious problems, loss of health, and possibly worsening of certain diseases.

Finally, we have learned of various nutrition and exercise therapies that not only help maintain muscle mass, but also help make it more functional as it relates to strength and power.

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