

NIH Workshop on
THE ROLE OF
DIETARY SUPPLEMENTS
FOR
PHYSICALLY ACTIVE
PEOPLE

Program and Abstracts

Office of the Director
National Institutes of Health

Workshop on the Role of Dietary Supplements for Physically Active People

A workshop sponsored by the NIH Office of Dietary Supplements, in conjunction with the American Society for Clinical Nutrition and the American Institute of Nutrition.

June 3-4, 1996

Natcher Conference Center
National Institutes of Health
Bethesda, Maryland

Cosponsored by the National Institute on Aging, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institute of Child Health and Human Development, National Institute on Deafness and Other Communication Disorders, National Institute of Dental Research, National Institute of Diabetes and Digestive and Kidney Diseases, National Heart, Lung, and Blood Institute, National Institute of Mental Health, Office of Alternative Medicine, Office of Research on Women's Health, and the NIH Division of Nutrition Research Coordination.

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Introduction to the Workshop on the Role of Dietary Supplements for Physically Active People

Scientific research linking dietary supplements to life span health can be viewed as a relatively new area of research. In the early part of this century, nutrition sciences and dietary recommendations were focused on the identification and treatment of nutritional deficiency diseases. Although the American people have been consuming vitamin and mineral supplements for decades, the direct relationship between diet and health and, therefore, the potential role for nutrients beyond the minimum required to avoid deficiency, has become apparent only within the last 15 years. The possible roles of other food components and natural product derivatives in promoting health and preventing disease are also now being recognized. The publication of the Surgeon General's Report on Nutrition and Health and the Diet and Health report from the National Academy of Sciences further highlighted the breadth of understanding of the diet–health relationship. Scientific research on the characterization of the potential roles of individual nutrients and compounds as dietary supplements has grown dramatically in the 1990's.

Dietary supplements in the United States are usually defined as comprising plant extracts, enzymes, vitamins, minerals, and hormonal products that are available without prescription and may be consumed in addition to the regular diet. Considerable research on the effects of dietary supplements has been conducted in Asia and Europe, where plant products have a long tradition of use. The overwhelming majority of supplements have not been studied scientifically and it is, therefore, important to conduct research to determine the benefits and risks of promising dietary supplements and to interpret current data for the public.

One strong and continuing public health message to the American people, based on such scientific information, is that moderate exercise should become a part of their daily lives. Physical activity has been shown to reduce the risk of cardiovascular disease through its effects on high blood pressure, high blood cholesterol, diabetes mellitus/insulin resistance, and overweight. Americans need to heed the advice of health professionals and adopt a more physically active lifestyle that includes a planned exercise component. This scientific workshop will focus on the role of dietary supplements for physically active people who are interested in health promotion, in improving their personal performance in recreational sports, or in general fatigue reduction. The goal of the meeting is to develop a research agenda that will identify key areas warranting further investigation.

The workshop will bring together specialists in aging, anatomy, child development, clinical nutrition, cognitive science, dietary supplements, dietetics, endocrinology, exercise physiology, exercise science, growth and development, kinesiology, medicine, nutrition, nutritional biochemistry, pediatrics, physiology, sports medicine, and women's health issues. These scientists will present reviews of the current state of scientific knowledge regarding selected dietary supplements and physical activity. Although scientific studies in many of the areas to be addressed in this workshop have often necessarily included primarily elite athletes, the focus of this workshop is on the more typical healthy person who is physically active.

GENERAL INFORMATION

Workshop sessions will be held in the Natcher Conference Center (Building 45), National Institutes of Health, 9000 Rockville Pike, Bethesda, Maryland. Sessions will run from 8:00 a.m. to 5:00 p.m. on Monday and 8:00 a.m. to 5:30 p.m. on Tuesday. The telephone number for the message center is 301-496-9966.

CAFETERIA

The cafeteria is located on the lobby level and is open daily from 7:00 a.m. to 3:00 p.m.

CONTINUING EDUCATION CREDIT

For Physicians

The purpose of this workshop is to review current knowledge regarding selected dietary supplements in relation to exercise, in improving performance, and in general fatigue reduction and to develop a research agenda that will identify key areas warranting further investigation.

The workshop will present in open, public sessions state-of-the-art scientific information regarding dietary supplements and their role in physical activity and will inform the biomedical research and clinical practice communities as well as the general public of the research recommendations of the workshop.

The National Institutes of Health is accredited by the Accreditation Council for Continuing Medical Education to sponsor continuing medical education for physicians.

The National Institutes of Health designates this continuing medical education activity for a maximum of 17 credit hours in Category I of the Physician's Recognition Award of the American Medical Association.

For Dietitians

Participation in this workshop meets the American Dietetic Association's requirement for continuing education criteria for 17 credit hours.

SPONSORS

The primary sponsor for this workshop is the NIH Office of Dietary Supplements, in conjunction with the American Society for Clinical Nutrition and the American Institute of Nutrition. The workshop is cosponsored by the National Institute on Aging, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institute of Child Health and Human Development, National Institute on Deafness and Other Communication Disorders, National Institute of Dental Research, National Institute of Diabetes and Digestive and Kidney Diseases, National Heart, Lung, and Blood Institute, National Institute of Mental Health, Office of Alternative Medicine, Office of Research on Women's Health, and the NIH Division of Nutrition Research Coordination.

**THE ROLE OF DIETARY SUPPLEMENTS FOR
PHYSICALLY ACTIVE PEOPLE**

June 3–4, 1996
Natcher Auditorium
National Institutes of Health
Bethesda, Maryland

Monday, June 3, 1996

8:00AM-5:00PM

8:00 Welcome and Introductions
Ruth L. Kirschstein, M.D., Deputy Director
National Institutes of Health, Bethesda, Maryland

Opening Remarks
C. Everett Koop, M.D., Sc.D., Chairman and Founder
Shape Up America!, Bethesda, Maryland

Conference Overview and Issues
Bernadette M. Marriott, Ph.D., Director, Office of Dietary Supplements
National Institutes of Health, Bethesda, Maryland

SESSION I. DETERMINING THE METABOLIC BASIS OF SUPPLEMENTATION

(Each of the following presentations will last 20 min. and be followed by 10 min. of discussion)

Moderator: Janet C. King, Ph.D.

USDA Western Human Nutrition Research Center, San Francisco, California

8:30 Is There a Metabolic Basis for Dietary Supplements?
Steven H. Zeisel, M.D., Ph.D.
University of North Carolina, Chapel Hill, North Carolina

9:00 Physical Activity as a Metabolic Stressor
Edward F. Coyle, Ph.D.
University of Texas at Austin, Austin, Texas

9:30 Overview of Diet and Activity as Modifiers of Growth and Adolescent Development
Alan D. Rogol, M.D., Ph.D.
University of Virginia, Charlottesville, Virginia

10:00 Aging as a Modifier of Metabolism
Robert M. Russell, M.D.
USDA Human Nutrition Research Center on Aging, Tufts Univ., Boston, Massachusetts

10:30 Modifiers of Metabolism: Overnutrition, Undernutrition, and Disease States
F. Xavier Pi-Sunyer, M.D., M.P.H.
St. Luke's-Roosevelt Hospital Center, Columbia Univ., New York City, New York

11:00 Supplements: Food, Special Formulas, Pills, and Intravenous Infusions
Peggy R. Borum, Ph.D.
University of Florida, Gainesville, Florida

Monday, June 3, 1996 (continued)

11:30 Methodological Issues of Measuring Physical Activity and Physical Fitness When Evaluating the Role of Dietary Supplements for Physically Active People
William L. Haskell, Ph.D.
Stanford University, Palo Alto, California

12:00 Moderated Discussion and Questions

12:30 Lunch

SESSION II. MACRONUTRIENTS AND AMINO ACIDS

(Each of the following presentations will last 20 min. and be followed by 10 min. of discussion)

Moderator: Gail E. Butterfield, Ph.D., R.D.
Veterans Affairs Medical Center, Palo Alto, California

1:30 Exercise and Protein Supplements
Robert R. Wolfe, Ph.D.
Shriners Burn Institute, Galveston, Texas

2:00 Carbohydrate Supplements as Potential Modifiers of Physical Activity
W. Michael Sherman, Ph.D.
The Ohio State University, Columbus, Ohio

2:30 Lipid Metabolism During Exercise
Samuel Klein, M.D.
Washington University School of Medicine, St. Louis, Missouri

3:00 Fluid and Electrolyte Supplement for Exercise-Heat Stress
Michael N. Sawka, Ph.D.
United States Army Research Institute for Environmental Medicine, Natick, Massachusetts

3:30 Nutritional Effects on Central Fatigue
J. Mark Davis, Ph.D.
Blatt-Center, University of South Carolina, Columbia, South Carolina

4:00 Glutamine and Other Amino Acids as Supplements-A Physiological Case
Michael J. Rennie, Ph.D., FRSE
University of Dundee, Dundee, Scotland

4:30 Moderated Discussion and Questions

5:00 Adjourn

The National Nutritional Foods Association will host a reception in honor of the new NIH Office of Dietary Supplements from 6:00 p.m. to 7:30 p.m., Monday, June 3 at the Hyatt Regency Bethesda, one Metro stop from the NIH campus. All conference participants are cordially invited to attend.

SESSION III. MINERALS

(Each of the following presentations will last 20 min. and be followed by 10 min. of discussion)

Moderator: Joanne L. Slavin, Ph.D., R.D.

University of Minnesota, St. Paul, Minnesota

8:00 Meeting Optimal Calcium Requirements

Connie M. Weaver, Ph.D.

Purdue University, West Lafayette, Indiana

8:30 Magnesium, Zinc, and Chromium

Henry C. Lukaski, Ph.D.

USDA Grand Forks Human Nutrition Research Center, Grand Forks, North Dakota

9:00 Iron Nutrition and Exercise

John L. Beard, Ph.D.

Penn State University, University Park, Pennsylvania

9:30 Moderated Discussion and Questions

**SESSION IV. OTHER SUPPLEMENTS OF POTENTIAL INTEREST FOR THE
PHYSICALLY ACTIVE**

(Each of the following presentations will last 20 min.)

Moderator: E. Wayne Askew, Ph.D.

University of Utah, Salt Lake City, Utah

10:00 The Effect of Physical Activity on Thiamin, Riboflavin and Vitamin B-6 Requirements

Melinda M. Manore, Ph.D., R.D.

Arizona State University, Tempe, Arizona

10:20 Does Dietary Creatine Supplementation Have a Role to Play in Exercise Metabolism?

Paul L. Greenhaff, Ph.D.

Queens Medical Center, Nottingham, England

10:40 Supplemental Carnitine and Exercise

Eric Brass, M.D., Ph.D.

Harbor UCLA Medical Center, Torrance, California

11:00 Effects of Choline on Athletic Performance and Fatigue

Bobby W. Sandage, Jr., Ph.D.

Interneuron Pharmaceuticals, Inc., Lexington, Massachusetts

11:20 Selected Herbals and Exercise Performance

Luke Bucci, Ph.D.

Weider Nutrition Group, Salt Lake City, Utah

Tuesday, June 4, 1996 (continued)

11:40 Roundtable Discussion: What is the conclusion for what research is needed?

12:30 - 1:15 LUNCH

SESSION V. ANTIOXIDANTS

(Each of the following presentations will last 20 min. and be followed by 10 min. of discussion)

Moderator: Mitchell Kanter, Ph.D.

Quaker Oats Company, Barrington, Illinois

1:15 Antioxidants: What Are They and What Role Do They Play in Physical Activity and Health?

Priscilla M. Clarkson, Ph.D.

University of Massachusetts, Amherst, Massachusetts

1:45 Exercise and Oxidative Stress: Effect of Vitamin E and Aging

William J. Evans, Ph.D.

Penn State University, University Park, Pennsylvania

2:15 Selenium and Other Antioxidant Issues

Carl L. Keen, Ph.D.

University of California at Davis, Davis, California

2:45 Physical Exercise and Thiol Homeostasis: Possible Implications

Chandan K. Sen, Ph.D. and Lester Packer, Ph.D.

University of California at Berkeley, Berkeley, California; University of Kuopio, Kuopio, Finland; University of California at Berkeley, Berkeley, California

3:15 Antioxidant Methodology: A Critique

Robert R. Jenkins, Ph.D.

Ithaca College, Ithaca, New York

3:45 Moderated Discussion and Questions

4:00 **Session moderators review of issues followed by general discussion**

Janet C. King, Gail E. Butterfield, Joanne L. Slavin, Elden W. Askew, Mitchell Kanter

Discussants: Karl Freidl, DoD; Robert Moore, FDA; Marilyn Schorin, PepsiCo International; William C. Wenger, Bayer Corporation

5:30 Adjournment

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Abstracts

Abstracts

The following abstracts of presentations to the workshop, “The Role of Dietary Supplements for Physically Active People,” were furnished by presenters in advance of the workshop. This book is designed for the use of the workshop participants and as a pertinent reference document for anyone interested in the workshop topic. We are grateful to the authors who have summarized their materials and made them available in a timely fashion.

Bernadette M. Marriott, Ph.D.
Director, Office of Dietary Supplements
Office of Disease Prevention
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Is There a Metabolic Basis for Dietary Supplements?

Steven H. Zeisel, M.D., Ph.D.

Dietary supplements that are efficacious must either provide a nutrient that is normally undersupplied to cells or exert a pharmacologic effect on cellular processes. In the first case, a nutrient is required by the organism, and a specific concentration of this nutrient results in optimal cell function. For a dietary supplement to exert a positive effect, normal availability of the supplied nutrient must be suboptimal. For example, a vitamin supplement increases low intracellular vitamin E levels so that optimal antioxidant protection is present in the cell. In the second case, the dietary supplement contains a constituent which is normally not required by the cell, but is capable of altering normal cell function. For example, foxglove contains the drug digitalis, which alters cardiac muscle function so that the force generated by a muscle contraction is enhanced. This talk focuses on the first example, in which a nutrient in a supplement corrects suboptimal nutrient concentrations.

The recommended daily intake of a nutrient is calculated based on the needs of the entire population. My colleagues and I recommend an amount that should permit optimal cellular function for most normal people. Because genetic, developmental, environmental, and pathological conditions differ, the actual nutrient requirements for individuals vary greatly from the recommended dietary intake. In most cases the recommendation exceeds needs, but in some cases it falls short of requirements. For example, exercise results in the production of oxygen derivatives that damage cells, and thereby create an increased demand for dietary antioxidants. Aged individuals produce less vitamin D in their skin and may benefit from supplemental vitamin D. Individuals with a genetic predisposition to overproduce free radicals might benefit from an antioxidant supplement. Complex interactions between nutrients in the diet also alter the requirement for a nutrient in a given individual. For example, a component of an earlier meal might activate metabolic pathways (such as p450 enzymes) in liver that destroy certain nutrients, thereby increasing the amount of the needed nutrient that must be ingested. An understanding of how the variation in nutrient requirements arises can help to identify which individuals are most likely to benefit from dietary supplements.

Physical Activity as a Metabolic Stressor

Edward F. Coyle, Ph.D.

Overview

It is becoming increasingly clear that a person's health and well-being are improved by physical activity as well as by a nutritious diet. Both physical activity and diet stimulate processes that, over time, alter the morphological composition and biochemical function of the body. Physical activity and diet are interrelated in that optimal adaptation to the stress of exercise-training usually requires a diet that is not lacking in various nutrients. Physical activity should therefore be viewed as providing stimuli that stress various systems of the body to differing degrees depending upon the type and intensity of exercise. Furthermore, the progressive adaptations to regular physical activity are very specific in response to the stress encountered during physical activity.

Definitions

Physical activity is defined as “any bodily movement produced by the contraction of skeletal muscle.”¹

Exercise, a type of physical activity, is defined as “a planned, structured, and repetitive bodily movement done to improve or maintain one or more components of physical fitness.”¹

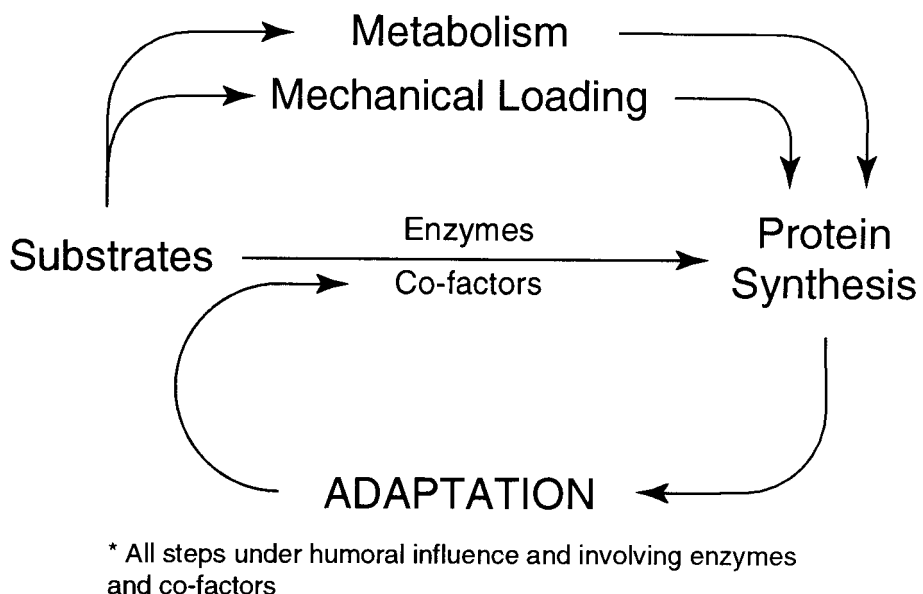
Physical fitness is defined as “a set of attributes that people have or acquire that relates to their ability to perform physical activity.”¹

Dietary supplements in the United States are usually defined as comprising plant extracts, enzymes, vitamins, minerals, and hormonal products that are available without prescription and are consumed in addition to the regular diet. In the present context, the addition of macronutrients and water to the diet can also be viewed as supplements.

Stress of Physical Activity

As shown in Figure 1, physical activity increases metabolism in the active muscles and other cells, and in the process of muscle contraction, produces mechanical loading. These processes of altered metabolism and mechanical loading are key physiological stimuli that serve to alter protein synthesis and degradation. This forms the basic scheme by which the body produces specific adaptations to exercise training and thus develops physical fitness. Substrates, obtained from the diet or endogenous production, supply the energy for metabolism and work and also provide the amino acids for protein synthesis. These processes are typically regulated by enzymatic activity requiring certain co-factors. Furthermore, all the steps shown are influenced by humoral factors as well as other effects.

FIGURE 1.



The stress of physical activity is typically categorized in two ways: (1) by the metabolic aerobic stress it places on the cardiovascular system when exercise is performed for several minutes or longer (Table 1) and (2) by the percentage of the individual's one-repetition maximum (1RM) for physical activity involving lifting weights for short periods of time (Table 2). Weightlifting is largely powered by anaerobic metabolism.

Classification	Relative Intensity			Absolute Intensity in METS ^a	
	% Max Heart Rate	% Max VO ₂ or %Heart Rate Reserve	RPE ^b	Young 20–29 years	Old 60–75 years
Very Low	<30	<25	<10	<3.0	<2.0
Low	30–49	25–39	10–11	3.0–4.7	2.0–3.1
Moderate	50–69	40–59	12–13	4.8–7.1	3.2–4.7
High	70–89	60–85	14–15	7.2–10.1	4.8–6.7
Very High	≥90	≥85	≥16	≥10.2	≥6.8
Maximum ^c	100	100	20	12.0	8.0

^aAbsolute intensity, measured in metabolic equivalent units (METs), is an approximate mean value for men. Mean values for women are approximately 1–2 METs lower than those for men.

^bBorg Rating of Perceived Exertion—original 7–20 scale.

^cMaximum values are mean values achieved during maximal exercise for healthy adults.

Intensity	Percentage of 1RM	Estimated Number of Repetitions Possible ^a
Very Heavy	95–100	1–2
Heavy	90–95	2–6
Moderately Heavy	85–90	3–8
Moderate	80–85	5–10
Moderately Light	75–80	7–12
Light	70–75	10–15
Very Light	65–70	15

^aThe estimated number of repetitions possible will depend upon the person's strength to endurance ratio.

Examples of the Stress of Various Types of Physical Activity and the Specific Adaptations

Low-intensity aerobic exercise such as walking (e.g., 25–40 percent VO_2 max) increases metabolism several fold above basal levels, with relatively large increases in free fatty acid mobilization and oxidation.³ The humoral responses are small but physiologically significant, and carbohydrate metabolism is stimulated slightly. Although the cardiovascular stress is mild, it may be sufficient to stimulate acute and chronic adaptations.

Aerobic exercise performed at moderate to high intensity (50–90 percent VO_2 max) for 30–60 minutes stimulates a large increase in carbohydrate metabolism and oxidation with a continued stimulation of fat metabolism.³ The humoral responses are large and characterized by increases in catecholamines and a reduction in insulin. After 1 week or more of chronic exercise training, the exercising musculature experiences sufficient mitochondrial biogenesis to increase oxidation of fat and carbohydrate oxidation with a reduced disturbance of cellular homeostasis.^{4,5} Cardiovascular adaptations include an increase in plasma volume and stroke volume during exercise.

Moderate to heavy weightlifting stimulates neuromuscular recruitment to very high levels. A sufficient number of repetitions produces brief alterations in metabolic homeostasis within muscle and a postcontraction hyperemia. Chronic activity promotes muscle fiber hypertrophy, possibly from the stretch-overload activation of a promoter of protein synthesis (α -actin),⁶ which requires certain humoral agents.⁷

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Overview of Diet and Activity as Modifiers of Growth and Adolescent Development

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The normal linear growth of a child is an expression of adequate nutrition and freedom from major illness; however, there is a remarkable *range* of what is considered normal. At each growth period—infancy, childhood, and adolescence—the growth rate results from the dynamic interplay of nutrition, physical activity, and hormonal processes upon the genetically determined template. Linear growth velocity decelerates rapidly from 30 cm/year during the first few months of life to approximately 9 cm/year at age 2 to 7 cm/year at age 5. Linear growth then continues at approximately 5.5 cm/year before slowing slightly just before puberty (preadolescent “dip”). For an average girl, the growth velocity increases sharply at approximately age 10, reaches a peak of approximately 10.5 cm/year at age 12, and decelerates toward zero as epiphyseal fusion occurs around age 15. For males, who follow a typical growth curve, the pubertal spurt begins around age 12, reaches a peak velocity of 12 cm/year at age 14, and then decelerates toward zero around age 17. The total growth at puberty is approximately 25 cm for girls and 28 cm for boys. If one adds the 2 extra years of prepubertal growth for boys, one has the 13 cm (5+5+3) difference in the mean height between men and women.

The overall contribution of heredity to adult size and body configuration varies with environmental circumstances, and the two continuously interact throughout the entire period of growth. The genetic control of the *tempo* of growth is apparently independent of that for size and configuration.

There has been a secular trend toward additional height and earlier sexual development documented at least over the past 150 years, with children of average economic status increasing their height by 1 to 2 cm per decade. During the 20th century, the age of menarche in Western Europe and the United States has decreased 2 to 3 months per decade, now averaging 12.8 years in middle class communities.¹

Infancy

Size at birth is determined more by intrauterine and placental factors and maternal nutrition than by genetic growth potential. Although length at age 2 and adult height have a correlation coefficient of 0.80, the correlation is but 0.25 at birth, reflecting those factors noted above.² Growth during the first 2 years is characterized by gradual deceleration in both linear growth velocity and rate of weight gain. Most infants will cross growth percentile lines as they “catch-up” or “lag-down” toward their genetically determined target.³ In addition, body shape changes toward a more linear one as fat accumulation wanes and the child becomes more muscular.

Childhood

Growth during childhood is a relatively stable process as the infancy shifts in growth channel are complete and the child follows the trajectory previously attained and grows at an average rate of 5 to 6 cm/year.^{1,4} During this stage, growth primarily depends on the thyroid hormones, those of the GH/IGF-I axis and insulin.

Pubertal Growth

The onset of puberty corresponds to a skeletal (biological) age of approximately 11 years in girls and 13 years in boys.⁵ Most methods for detecting skeletal age use a single radiograph of the left hand and wrist. On average girls enter and complete each stage of puberty earlier than boys, but there is significant intra- and interindividual variation in the *timing* and *tempo* of puberty. One of the hallmarks of puberty is the adolescent growth spurt, often preceded by slowing of prepubertal growth, also known as the “pre-adolescent dip.” Girls gain an average of 25 cm and boys 28 cm during pubertal growth.^{6,7} Boys enter puberty 2 years later than girls; the longer duration of prepubertal growth in combination with greater peak height velocity results in the average adult height difference of 13 cm between men and women.

Marked changes in body composition occur during puberty and result in the android and gynoid patterns of fat distribution in the adult. The hormonal regulation of growth and alterations in body composition increasingly become dependent on gonadal steroid hormones (driven by central gonadotropin secretion) and a dramatic activation of the GH/IGF-I axis, especially the *interaction* between them.⁸ Estrogens either secreted directly or converted peripherally from androgen precursors affect hormonal secretion of the GH/IGF-I axis and are responsible for skeletal maturation and the ultimate fusion of the epiphyseal plates.⁹

Variations of Normal Growth

There is wide variation in the *timing* and *tempo* of puberty, even among healthy children. When one determines the appropriateness of a particular growth velocity, the child's degree of biological maturation must be considered. Skeletal or pubertal maturation may be used to determine the child's degree of biological development. The bone age is determined as the “mean” of the skeletal ages of a number of the small bones of the hand and wrist. Pubertal maturation status is based on development of breasts and pubic hair in girls and pubic hair and genitals in boys. This range of normal variability is expanded to an even greater degree by alterations in energy intake and expenditure. Although moderate activity is associated with cardiovascular benefits and favorable changes in body composition, excessive physical activity during childhood and adolescence may negatively impact growth and adolescent development. Sports that emphasize strict weight control in the setting of high energy output are of particular concern—especially among scholastic wrestlers and female gymnasts and dancers, although selection criteria for certain body types make selection bias a confounding variable in the assessment of the impact of training on growth and adolescent development.^{10,11} One must consider that some of these changes are transient, at least in the wrestlers. The same markers of growth and body composition that were slowed during training (in season) were accelerated after the season, permitting “catch-up” to control children and left no permanent growth reductions.¹⁰

Perhaps it is important to establish an active lifestyle that may carry over to adult life during the childhood years because of the later health benefits (lessened coronary artery disease, hypertension, obesity) rather than for the immediate childhood–adolescent time frame. There are, however, data from a properly controlled study that indicate that moderate activity does not negatively impact growth and adolescent maturation in boys.¹²

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Aging as a Modifier of Metabolism

Robert M. Russell, M.D.

Background

The 1989 edition of the U.S. Recommended Dietary Allowances (RDAs) divides adults into two age groups: the younger adults ages 23–50 and older adults age 51 and older. In 1989, the United States RDA Committee concluded that the data were insufficient to establish separate RDA subdivisions of healthy older people (for example, those ages 51–70 and age 70 and older).

In the U.S. edition of RDAs, the only micronutrients that account for differences between the groups ages 23–50 and 51 and older are thiamine, riboflavin, niacin, and iron; that is, the RDAs for thiamine, riboflavin, niacin, and iron (in females) for people age 51 and older are lower than those for younger adults ages 23–50. The decrements for thiamine, riboflavin, and niacin are due to low observed intakes of calories and protein in older adults, and the fact that these nutrients participate in metabolic processes involving energy expenditure and protein utilization. In the case of iron, the decrement for females is due to the lack of menstrual periods in the older age group.

It is now recognized that the elderly cannot be regarded simply as older versions of young adults. Results of experiments performed on younger adults indicate that elderly people have distinctly different metabolic processes that do not allow for easy extrapolation of nutrient needs. For example, Roberts et al. have recently shown that elderly people continue to underfeed themselves after a period of enforced underfeeding, whereas the younger adult immediately increases his or her dietary intake after a period of enforced underfeeding.

This paper focuses on how age modifies metabolism and requirements for specific vitamins and minerals, since protein requirements do not appear to change with age (i.e., 0.8 mg/kg body weight is adequate for both younger and older adults).

Vitamins

Riboflavin

The riboflavin requirement has now been shown to be the same for both young adults and elderly people. Thus, the lower riboflavin RDA for elderly people is not warranted.

Folate

From a cross-sectional analysis of elderly participants in the Framingham study, the amount of dietary folate needed to ensure normal blood homocysteine values is approximately twice (400 μ g) the present RDA. This implies a greater need for dietary folate for both younger and elderly adults.

Vitamin D

1. There is a decreased ability to form previtamin D₃ in skin upon ultraviolet light exposure in elderly people versus young people. Further, there is decreased absorption of vitamin D with age and decreased synthesis of 1-25 dihydroxy vitamin D by the kidney upon parathyroid hormone stimulation.
2. It has been shown that significantly less spinal bone loss occurs in elderly women supplemented with vitamin D (400 i.u./day) than in placebo-treated volunteers. The present RDA for vitamin D (200 i.u.) is too low.

Vitamin B6

Vitamin B6 requirements for the elderly have been shown to be elevated, although the mechanism for this is uncertain. The evidence that vitamin B6 requirements are elevated in aging comes both from depletion/repletion design studies and from epidemiologic studies on the amount of dietary vitamin B6 needed to insure normal blood homocysteine levels.

Vitamin B12

Vitamin B12 requirements may be increased in a large number of elderly people who have atrophic gastritis. Although most digestive and absorptive functions are well preserved during the aging process, atrophic gastritis occurs among elderly people with a prevalence of 20–50 percent, depending on how the diagnosis is made. The increased vitamin B12 requirement with aging is due to impaired digestion of cobalamin from food protein from lack of acid pepsin digestion, as well as bacterial uptake of vitamin B12 in the proximal small intestine.

Given the potentially devastating effects of vitamin B12 deficiency on the nervous system, the new uncertainties about how best to define vitamin B12 nutritional status and the high prevalence of a condition (atrophic gastritis) that can affect vitamin B12 metabolism in elderly people, it seems imprudent to have lowered the 1989 RDA for vitamin B12 in those age 51 and older. Until more data are available, an RDA of 3.0 µg seems safer for elderly people.

Vitamin A

Vitamin A requirements may be lower in the elderly than in younger people because of decreased clearance of the vitamin by hepatic and other peripheral tissues, and possible increased absorption from the gastrointestinal tract. There is no evidence that carotenoid metabolism is affected by age.

Minerals

Calcium Absorption

Calcium absorption efficiency falls with advancing age. Studies on calcium supplementation alone seem to show that calcium intakes of more than 800 mg/day will not result in preservation of bone mineral. However, in combination with vitamin D supplementation, calcium intakes in the range of 1–1.5 g/day, have been shown to be of benefit in both hip and spine sites (in terms of preservation of bone mineral).

Other Minerals

There is no evidence that other mineral requirements (except for lower iron requirements in postmenopausal females) are different in elderly versus younger individuals.

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Modifiers of Metabolism: Overnutrition, Undernutrition, and Disease States

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Human energy expenditure can be divided into three components: basal or resting metabolic rate (RMR), the thermic effect of food (TEF), and the thermic effect of activity (TEA). The stoichiometric relationships between oxygen consumption and the heat release that occurs with biologic substrate oxidations are similar to those seen in chemical combustion. As a result, the rate of energy expenditure and substrate oxidation can be determined by measuring heat losses (direct calorimetry) or by measuring oxygen consumption and carbon dioxide production.

RMR has been operationally defined as the calories expended per unit time by a relaxed person who is in a thermoneutral environment and who has been fasting for 12 to 18 hours. The RMR defines that energy which is necessary for the basic maintenance of the body. This includes energy utilized for the movement of the heart and respiratory muscles, for maintenance of ionic gradients between cells and the body fluids, for synthesis of new protein, and for the maintenance of body temperature. The TEF is defined as the elevation of metabolic rate occurring after food ingestion. It includes the cost of the absorption, metabolism, and storage of the food within the body. The TEA is the energy expended with activity and exercise.

Obesity

The increase of caloric intake over expenditure leads to the accretion of fat and an increase in weight. The increase in fat is accompanied by a proportional increase in lean body mass. Thus, for every pound of excess weight added to the body, about two-thirds is fat and one-third is lean. Although the predominant increase in fat-free mass (FFM) is in muscle, other organs are also involved. It has been shown that the RMR is related to the FFM. As a result, as individuals gain weight, they increase their RMR.

On the other hand, what happens to the TEF is more complex. Some studies in obese persons have shown a decrease in TEF whereas others have not. This is most likely related to the insulin sensitivity of the subjects. The more insulin resistant an obese individual is, the more trouble he/she will have in glucose oxidation and disposal, so that postprandial thermogenesis will be decreased. Obese individuals with normal glucose tolerance will have normal TEF, whereas those with impaired glucose tolerance will tend to have decreased TEF.

The TEA is increased in obese individuals per unit of activity. That is, for a given activity, obese persons expend more energy because they are carrying around a greater weight. However, obese persons tend to be very sedentary, so that they actually are likely to spend fewer minutes per day in any type of activity.

Overall, in either room calorimeters or using doubly labeled water in the free-living state, obese persons expend more total 24-hour energy than age- and sex-matched nonathletic normal weight persons.

Starvation

The best known study of metabolism during starvation was conducted by Ancel Keys and his coworkers at the University of Minnesota in the late 1940's. They studied 32 young male volunteers, who were placed on a diet that provided about two-thirds of their usual calories for 24 weeks. The young men lost more than 70 percent of their fat and about 24 percent of their FFM. The RMR of these volunteers decreased by 40 percent after the 24 weeks of starvation. This decreased RMR can be ascribed primarily to the decrease in lean body mass (LBM). However, the RMR also decreased if expressed per unit of remaining lean tissue, suggesting that other hormonal changes had an important impact. The TEF also decreased, partly because smaller meals were being eaten by the subjects, although the influence of hormonal changes could also have played a role. In addition, TEA decreased, both because the men moved about much less and were moving a much lighter total body, requiring less work and caloric output.

This study has been replicated (less elegantly) in many other studies around the world on under-nourished populations. A great deal of information also exists on obese individuals placed on hypocaloric diets for weight loss. Even at weights that are above the normal, hypocaloric diets will induce a drop in RMR. This seems to be in proportion to the loss in LBM. In addition, there is an important drop in nonresting energy expenditure.

Cancer

One of the first manifestations of cancer is loss of weight. This has been primarily ascribed to a loss of appetite and decreased food intake. The net effect of such a hypocaloric diet is to lower energy expenditure. Despite the decreased energy expenditure, energy balance is not maintained. As the imbalance continues or exacerbates, severe undernutrition, called cancer cachexia, can result. Some studies have suggested that cancer patients have an increased RMR. These studies have often expressed RMR as kcal/kg of weight and compared the cancer patients with normal weight patients. Clearly, however, as already mentioned above in the Minnesota study, as one loses weight, one loses more fat than LBM. Since the kcal/kg of fat are much lower than the kcal/kg of LBM, losing proportionally greater fat will leave an individual with a higher kcal/kg of total weight. Overall, the available evidence suggests that an increased RMR contributes very little to the loss of weight in cancer patients, whereas decrease in food intake is key.

Infection

Infections are often manifested by fever. Fever is an elevation of body temperature above normal to more than 37.5_C and is a marker of inflammation. The infection may be obvious, with pain, redness, and inflammation at a site, or it may be a fever of unknown origin, such as bacterial endocarditis. In humans, for each temperature increment of 0.6_C (1_F), RMR increases by approximately 10 percent. Thus, a considerable increase of energy expenditure can occur with even a mild elevation of temperature. Cytokines such as tumor necrosis factor, IL-6, and IL-1 have been implicated in this process, probably working through prostaglandins, and re-setting the hypothalamic thermoregulatory center.

AIDS

There have been a number of studies suggesting that RMR is elevated in patients with AIDS, and that this may contribute to their weight loss and eventual demise. The issue is complicated, as with cancer, in that appetite is also decreased. Also, gastrointestinal symptoms are very prominent in many patients. Studies to date have generally observed an increase of about 10 percent in RMR in relation to the LBM, with a great deal of variation. This is probably explained as an infection effect, discussed above. However, studies of total energy expenditure using doubly labeled water suggest that 24-hour energy expenditure is decreased, related to the fact that these patients feel very ill and as a result are very inactive.

Anorexia Nervosa

The weight loss that occurs in anorexia nervosa because of the patients' unwillingness to eat appropriate amounts of calories leads to a decrease in RMR, similar to that which occurs in any other starved individual. However, anorexia nervosa patients are generally overactive, so that their 24-hour energy expenditure tends to be higher than one would predict on the basis of their RMR.

Rheumatoid Arthritis

Rheumatoid arthritis is an inflammatory disease in which cytokine production is increased. A recent study has reported that RMR is 12 percent higher in this disease than would be predicted. This is probably modulated by increased levels of IL-1 beta and TNF-alpha. In contrast, TEA is much lower because general activity and certainly exercise is greatly decreased in patients with the disease.

Surgery and Trauma

Energy expenditure is increased in response to surgery and trauma. The stress that occurs leads to increased levels of catecholamines, cortisol, and glucagon. These, particularly catecholamines, are thermogenic hormones. Also, some cytokine effects lead to fever and anorexia. Energy expenditure tends to be increased proportionate to the degree of injury. A catabolic response occurs that can rapidly deplete muscle mass, again mediated by hormonal response to injury.

Pulmonary Disease

Patients with chronic obstructive pulmonary disease and emphysema tend to be very thin. Studies that have been published on their RMR suggest that it is elevated. This has been ascribed to the increased energy cost of breathing. TEA is decreased in these patients because of their difficulty breathing. Therefore, generally, their total 24-hour energy expenditure may be low, normal, or high, depending on the balance between these two conditions.

Diabetes Mellitus

When diabetes is out of control, with high fasting and postprandial blood glucose levels, energy expenditure is increased above the predicted level for the individual because of an increased RMR. Such an increased RMR has been ascribed primarily to the protein catabolism that occurs in this condition. The protein that is broken down needs to be replaced so that protein synthesis can be increased. This increased protein turnover is metabolically costly and raises the energy expenditure, which returns to normal with diet and drug therapy, as glucose metabolism comes under control.

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Supplements: Food, Special Formulas, Pills, and Intravenous Infusions

Peggy R. Borum, Ph.D.

Supplement Information Is Confusing

Broadcast and print media, the Internet, product information, and scientific literature frequently contain statements concerning supplements. The audiences span the full spectrum of demographics, and yet their common reaction to these statements is that they are receiving mixed messages. The lack of data needed to address the issues being discussed is one of several sources of confusion that will require great effort to eliminate. However, one source of confusion could be greatly reduced today if each statement concerning supplements always defined specific parameters, which should be available from the study on which the statement is based. Elimination of this source of confusion requires that the creators of all types of information concerning supplements define these parameters or that their audiences ignore information that does not include the needed definitions.

Compounds Are Presented to the Body in Many Ways

The same chemical compound may be both a nutrient and a drug. Niacin has long been recognized as an important nutrient in the diet that has been added to fortified flour, has been a component of daily vitamin pills, and has been used as a medication in many patients. Although all these uses of niacin are often termed supplementation, their effects on the recipient are not the same.

At the beginning of this century, compounds were presented to the body in the form of food. With the discovery of the critical role of micronutrients in metabolism and the devastating deficiency diseases that result when the diet does not contain them in adequate quantities, vitamin supplements began to be presented to a large number of recipients. Complete liquid formulas for infants not receiving breast milk and complete liquid low-residue formulas for the space program were developed. Using this technology, researchers developed complete formulas and new enteral methods of presentation using tubes for patients who for medical reasons could not consume a diet in the usual manner. Today, messages from the public media suggest benefits for many healthy adults who consume some of these complete formulas as a supplement to their usual diet. During the last third of this century, special formulas and administration techniques were developed to permit total parenteral nutrition for many years in individuals whose gastrointestinal tract either was nonfunctional or had been surgically removed. Thus dietary compounds or nutrients are being presented to the body in many ways.

A growing body of data suggests that a compound required in the diet at a certain quantity to prevent well-described deficiency diseases may be presented to the body in higher quantities to prevent or to treat other undesired conditions. Therefore, the compound of interest has been added (in quantities greater than frequently consumed by healthy populations) to presentations known as functional foods, megadose vitamins, complete formulas for specific diseases, oral drugs, and intravenous drugs.

Table 1 lists the different modes of presentation used to “supplement” a recipient with compound C. Clearly the same compound is being presented by different routes, in different matrices, and in

different quantities to recipients who are in different physiological states. However, each of these presentations has been termed C supplementation. It is not surprising that statements concerning C supplementation based on these different presentations of C and the accompanying different effects on the recipient do indeed give their audiences mixed messages.

TABLE 1. Different Presentations of Compound C to the Body				
Mode	Route	Matrix	Quantity	Example
Cf	Enteral	Very complex	Dietary quantities	Food
Cs	Enteral	Simple	Dietary quantities	Daily vitamins
Ce	Enteral	Complex	Dietary quantities	Complete formula
Cp	Parenteral	Simple	Dietary quantities	Parenteral nutrition
C↑f	Enteral	Very complex	>Dietary quantities	Functional food
C↑s	Enteral	Simple	>Dietary quantities	Daily vitamin with C↑
C↑e	Enteral	Complex	>Dietary quantities	Formula with C↑
C↑p	Parenteral	Simple	>Dietary quantities	Parenteral nutrition with C↑ and IV drug

Note: C = Compound under investigation; C↑ = Elevated concentration of compound

Warning: Extrapolation of data from one presentation of a compound to the body to predict the result of another presentation of a compound to the body may be harmful to your health.

Carnitine

This discussion is applicable to many compounds, but because my colleagues and I have studied carnitine for nearly 25 years, it is used for illustrative purposes. Carnitine biosynthesis in the body requires two essential amino acids and several essential micronutrients. Dietary carnitine is found predominately in animal products and is known to facilitate beta-oxidation of long chain fatty acids by transporting the fatty acids into the mitochondrial matrix where the enzymes of beta-oxidation are located. However, many other functions of carnitine are being investigated, and some of these may be more critical to many recipients receiving carnitine supplementation than its role in facilitating fatty acid oxidation. Exogenous carnitine is not required by healthy adults to maintain health and thus is not considered an essential nutrient. However, carnitine is being vigorously investigated as a conditionally essential nutrient because exogenous carnitine does appear to be required during certain physiological conditions such as during the newborn stage and during a variety of pathologies. The evidence that carnitine is a conditionally essential nutrient for the newborn led infant formula manufacturers more than a decade ago to add carnitine at concentrations found in breast milk to formula not containing endogenous carnitine. However, none of the parenteral nutrition solutions available in the United States and frequently used in preterm neonates contains carnitine. Vegan diets contain little or no carnitine. The typical U.S. diet contains less than 5 mg/day of carnitine, but many patients receive 100 mg/kg per day of exogenous carnitine. Pharmaceutical grade carnitine is commercially available in the United States for both enteral and parenteral administration. The Internet and the print media currently advertise a multitude of products that contain carnitine with the suggestion that carnitine supplementation is beneficial to many different types of people. A large portion of these advertisements suggest that carnitine will either enable the recipient to lose weight or to improve physical performance.

Route of Presentation

A compound presented to the body via the gastrointestinal tract will be absorbed by a completely different mechanism than one presented to the body intravenously. However, other differences in metabolism of the compound may occur if the orally ingested compound is metabolized by enterocytes or metabolized during its first pass through the liver. Several amino acids that are not essential in an oral diet become essential during parenteral nutrition. Drugs administered orally or intravenously do not demonstrate the same pharmacokinetics with both routes of administration.

Matrix of Presentation

The absorption and metabolism of a compound may be significantly altered by the presence or absence of other compounds. There are many well-documented examples of the absorption of a micronutrient being decreased or increased by the chemical composition of the liquid consumed with the micronutrient. For example, the iron in a serving of liver will be absorbed quite differently from the iron in a vegan diet. Also, the iron in an iron supplement capsule will be absorbed quite differently if it is taken at breakfast with a cup of tea or with a glass of orange juice. Food, complete formulas for specific disease states, and oral pills clearly present a compound to the body in very different matrices. However, the data from one of these presentations are frequently used to suggest what should be expected from one of the other presentations.

Quantity of Compound Presented

Carnitine is transported across the gastrointestinal tract by both active and passive processes, and the percentage of carnitine absorbed versus excreted is greatly modified by the quantity being presented to the gastrointestinal tract. In addition, metabolism of carnitine by the enterocytes appears to be altered by the quantity of carnitine being presented. A large number of studies have evaluated the potential beneficial effect of carnitine on symptoms such as anemia and exercise tolerance in renal patients on hemodialysis. Many questions remain unanswered including what quantity of carnitine should be presented. Some investigators have used quantities greater than those consumed in the diet of most populations with reasonably beneficial results, whereas other investigators have found that quantities closer to the amount consumed in the diet by many healthy populations to be more beneficial. However, the data from one of these presentations are frequently used to suggest what should be expected from one of the other presentations.

Purity of Compound Presented

If a 1-gram capsule of carnitine is not pure, there are two obvious potential problems. The recipient is not receiving the needed 1 gram of carnitine and is receiving an unknown compound, which may cause harm. Most recipients assume that any compound being administered to them is pure. For pharmaceutical grade compounds, there are required documented quality control checks to support that assumption, but these checks do not exist for compounds that are not pharmaceutical grade. As with many other compounds, the nonpharmaceutical grade carnitine products have been shown to vary widely

in purity from 100 percent pure to undetectable levels. With some products, the capsules within a single bottle show varying levels of purity. However, statements concerning the supplementation of a compound such as carnitine often do not include any reference to the purity of the compound being used.

Physiological State of Recipient

One reason that compounds are presented to recipients in different quantities and matrices and by different routes is that the physiological states of the recipients differ; therefore, so do their needs. Even if one assumes that the preterm neonate, the child with an inborn error of metabolism, the renal patient on hemodialysis, and the weekend athlete would all benefit from exogenous carnitine, one would not expect their needs or their metabolism of the supplemented carnitine to be the same. However, the data from a recipient in one physiological state are frequently used to suggest what should be expected from supplementation of a recipient in a very different physiological state.

Suggested Approach

The following recommendation could be implemented today by the creators of all information concerning supplements.

Any statement concerning supplementation with a compound should define route of presentation, matrix of presentation, quantity of compound, purity of compound, and physiological condition of the recipient.

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Methodological Issues of Measuring Physical Activity and Physical Fitness When Evaluating the Role of Dietary Supplements for Physically Active People

William L. Haskell, Ph.D.

Dietary supplements may be used by physically active people to increase their physical performance (physical fitness), improve their health, or reduce the potential negative consequences of physical activity (injury, suppressed immune function, etc.). To appropriately assess these effects, it is essential that reliable and accurate measures of physical activity, physical fitness, and health-related outcomes be used. All of these outcomes are complex entities consisting of a number of different characteristics or components that need to be differentially considered depending on the specific scientific/clinical questions being addressed. This presentation focuses on some of the key issues that need to be considered in the measurement of physical activity and physical fitness when the effects of dietary supplements in physically active people are assessed.

Physical Activity

Physical activity is a very complex and not easily measured set of behaviors. Numerous different approaches have been used to assess physical activity or change in activity in studies where health and performance status are the primary outcomes. Most frequently used are self-reported surveys, but other measures have included job classification, behavioral observation, motion sensors, and physiologic markers. The strength of the relation between physical activity and health or performance is highly dependent on the effectiveness of the measurements used. Of particular concern is the accuracy and reliability of the measurements of physical activity and their appropriateness for documenting the primary outcome of the study. The methods used for measuring various aspects of physical activity have become highly developed, but still need additional standardization for use with specific populations, especially in older persons, women, and ethnic minorities.

Whether one assesses the effects of dietary supplements on exercise performance, the effects of dietary supplements on the positive or negative health consequences of exercise, or the interaction of dietary supplementation and exercise on health or performance parameters, it is critical to carefully define and measure the exercise characteristic(s) of interest. These characteristics include the total amount or volume of exercise performed and the intensity, frequency, and duration of each bout or combined bouts.

Amount

When dietary supplementation is considered, the total amount of activity performed, expressed as the total amount of energy expended (kilo joules or kilo calories) is likely one of the more important characteristics to assess accurately. The requirements for certain dietary constituents may be related to a person's total energy expenditure and thus closely tied to body size and the total amount of activity performed. There are increasing data indicating that some of the health benefits of exercise are related to total energy expenditure during exercise performed at moderate intensity or higher. Total activity or energy expenditure has been measured by questionnaires such a diary or recall and by doubly labeled

water. Questionnaires can be used for large groups of subjects over extended periods of time but lack precision in estimating the energy expenditure for an individual. Doubly labeled water appears quite accurate and reliable for estimating total energy expenditure over days or weeks, but is limited in use by high isotope costs and expensive analysis equipment.

Intensity

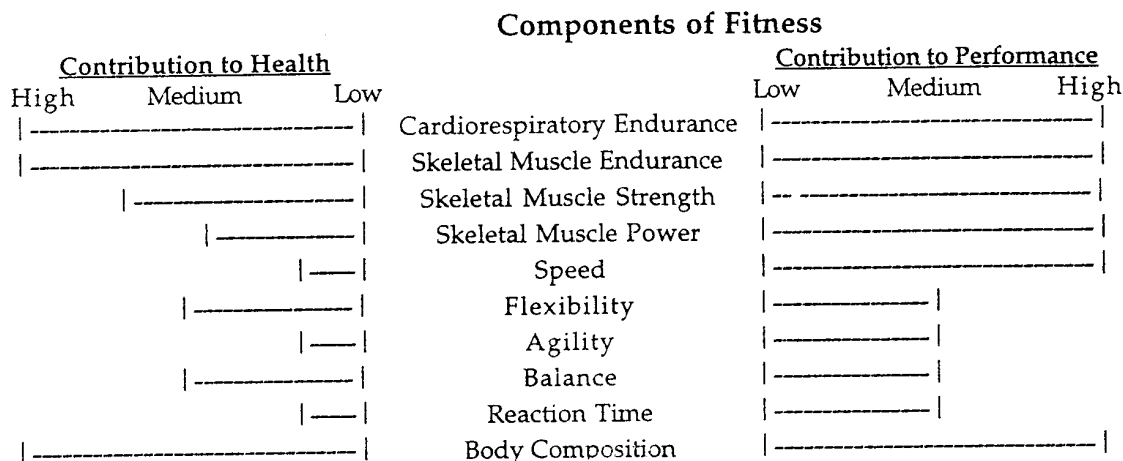
Intensity of an activity can be described in both absolute and relative terms. In *absolute* terms, intensity usually is expressed as either the increase in energy expenditure required to perform the activity or the force produced by the muscle contraction. The intensity of endurance activity usually is expressed in units of oxygen or converted to a measure of heat (calories) or a measure of energy expenditure (joules). The force of the muscle contraction is usually measured by how much weight is being moved or the force exerted against an immovable object. In *relative* terms, the intensity of the activity is expressed in relation to the capacity of the person performing the activity. For energy expenditure, the intensity usually is expressed as a percent of the person's aerobic power (percent of maximal oxygen uptake). Intensity is an important characteristic of exercise because certain performance changes are highly related to intensity of activity, including increases in aerobic power in response to endurance training and muscle strength in response to resistance training. Utilization of carbohydrate, fat, and protein as energy substrate during exercise is substantially influenced by the intensity of the exercise relative to the person's capacity. Also, intensity of activity is a major factor contributing to overuse injuries. High-intensity exercise can be measured accurately and reliably by a variety of questionnaires, heart rate monitors, and for certain types of exercise by motion sensors or accelerometers. Doubly labeled water does not provide accurate information on the "intensity profile."

Physical Fitness

To define more accurately the outcomes of physical activity programs for improving health rather than maintaining or enhancing physical or athletic performance, the concept of performance-related fitness versus health-related fitness has evolved. Although it has been proposed that there is a clear separation between the health- and performance-related components of physical fitness, this is clearly not always the case. For example, cardiorespiratory endurance and muscle strength are highly important components of both. In Figure 1, the contribution of each of the components to health- and performance-related fitness is qualitatively rated.

This figure is designed to show that most components of physical fitness contribute to both performance and health status. The magnitude of the contribution of any one component will depend on the specific objective. When the effects of dietary supplementation on "physical fitness" are evaluated or when an interaction between supplementation and fitness is being investigated, the various components to be included as dependent variables need to be considered. As discussed by other conference speakers, there is some evidence, or at least claims by numerous athletes and the general public, that nutrition supplementation enhances various components of fitness, especially muscle strength and endurance, muscle power, and aerobic power.

FIGURE 1. Components of Physical Fitness and Their Relation to Physical Performance and Health



Note: The magnitude of the contribution will vary depending on the specific sport or activity being performed or the specific measure of health being considered.

Cardiorespiratory Endurance

The “gold standard” or criterion measure of cardiorespiratory fitness is maximal oxygen uptake or aerobic power (VO_2 max). Measured in healthy persons during large muscle, dynamic activity such as walking, running, or cycling, it is primarily limited by the oxygen transport capacity of the cardiovascular system. The most accurate assessment of VO_2 max is determined by measuring expired air composition and respiratory volume during maximal exertion. This procedure requires relatively expensive equipment, highly trained technicians, and time and cooperation from the subject, all of which make it difficult to use in large-scale studies.

Because the interindividual variation in mechanical and metabolic efficiency is quite low in adults during activities that do not require much skill such as walking or running on a motor-driven treadmill or cycling on a stationary ergometer, oxygen uptake can be quite accurately estimated from the rate of work (speed, grade, and resistance). Thus, VO_2 max can be estimated from the peak exercise intensity during a maximal exercise test without measuring respiratory gases. This procedure requires the use of an accurately calibrated exercise device, careful adherence to a specific protocol, and good cooperation by the subject.

Having a subject perform any maximal test to assess cardiorespiratory fitness carries a substantial burden for both the subject and examiner. The burden for the subject includes time, effort, and risk. To reduce this burden, various submaximal exercise testing protocols have been developed and used in numerous observational and intervention studies for evaluating the relationship of physical activity, cardiovascular fitness, and cardiovascular health. In most protocols, the estimate of cardiovascular fitness is made from the heart rate response to a set workrate or workloads and data from the submaximal response are used to extrapolate to a predicted VO_2 max.

Another approach to assessing cardiorespiratory fitness has been the use of field testing, where subjects usually perform a walk, jog, or run of a specified time or distance and their performance is converted to an estimate of VO_2 max or aerobic power. In many cases, these tests require maximal or near-maximal effort by the subject and thus have not been used for older persons or those at increased risk

for cardiovascular disease. The advantage is that large numbers of subjects can be tested rapidly at low cost, but to obtain an accurate evaluation, subjects must be willing to exert themselves and know how to set a proper pace.

Muscle Endurance

Muscle endurance is specific to each muscle group, whereas cardiorespiratory endurance is general (i.e., not specific to any muscle group). Few tests for use in the general population are pure measures of muscle endurance, as most tests of muscular endurance are also tests of muscle strength. Tests of muscular endurance and strength include situps, pushups, bent arm hang, and pullups. These tests need to be properly administered and may not discriminate well in some populations (e.g., pullups are not good for many populations because a percentage of those tested will have 0 scores). Few laboratory tests of muscle endurance have been developed. Such tests usually involve having the subject perform a series of contractions at a set percentage of maximal strength and at a constant rate until the person can no longer continue at that rate. The total work performed or the test duration is used as a measure of muscle endurance.

Muscle Strength

Muscle strength can be measured during performance of either static or dynamic muscle contraction. Like muscle endurance, strength is very specific to the muscle group, so the testing of one group (e.g., using hand grip) does not provide accurate information about the strength of other muscle groups. Thus, to be effective, strength testing must involve at least several major muscle groups, including the upper body, trunk, and lower body. Standard tests have included the bench press, leg extension, and biceps curl using free weights. The heaviest weight a person can lift only one time through the full range of motion is considered the person's maximum strength.

Flexibility

Flexibility is a difficult component to measure accurately and reliably because it is specific to the joint being tested; no one measure provides a satisfactory index of an individual's overall flexibility. Field testing of flexibility frequently has been limited to the sit-and-reach test, which is considered to be a measure of lower back and hamstring flexibility. Other tests have been used to determine the flexibility of the shoulder, hip, knee, and ankle. The criterion method for measuring flexibility in the laboratory is goniometry, which is used to measure the angle of the joint at both extremes in the range of motion.

Balance, Agility, and Coordination

There are no generally accepted standard techniques for measuring balance, agility, and coordination, especially in older persons. Field methods have included various "balance stands" (one foot stand with eyes open, and with eyes closed; standing on a narrow block, etc.) and "balance walks" on a narrow line or rail. In the laboratory, computer-based technology is now being used to evaluate balance measured on an electronic force platform or by analysis of a videotape of the subject walking. Agility or coordination is measured most frequently using a field test such as an agility walk or run, and while in the labora-

tory, coordination or reaction/movement time is determined using electronic signaling and timing devices. More test development is needed to establish norms using standardized tests for the measurement of balance, agility, and coordination of older persons.

Body Composition

In most population-based studies that have provided information on the relation between physical activity and morbidity or mortality, body composition has been estimated by measuring body height and weight and calculating body mass index (weight/height²). The preferred method for determining fat and lean body mass in exercise training studies has been hydrostatic or underwater weighing. This method has been considered the criterion for estimating fat and lean body mass in clinical studies, but it lacks accuracy in some populations, including older persons. Various anthropometric measurements (i.e., girths, diameters, and skinfolds) have been used to calculate percent body fat with varying degrees of accuracy and reliability. Usually the measurements and prediction equations are age and sex specific and the equations need to be quadratic rather than linear to reduce individual estimation errors. Data now exist demonstrating that the distribution of body fat, especially accumulation in the abdominal area, as well as total body fat is a significant risk factor for cardiovascular disease and diabetes. The magnitude of this abdominal or central obesity has been determined by the waist-to-hip circumference ratio or by new electronic methods that can image regional fat tissue. New technologies that have been used to determine body composition include total body electrical conductivity; bioelectrical impedance; magnetic resonance imaging, which has the potential for assessment of regional body composition; and dual photon absorptiometry. None of these new procedures has yet produced data that have influenced our understanding of the relation between physical activity and morbidity or mortality, but the procedures have substantial potential to provide new information on how changes in physical activity along with the use of dietary supplementation effects body composition.

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Exercise and Protein Supplements

Robert R. Wolfe, Ph.D.

Optimal muscle strength and function lie at the heart of almost all athletic endeavors. However, within that broad context, considerable differences exist in the goals of training that are dependent on the particular sport. For example, in weight lifting, muscle size and strength are most important. In distance running, on the other hand, not only is endurance the major goal, but also increased muscle size is a detriment because of the extra body weight. Goals may differ even within a general sport category. Thus, weightlifting may be done for power lifting, which is judged by the amount of weight lifted; for body building competition, in which appearance is most important; or for conditioning for another sport, in which case the goal depends on the particular sport. Consequently, it must be recognized that the response of muscle protein to all possible types of training, as well as to the protein supplements in these various circumstances, may be diverse. Unfortunately, sufficient data do not exist to distinguish the diverse responses in different types of exercise. Furthermore, the responses of trained versus untrained subjects may differ, but current data are insufficient for resolving this issue also.

It is impossible to form a consensus position regarding the benefit of protein/amino acid supplements in exercise training. Apparently credible review articles have been written extolling the benefits of specific supplements,¹ whereas others have dismissed the use of all protein/amino acid supplements as being “worthless.”² Determining whether supplements provide any benefit has probably been hampered by the failure to select appropriate endpoints for evaluation of a positive effect. However, studies focused at a more basic level have failed to agree on the response of protein metabolism in exercise, and thus the metabolic basis for protein. Therefore, it has been shown that whereas nitrogen balance may become negative on a fixed protein intake at the onset of an endurance-training program,³ urea production is not stimulated during exercise, regardless of the level of protein intake.⁴

An often ignored but important complication of dietary studies is the level of energy intake. Thus, during training the effectiveness of a given amount of protein intake to spare endogenous protein will be affected by the level of energy intake.⁵ Because of these and other complications, studies at the whole body level have not yielded a clear picture of the need for, or response to, dietary protein/amino acid supplements. Consequently, this issue needs to be examined at the tissue level.

Both muscle protein breakdown and synthesis are increased in response to exercise in untrained subjects.⁶ Thus, muscle protein turnover is increased by exercise, and there may be a relation between muscle protein turnover and strength. Amino acid intake further stimulates muscle protein turnover after exercise. The effect of amino acids after exercise is greater than the stimulatory effect of amino acids on muscle protein synthesis when given at rest.⁷ These data suggest that not only may the exact composition and amount of a supplement be important, but also the timing of the intake of the supplement in relation to the performance of exercise factors must be considered in designing future studies to evaluate the efficiency of supplements.

In summary, an elevated rate of muscle protein breakdown occurs in a variety of forms of exercise in untrained subjects, providing the rationale for protein supplements when training. On the other hand, evidence demonstrating an unequivocal beneficial effect of supplements is lacking, thereby documenting the need for further studies in this area in which physiologically significant, quantifiable endpoints are evaluated.

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Carbohydrate Supplements as Potential Modifiers of Physical Activity*

W. Michael Sherman, Ph.D.

The energy required for physical activity requiring a low amount of effort that can be maintained for hours is provided primarily by the metabolism of fat. The energy required for physical activity requiring a moderately high amount of effort that can be maintained for 90–120 minutes is provided by the metabolism of both fat and carbohydrate. The energy required for physical activity requiring intense effort lasting 60 seconds is provided by the anaerobic metabolism of muscle glycogen and blood glucose.

Muscle glycogen can be reduced and fatigue can occur while one is engaging in 30–75 minutes of intermittent physical activity requiring very intense effort. Also, fatigue will occur for moderately high physical activity lasting longer than 90 minutes when muscle glycogen and blood glucose reach low levels, are lowered by some amount, or reach some critical threshold. Physical activity requiring a low effort can last for hours, and fatigue is more likely to be related to dehydration, hyperthermia, orthopedic problems, or boredom.

Under normal conditions, protein is metabolized in very small amounts during exercise and the capacity for the oxidation of protein will rarely determine performance capability. Additionally, under normal circumstances, body fat stores are almost always adequate to support long-duration exercise of moderate and high intensities; thus, fat availability rarely determines performance capability. On the other hand, the levels of bodily carbohydrates play an important role in the level of physical activity that can be maintained at moderate and moderately high intensities. Thus, it is important to replenish bodily carbohydrate reserves to maintain their concentration at “optimal” levels.

Normalization of bodily carbohydrate stores between sessions of physical activity is at minimum, essential to “optimally” influence training and performance capabilities. Bodily carbohydrate reserves can most likely be recovered in 24 hours after moderately high-intensity physical activity provided between 500–600 g of carbohydrate are consumed over the 24 hours following the activity (i.e., 7–10 g carbohydrate/kg body weight). On the other hand, it is likely that bodily carbohydrate reserves can be recovered in 24 hours after moderate-intensity physical activity, provided that about 300–350 g of carbohydrate are consumed over the 24 hours after the activity (i.e., 5 g carbohydrate/kg body weight).

Recovery of bodily carbohydrate reserves is apparently affected by the glycemic index of the consumed carbohydrate. It appears that carbohydrates that produce a high glycemic index and insulin response promote more rapid recovery of carbohydrate reserves than do those carbohydrates that produce a low glycemic index and insulin response.

Carbohydrate that is ingested before and during exercise provides an alternate source of muscle fuel that can support moderate and moderately high-intensity physical activity. Concurrently, the ingestion of carbohydrate at these times reduces the mobilization and oxidation of fats. Importantly, this reduction in the metabolism of fats does not impair performance; rather, the provision of the ingested carbohydrate usually improves performance.

*Adapted in part from: Coyle, EF. *Substrate utilization in active persons*. In: *New dimensions in carbohydrates*. A symposium sponsored by the American Society for Clinical Nutrition, Inc., and the Sugar Association, Washington, DC, December 1993.

Although the training endurance athlete and the athlete who trains intensely using intermittent exercise will necessarily rely on adequate carbohydrate consumption to optimize training and thereby performance capability, the recreational athlete can most likely undertake leisure-time physical activity by consuming a healthy diet (less than 30 percent of energy from fat, 12–15 percent of energy from protein, and the balance of energy from carbohydrate). Nevertheless, both types of sportspersons must assure adequate hydration and minimize environmental stress during physical activity. Table 1 provides more specific information on carbohydrate supplementation and physical activity.

TABLE 1. Summary of Perspectives on Carbohydrate Supplementation and Physical Activity
<p><i>For Recreational Athletes</i></p> <ul style="list-style-type: none"> • Consume a healthy diet that contains less than 30 percent of energy as fat, 10–12 percent of energy as protein and the balance as carbohydrate. • During exercise that produces sweating, consume fluid to avoid dehydration—the fluid can contain carbohydrate to enhance palatability and enhance fluid consumption. <p><i>For Endurance Athletes</i></p> <ul style="list-style-type: none"> • Presumably to optimize daily training that should result in improved performance, consume 500–600 g carbohydrate/day (7–10 g carbohydrate/kg body weight) of moderate to high glycemic foods, or supplement foods with carbohydrate beverages. • During the hours before endurance exercise limited by carbohydrate availability, consume 4–5 g carbohydrate/kg body weight 3–4 hours before exercise or consume up to 2 g carbohydrate/kg body weight 1–2 hours before exercise. • During endurance exercise limited by carbohydrate availability, consume 0.1 to 0.2 g carbohydrate/kg body weight per 15–20 minutes intervals of a solution containing between 5–10 percent carbohydrate. • During the hours after endurance exercise, consume carbohydrate immediately and at 2-hour intervals after exercise or consume as much as 1.2 g carbohydrate/kg body weight per 15 minutes for 4 hours. <p><i>For Sprinting Athletes</i></p> <ul style="list-style-type: none"> • Maintain at least normal body carbohydrate reserves by consuming 5–8 g of carbohydrate/kg body weight/day. • Ingest carbohydrate-containing beverages during intermittent exercise such as soccer and ice hockey.

The above perspectives probably apply to all sportspersons regardless of age, gender, or ethnicity. There is the suggestion that women have a lower capability to increase muscle glycogen compared with men and that phases of the menstrual cycle influence carbohydrate and fat metabolism; however, these perspectives require much additional research before they can be accepted as principles.

Very little research exists on the applicability of these findings to wheel-chair bound individuals. Such individuals will use a much smaller muscle mass to produce movement; consequently, it is likely that these recommendations are especially applicable to these individuals.

Lipid Metabolism During Exercise

Samuel Klein, M.D.

Endogenous triglycerides represent the largest fuel reserve in the body. Most triglycerides (~17,500 mmol in a lean adult man) are compactly stored in adipose tissue as an oil. Triglycerides are also present in skeletal muscle (~300 mmol) and in plasma very low-density lipoproteins (~0.5 mmol). The total amount of energy stored as triglyceride (~ 135,000 kcal) is 65-fold greater than the amount of energy stored as glycogen (~ 2,000 kcal). Therefore, the use of fat as a fuel during endurance exercise permits sustained physical activity and delays the onset of hypoglycemia. The relative contribution of different endogenous fat depots for energy production during endurance exercise is not precisely known because of methodological limitations. The major source of fatty acids oxidized during prolonged exercise is derived from adipose tissue. It has been estimated that intramuscular triglycerides comprise 5–50 percent of the fat oxidized, whereas the contribution from circulating lipoproteins is minimal.¹

The use of triglyceride as a fuel requires hydrolysis to free fatty acids (FFA) and glycerol and subsequent oxidation of FFA by working muscles. Therefore, the level of FFA and glycerol in plasma has been used as an index of lipolysis. However, plasma FFA and glycerol concentration represent a balance between FFA and glycerol release into plasma and their uptake by peripheral tissues. Therefore, plasma FFA and glycerol concentrations may not accurately reflect lipolytic activity. For example, we have found that the relationship between plasma FFA concentration and lipolysis can vary markedly during different physiological conditions.² Plasma FFA concentrations during exercise correspond to a much greater rate of lipolysis than do the same plasma FFA concentrations during epinephrine infusion. Therefore, the use of isotope tracer methodology to measure free fatty acid and glycerol rates of appearance (Ra) in plasma represents the best approach for studying lipid kinetics during exercise.

Glycerol Ra, an index of whole body lipolysis, and FFA Ra, an index of FFA availability, increase progressively during endurance exercise,³ primarily because of an increase in catecholamine stimulation of beta-adrenergic receptors. In fact, strenuous exercise is the most potent physiologic stimulus for lipolysis. Glycerol Ra during high-intensity exercise⁴ represents the highest values reported in humans and is threefold higher than those reported during critical illness⁵ or after 84 hours of starvation.⁶ The increase in lipolysis in conjunction with an increase in skeletal muscle energy requirements is responsible for the marked increase in fatty acid oxidation observed during exercise. The rate of triglyceride–fatty acid cycling changes dramatically during exercise because of differences in the relative increase in fatty acid oxidation and lipolysis. In one study, approximately two-thirds of FFA released into plasma were reesterified during resting basal conditions, whereas only one-fourth of FFA released was reesterified during prolonged moderate intensity exercise.⁷

The rate of lipolysis depends on the intensity and duration of the exercise bout, previous exercise training, and recent dietary intake. Modifications in dietary intake before exercise can cause changes in lipid metabolism during exercise. Plasma FFA and glycerol concentrations are higher at rest and increase more rapidly during exercise following a low-carbohydrate diet or short-term fasting.^{8,9} Endurance training has been reported to decrease lipolytic rates during exercise but increase total fat oxidation, presumably because of an increase in intramuscular triglyceride oxidation.¹⁰

Performance during exercise depends, in part, on the provision of adequate fuel to working muscles. Therefore, athletes often ingest carbohydrate during intense endurance exercise to support plasma glucose concentrations and spare muscle glycogen oxidation. Ingestion of typical dietary fat is not a useful approach for providing fuel during exercise because it may take several hours for the long-chain fatty acids to be oxidized. Long-chain triglycerides are emptied slowly from the stomach, packaged into chylomicrons in the small intestine, and secreted into the lymphatic system before entering the bloodstream. Only a portion of triglycerides present in circulating chylomicrons ultimately provide fatty acids to muscle. In contrast, medium chain triglycerides (MCTs) have been proposed as a potential ergogenic fuel during exercise and are currently present in several commercially prepared sport bars. Medium-chain triglycerides are emptied rapidly from the stomach,¹¹ rapidly absorbed and hydrolyzed by the small intestine, and secreted directly into the systemic circulation. Furthermore, medium-chain fatty acids do not require the acylcarnitine transferase system to cross the inner mitochondrial membrane in liver and muscle for oxidation. However, several studies have demonstrated that oral supplementation with MCTs is unlikely to improve performance during endurance exercise. The amount of MCTs that can be given orally is limited to approximately 25–30 grams because diarrhea and other gastrointestinal side effects are common with higher doses. Furthermore, although orally administered medium-chain triglycerides are readily oxidized,^{12–14} they do not spare muscle glycogen during either moderate or high-intensity exercise.^{12–16}

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Fluid and Electrolyte Supplementation for Exercise-Heat Stress²

Michael N. Sawka, Ph.D., Scott J. Montain, and William A. Latzka

Introduction

Depending on the climatic conditions, the relative contributions of evaporative and dry (radiative and conductive) heat exchange to the total heat loss will vary. The hotter the climate, the greater the dependence on evaporative heat loss and, thus, on sweating. Therefore, a substantial volume of body water may be lost via sweating to enable evaporative cooling in hot climates. Generally, the individual dehydrates during exercise because of fluid nonavailability or a mismatch between thirst and body water requirements. In these instances, the individual starts the exercise task as euhydrated but incurs an exercise-heat mediated dehydration over a prolonged period of time.

Fluid and Electrolyte Needs

A person's sweating rate is dependent on the climatic conditions, clothing worn, and exercise intensity. Persons in desert climates often have sweating rates of 0.3–1.2 L·h⁻¹ while performing occupational activities. Persons wearing protective clothing often have sweating rates of 1–2 L·h⁻¹ while performing light-intensity exercise. Likewise, athletes performing high-intensity exercise commonly have sweating rates of 1.0–2.5 L·h⁻¹ while in the heat. Fluid requirements will vary in relation to climatic heat stress, clothing worn, acclimation state, and physical activity levels. Daily fluid requirements might range (for sedentary to very active persons) from 2–4 L_{day}⁻¹ in temperate climates and from 4–10 L_{day}⁻¹ in hot climates. Electrolytes, primarily sodium chloride and to a lesser extent potassium, are lost in sweat. Sweat sodium concentration averages approximately 40 mEq_L⁻¹ (range = 10–100 mEq_L⁻¹) and varies depending on diet, sweating rate, hydration, and heat acclimation level. Heat-acclimated persons have relatively low sodium concentrations (greater than 50 percent reduction) in sweat.

During exercise-heat stress, a principal problem is to avoid dehydration by matching fluid consumption to sweat loss. This is a difficult problem because thirst does not provide a good index of body water requirements. Thirst is probably not perceived until an individual has incurred a water deficit of approximately 2 percent of body weight. Numerous investigators report that ad libitum water intake results in incomplete water replacement or “voluntary” dehydration during exercise and/or heat exposure. The flavoring and cooling of ingested fluid increase its palatability and can help to minimize “voluntary” dehydration. Heat acclimation status may also influence the voluntary dehydration incurred during exercise in the heat. Although heat acclimation improves the relationship between thirst and body water needs, voluntary dehydration still occurs. Since thirst provides a poor index of body water needs, persons will dehydrate by 2–8 percent of their body weight during situations of prolonged sweat loss.

Hypohydration and Temperature Regulation

Hypohydration (less than normal total body water) increases core temperature responses during exercise in temperate and hot climates. A critical deficit of 1 percent of body weight elevates core temperature during exercise. As the magnitude of water deficit increases, there is a concomitant graded

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elevation of core temperature during exercise heat stress. The magnitude of core temperature elevation ranges from 0.10 to 0.23 °C for every percent body weight lost, and this elevation is greater during exercise in hot than in temperate climates. Hypohydration not only elevates core temperature response, but it also negates the core temperature advantages conferred by high-aerobic fitness and heat acclimation. Therefore, heat-acclimated persons (who have increased sweating rates) who do not drink adequately may more rapidly experience the adverse effects of hypohydration than their nonacclimated counterparts. Recent studies at our laboratory indicate that the core temperature elevation is greater with increased exercise intensity at low (3 percent body weight loss) but not higher (5 percent body weight loss) hypohydration levels.

Hypohydration impairs both dry and evaporative heat loss (or, if the air is warmer than the skin, dehydration aggravates dry heat gain). Hypohydration delays sweating onset and skin vasodilatation. It also reduces sweating sensitivity. Hypohydration may be associated with either reduced or unchanged sweating rates at a given metabolic rate in the heat. The physiological mechanisms mediating the reduced dry and evaporative heat loss from hypohydration include both the separate and combined effects of plasma hyperosmolality and reduced blood volume.

Hypohydration and Fatigue

A common complaint of hypohydrated persons is skeletal muscle fatigue; however, little research had been conducted to address whether hypohydration reduces skeletal muscle performance (in absence of heat stress). Recent research at our laboratory demonstrated that, in temperate conditions, hypohydration (4 percent body weight loss) reduced single-leg knee endurance time by 18 percent compared with euhydration. The mechanism(s) responsible for this are unclear, as hypohydration does not seem to markedly alter skeletal muscle glycogen utilization. To study possible mechanism(s), subjects are repeating these exercise experiments inside of a nuclear magnetic resonance (NMR) magnet and ³¹P spectra are being collected. It is hypothesized that hypohydration might accelerate depletion of adenosine triphosphate (ATP) and PCr or impair the ability to buffer hydrogen and P_i ions produced during exercise.

Hyperhydration

Hyperhydration, or greater than normal body water, has been suggested to improve, above euhydration levels, thermoregulation and exercise-heat performance. The concept that hyperhydration might be beneficial for exercise performance arose from the adverse consequences of hypohydration. It was theorized that body water expansion might reduce the cardiovascular and thermal strain of exercise by expanding blood volume and reducing blood tonicity, thereby improving exercise performance. Studies that have directly expanded blood volume (e.g., infusion) have usually reported decreased cardiovascular strain during exercise, but have reported disparate results on heat dissipation and exercise-heat performance. Studies that have attenuated plasma hyperosmolality during exercise-heat stress generally report improved heat dissipation, but have not addressed exercise performance.

Ten studies have been published that evaluated hyperhydration effects on thermoregulation in the heat. Briefly, 6 of 10 studies observed smaller core temperature increases during exercise with hyperhydration. Together, these studies support the notion that hyperhydration can provide a thermoregulatory benefit; however, most of these studies suffer from serious experimental design flaws. Examina-

tion of their data indicates that “control” conditions generally do not represent euhydration, and that there may have been order problems so that subjects were more heat acclimated during hyperhydration trials. Recent studies at our laboratory have controlled for these confounding factors, and observed no thermal advantage with either water hyperhydration or glycerol hyperhydration during exercise-heat stress.

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Nutritional Effects on Central Fatigue

J. Mark Davis, Ph.D.

Fatigue during voluntary muscular effort is a complex phenomenon involving the central nervous system (CNS) as well as the muscle itself. The mechanisms of fatigue within muscle (peripheral fatigue) are well studied and include impairments in neuromuscular transmission and propagation down the sarcolemma, dysfunction within the sarcoplasmic reticulum involving calcium release and uptake, availability of metabolic substrates and accumulation of metabolites, and actin-myosin cross bridge interactions.¹ Optimal nutritional strategies to delay peripheral fatigue are well documented.^{2,3} Conversely, CNS influences on fatigue (central fatigue) have been largely ignored even though the inability/unwillingness to generate and maintain central activation of muscle is the most likely explanation of fatigue for most people during normal daily activities.^{1,4}

Recently, central fatigue has begun to receive more attention with the development of several theories that may provide a clue to the biological mechanisms. Some theories have focused on the possibility that alterations in various neurotransmitters, including serotonin (5-hydroxytryptamine [5-HT]), dopamine, and acetylcholine may be responsible for heightened perception of effort and/or depressed central activation of muscle. An intriguing aspect of these theories involves the likelihood that nutritional strategies may affect the synthesis of these neurotransmitters by altering the availability of their amino acid precursors.⁵⁻⁸

The most well studied of these theories involves an increase in brain 5-HT concentration and the associated deterioration in sport and exercise performance.^{6,7} Good evidence suggests that increases and decreases in brain 5-HT activity during prolonged exercise hasten and delay fatigue, respectively. Several studies also show that nutritional manipulations involving carbohydrates and branched-chain amino acids that are designed to attenuate brain 5-HT synthesis can improve endurance performance.^{6,7} However, some studies show no performance benefits and studies often suffer from methodological flaws.³ Even less is clear about the proposed benefits of supplementation with tyrosine or choline, both of which are thought to increase dopamine and acetylcholine, respectively.^{9,10}

Unfortunately, progress in this area as a whole is seriously hampered by the lack of good methodologies to measure specific alterations in neurotransmitters during fatiguing exercise in humans or to distinguish central from peripheral mechanisms of fatigue during dynamic whole body exercise. Therefore, although good theoretical reasons exist for believing that proper nutrition might delay central fatigue and thereby enhance physical performance, the scientific data to support the theory are tenuous at this time. The exciting possibility that important relationships exist among nutrition, brain neurochemistry, and physical performance is likely to develop into a new frontier in nutrition research. However, although the evidence is intriguing and makes good intuitive sense, our knowledge in this area is rudimentary at best.

Finding the cause(s) and possible treatments for central fatigue is important not only for sports competitors but also for the general population in which “generalized fatigue” is the primary cause of lost productivity at home and in the workplace, and for those who are sick from infection or other diseases in which debilitating fatigue is often a primary symptom.

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Glutamine and Other Amino Acids as Supplements—A Physiological Case

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The strict division of amino acids into categories of essentiality (nondispensability) and nonessentiality (dispensability) is now recognized as being too crude to be useful in attempting to understand completely the ways in which amino acids are metabolized in health and disease. No amino acid fulfills the new criterion of being “conditionally essential” better than glutamine.

Glutamine is involved in a large variety of metabolic processes as a precursor (e.g., for nucleobases); as a component of acid-base homeostasis; in regulation of cell volume as a regulator of the balance between anabolism and catabolism of fat, carbohydrate, and protein; as a fuel for the gut and cells of the immune system; and of course as a component of protein. Over the past 15 years it has become apparent that there are many situations in which the input to the free pool of glutamine (i.e., from the diet, from protein breakdown, and by depletion of the intracellular stores in muscle) are not always able to satisfy the requirements for glutamine. In such circumstances glutamine is a conditionally essential amino acid without which the body operates suboptimally.

There are now a number of well-defined circumstances in which delivery of exogenous glutamine is able to confer benefit upon patients whose requirement has increased. Examples of these are presented. We still do not fully understand the mechanisms by which glutamine confers its beneficial effects in all cases. A number of major puzzles remain, e.g., why glutamine appears to be conditionally essential when there is rarely a fall in blood glutamine in circumstances where benefit can be shown after supplementation. To what extent the lessons we have learned from clinical situations can be applied in physically active subjects is not yet known.

Exercise and Amino Acid Metabolism

The controversy regarding the extent to which protein is used as a fuel is a very old one. Strong evidence now indicates that as long as energy requirements are met, given a diet of normal composition, high rates of physical activity will not predicate the greater requirement for protein. Although some argue that in order to maximize protein accretion or adaptation of muscle architecture (e.g., to increase mitochondrial capacity) consumption of dietary protein at high rates is beneficial, the evidence for this position is not strong.

If there is no general need for extra protein, does physical activity confer an extra requirement for glutamine? If so, can glutamine be used as a protective agent (i.e., preventing damage) and also as what might be called a rescue agent, i.e., in speeding recovery from damage associated with exercise?

Although a number of tantalizing possibilities exist, the evidence is rather sparse. For the hypothesis that regular, vigorous physical exercise causes increased glutamine requirements to be true, exercise would have to cause a *fall* in glutamine stores or, at least, institute circumstances in which glutamine supplementation were beneficial, even if no fall in glutamine were observed.

Exercise at very high rates certainly depletes glutamate in skeletal muscle and (according to animal studies), if oxygen delivery is compromised, also in the heart. Normally glutamine concentrations in these tissues are sufficiently high such that measuring any diminution in glutamine pool size in muscle during exercise is rather difficult; certainly if glutamine fell below some critical level at which its ability

to supply metabolic processes in muscle or elsewhere were compromised then supplementation with glutamine *might* make sense. For example, there is good evidence that the ability to work at near maximal rates of oxygen consumption is limited by the constant draining of citric acid cycle intermediates, e.g., escape of succinate and malate. Glutamate is an obvious precursor for 2-oxoglutarate which could top up the citric acid cycle intermediates, but glutamate concentrations themselves can fall dramatically in strenuous exercise, suggesting that the glutamate pool size is insufficient. Glutamine is able to be converted to glutamate, very efficiently by phosphate-dependent glutaminase which is present in skeletal muscle and heart mitochondria, and as long as glutamine can get into them it should act as a precursor of 2-oxoglutarate. The problem is, if this is so why does muscle glutamine concentration not show a bigger fall? Furthermore, would *additional* glutamine (i.e., an amount over and above the residual store) have any benefit? These are ideas that need to be tested.

The ability to mount defenses against metabolic acidosis very much depends upon glutamine availability. To what extent this is important during physical exercise is not known.

Exercise is also said to be associated with an increase in free-radical induced damage, and there is good evidence that, paradoxically, glutamine may be a better precursor of intracellular glutamate for the synthesis of glutathione (e.g., in white cells and liver) than extracellular glutamate. Under such circumstances glutamine availability may help maintain the cellular defenses against oxidative damage.

There is a substantial amount of anecdotal evidence that highly trained subjects are more susceptible to infection, in particular upper respiratory tract infection. Whether this is because of a weakening of epithelial barriers against micro-organisms or because of an inability of the immune system to cope adequately is not known, but there is certainly a wealth of evidence to suggest that cells of the immune system are better able to cope when they are well supplied with glutamine. Evidence is emerging that glutamine may afford some benefit when given after a substantial bout of exercise, e.g., after a marathon or half marathon, but it is not known to what extent glutamine provision before exercise can act as a protective agent.

Glutamine is said to improve mood in some circumstances, but no very good data exist in healthy individuals.

It seems likely that glutamine itself is the missing magic ingredient in many cases, but a number of enthusiasts have espoused glutamate and 2-oxoglutarate, either on its own as the Na salt or as the ornithine or aspartate salts, but the benefit of such supplements has not been proven.

Other amino acids that have been thought to be conditionally essential under certain circumstances and that might be considered with respect to physical exercise are arginine, histidine, and cysteine. The arginine/nitric oxide story is well known, but I have been unable to discover any evidence of a beneficial effect of arginine with respect to physical activity. Histidine is said to have a role in mopping up free radicals, and cysteine may well contribute toward glutathione synthesis. However, the difficulties of delivering sulphur-containing amino acids as supplements are well known, and it seems unlikely that it would be possible to produce such supplements in a stable and palatable form.

Functional Foods and Oral Glutamine

It is still not known to what extent glutamine-containing proteins are broken down to free glutamine and then absorbed or whether absorption occurs as glutamine-containing peptides; nor is it known to what extent protein-bound glutamine appears as free glutamine in the hepatic portal vein or

whether glutamine is largely metabolized by the cells of the gastrointestinal tract. Nevertheless, a number of companies are working on so-called functional foods, which contain large amounts of protein-bound glutamine, principally from wheat and milk proteins. These may be more effective ways of delivering glutamine to the body. Glutamine can be given effectively in substantial doses, e.g., up to 8 g/330 mL in water and despite a substantial (probably unwarranted) amount of worry about glutamine degradation and toxicity of the product 5-oxoproline, it seems that packaging glutamine in sachets for mixing with soft drinks, yogurt, or milk-based drinks is a perfectly feasible way of supplementing dietary intake.

Future Research Needs

More research is needed to determine the way in which glutamine is handled by the gut, the specific requirements of particular metabolic processes for glutamine, and whether glutamine compartmentation limits the free exchange of the amino acid between different pools in the body. Better evidence is needed for the way in which glutamine has its beneficial effects in anabolism, the immune system, and in possibly combating free radical damage. Population groups that might benefit particularly, e.g., elite athletes and the elderly, need to be identified. Better information is also needed about the dose-response relationship between glutamine and its possible beneficial effects and also the possible downsides of giving large amounts of nonessential amino acids with an otherwise normal diet.

Meeting Optimal Calcium Requirements

Connie M. Weaver, Ph.D.

Calcium

Adequate dietary intake of calcium is important to bone health and has been associated with reduced risk of colon cancer and hypertension in some individuals. Unfortunately, calcium intake by many Americans, especially females, is less than the Recommended Dietary Allowance and the amounts recommended by the NIH Consensus panel on optimal calcium intake (Table 1).¹

Osteoporosis is a major health problem affecting more than 25 million people in the United States. Prevention of osteoporosis is the most cost-effective means for managing this disease. Optimizing peak bone mass during bone growth and consolidation and reducing the subsequent rate of bone loss are two strategies for keeping bone mass above the threshold for fracture. Adequate dietary calcium is a requisite to maximizing development of peak bone mass within an individual's genetic potential and to reducing bone resorption later in life. Approximately 90 percent of the total body bone mass in females is achieved by age 16.9, 95 percent by age 19.8, and 99 percent by age 26.2.²

Dairy products provide the most absorbable calcium of the food groups. Aside from calcium, dairy products provide many other nutrients important for bone health. For those individuals who cannot meet their dietary requirements for calcium from foods naturally containing this nutrient, it is important to consider which nutrients in addition to calcium may be beneficial in supplements. For example, vitamin D enhances calcium absorption and magnesium improves bone quality.

Group	Optimal Daily Intake (in mg of calcium)
Infants	
Birth–6 months	400
6 months–1 year	600
Children	
1–5 years	800
6–10 years	800–1,200
Adolescents/Young Adults	
11–24 years	1,200–1,500
Men	
25–65 years	1,000
Over 65 years	1,500
Women	
25–50 years	1,000
Over 50 years (postmenopausal)	1,000
On estrogens	1,500
Not on estrogens	1,500
Over 65 years	1,500
Pregnant and nursing	1,200–1,500

Exercise

Weight-bearing exercise has also been associated with positive benefits on bone mass or bone density. However, this relationship is not supported by the literature to the extent of dietary calcium and bone mass. Most previous exercise studies are potentially biased because they are nonrandomized, and others have an inadequate sample size to show statistical significance or are compromised by previous physical activity and other confounding factors. One of the few randomized exercise intervention studies was by Snow-Harter in premenopausal women.³ An 8-month weight training or jogging program resulted in a significant increase in lumbar spine bone mineral density. Weight training and jogging resulted in similar increases in bone density (1.2 vs. 1.3 percent, respectively) compared with a slight decrease in the control group.

Calcium and Exercise Interaction

Very few studies have examined the interaction of dietary calcium and exercise. The effects may be independent or additive. Recent studies have evaluated the effect of exercise in addition to calcium supplementation. Lohman et al. reported a positive effect of 18 months of resistance exercise training on femoral trochanter and spine bone mineral density in 56 women ages 28–39 while taking a supplement of 500 mg of calcium in addition to their diet, which averaged 1023 mg/day.⁴ Similarly, in 168 postmenopausal women randomized into one of four groups (placebo, calcium from milk powder, calcium supplements, and supplements plus weight-bearing exercise), exercise in addition to calcium was beneficial for preventing bone loss at the tibia and hip.⁵ Calcium plus exercise was more beneficial at the femoral neck than calcium alone. Thus, in both of these studies, calcium status should have been replete so that a true calcium by exercise effect remains unknown.

Needed Research

Well-designed randomized intervention trials are needed to establish the interactive effects of dietary calcium and exercise on bone health. The effect of type, duration, and intensity of exercise should be evaluated. Although long-term intervention trials through several bone remodeling cycles are of value in evaluating the effect of an intervention, it is also of practical value to determine the effect of cyclic starting and stopping exercise programs. The study of other life style factors that impinge upon both calcium and physical activity requirements for healthy bones is also needed.

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Magnesium, Zinc, and Chromium

Henry C. Lukaski, Ph.D.

In contrast to the macronutrients (proteins, carbohydrates, and fats) that are consumed daily in large amounts (tens and hundreds of grams), micronutrients, such as magnesium, zinc, and chromium, are ingested in much smaller amounts (thousandths and millionths of a gram). The relative paucity of these micronutrients, both in the diet and in the body, suggests their importance in the regulation of whole-body metabolism, including energy utilization and work performance.

The biological importance of magnesium, zinc, and chromium is revealed by the various metabolic processes in which these elements regulate biological function. Magnesium, a ubiquitous element that plays a fundamental role in many cellular reactions, is involved in more than 300 enzymatic reactions in which food is metabolized and new products are formed. Some important examples include glycolysis, fat and protein metabolism, adenosine triphosphate synthesis, and second messenger system. Magnesium also serves as a physiological regulator of membrane stability and in neuromuscular, cardiovascular, immune, and hormonal function.

Another intracellular cation, zinc, is required for more than 300 enzymes from many species. Zinc-containing enzymes participate in many components of macronutrient metabolism, particularly cell replication. In addition, some zinc-containing enzymes, carbonic anhydrase and lactate dehydrogenase, are involved in exercise metabolism, and another enzyme, superoxide dismutase, protects against free radical damage.

Recent attention has been directed to the element chromium. Trivalent chromium is required for the maintenance of normal glucose metabolism in animals; thus, chromium may act as a cofactor for insulin action. This insulinogenic characteristic of chromium has prompted the suggestion that chromium has an anabolic function.

Attempts to examine the relationships between physical activity and magnesium, zinc, or chromium nutritional status have been hampered generally by limitations in experimental design. Overall, the evidence to suggest that physically active individuals have either impaired magnesium or zinc status or improve performance with supplementation of these minerals is equivocal because nutritional status was not examined.

The effects of chromium supplementation on human physical performance are current topics of interest. Initial studies, in which collegiate football players were supplemented with 200 μ g chromium as chromium picolinate, reportedly found a significant increase in fat-free mass with a concomitant decrease in body fat during resistance training based on anthropometric assessment of body composition. Subsequent studies that used more accurate methods of body composition assessment failed to document beneficial effects of generalized chromium supplementation (200 μ g/d) on strength gain or body composition in young adults.

The indiscriminate use of mineral supplements may be hazardous. Ingestion of magnesium supplements in amounts exceeding 500 mg/d often results in gastrointestinal disturbances, particularly diarrhea, and may adversely affect phosphate balance. Supplemental zinc (50 mg/d) can inhibit absorption of copper from the diet. Consumption of supplemental zinc in amounts ranging from 17 to 50 mg/d can prevent an exercise-induced increase in high-density lipoprotein cholesterol (HDL) concentration.

Excessive intake of supplemental zinc (160 mg/d) can decrease HDL. Chromium supplementation (200 µg/d as chromium picolinate) may reduce transferrin saturation and induce iron deficiency. Therefore, the use of mineral supplements is not recommended unless under the guidance of a physician or a registered dietitian.

The effect of nutritional supplementation on physical performance has been examined in cross-over design experiments. Based on laboratory and field performance tests, there was no measurable effect of nutritional supplementation in well-nourished individuals. These findings support the hypothesis that there is no beneficial effect of nutritional supplements on performance when athletes consume diets adequate in essential nutrients.

Because of the importance of magnesium, zinc, and chromium for the maintenance of health and the development of physical fitness, physically active individuals should consume a balanced diet. If an individual is concerned about the nutritional quality of the diet, he or she should seek advice from a registered dietitian who is experienced in working with people with active lifestyles.

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Iron Nutrition and Exercise

John L. Beard, Ph.D.

Iron deficiency is the most common single nutrient deficiency disease in the world and is a major concern for approximately 15 percent of the world's population. The commonly used definition for anemia, for whatever cause, is a low hemoglobin concentration (Hb). If iron deficiency is an underlying etiology, then by definition the individual must have depleted iron stores, a low plasma ferritin or decreased stainable iron in bone marrow, and an inadequate delivery of iron to tissues, as characterized by a low transferrin saturation, a high erythrocyte protoporphyrin concentration, and an elevated transferrin receptor concentration.^{1,2}

Iron deficiency can be defined as that moment in time when body iron stores become depleted and a restricted supply of iron to various tissues becomes apparent.³ The process of depletion of iron stores can occur rapidly or very slowly and is dependent on the balance between iron intake and iron requirements. Clearly, iron intake is dependent on food composition and quantity of iron therein, with a number of inhibitors and a smaller number of enhancers of iron absorption now known to exist. Iron absorption increases in individuals who have depleted iron stores; it is this internal regulator of absorption that may be more important than any particular constituents of the food supply.⁴ Basal obligatory iron losses in humans are approximately 1 mg/day and must be replaced by an equivalent amount of iron derived from the diet. The typical Western diet provides an average of 6 mg of heme and nonheme iron per 1,000 kcals of energy intake.

Consequences of Poor Iron Status

Many organs show morphological, physiological, and biochemical changes with iron deficiency in a manner related to the turnover of essential iron-containing proteins. Sometimes this occurs even before there is any significant drop in Hb concentration.⁵ Iron deficiency is associated with altered metabolic processes; among them are mitochondrial electron transport, neurotransmitter synthesis, protein synthesis, organogenesis, and others.

It is also important to delineate whether exercise itself may alter iron status, and whether such alterations are detrimental to athletic performance or to the health of an athlete. Although a multitude of laboratories worldwide have contributed to a very broad-based accumulation of knowledge in these areas, an analysis of more than two decades of research illustrates four central points:

1. Reductions in heme and nonheme iron can detrimentally alter exercise performance.
2. Iron status is altered in certain populations of chronically exercising individuals.
3. Women may have an increased prevalence for exercise-related alterations in body iron.
4. It is reasonable to question whether these manifestations are specifically detrimental to the health or the athletic performance of the individual afflicted.

Iron and Exercise Performance

The role of heme and nonheme iron in biologic function and work performance has been elucidated through human and animal experiments, and several classic reviews have been published.^{6,7} It is not surprising that hemoglobin iron, when lacking, can profoundly alter physical work performance via a decrease in oxygen transport to exercising muscle. What is intriguing, however, is that although nonheme iron associated with enzyme systems comprises only 1 percent of total body iron, profound deficits of these components per se may have detrimental effects on athletic performance. Studies illustrate that maximal oxygen consumption is determined primarily by the oxygen-carrying capacity of the blood and is thus correlated to the degree of anemia. Endurance performance at reduced exercise intensities, however, is more closely related to tissue iron levels, since a strong association is seen between the ability to maintain prolonged submaximal exercise and the activity of the oxidative enzyme pyruvate oxidase. There is disagreement in results of human studies regarding the concept of a Hb threshold phenomenon. Research by Edgerton et al.⁸ suggests that the decrement in work performance in iron-deficient anemic subjects was a reflection of the level of anemia rather than other non-Hb related biochemical changes. Unlike the data of Perkkio et al.,⁹ these studies suggest a more linear relationship between Hb and work performance and, thus, do not necessarily support the presence of a Hb threshold phenomenon.

Several studies conducted as early as two decades ago documented altered iron status in athletes, yet questioned whether such alterations were physiologically detrimental. Wijn et al.¹⁰ measured Hb, packed cell volume, serum iron, and iron-binding capacity of selected athletes and compared these with the hematologic profile of officials during the 1968 Olympic games. These data illustrated an iron-deficient anemia in 2 percent of male and 2.5 percent of female athletes and a mild anemia without signs of iron depletion in 3 percent of the athletic population. Many other experiments have demonstrated a significant decrease in RBC number and a decrease in Hb, but often the runners were not affected. Subsequent investigations have supported these early studies and have demonstrated a reduction in Hb and HCT in certain athletic populations.

Several investigators have proposed mechanisms by which iron balance could be affected by intense physical exercise.¹¹ Explanations include increased gastrointestinal blood losses following running, and hematuria as a result of erythrocyte rupture within the foot during running.¹²

A growing body of evidence suggests that the prevalence of iron deficiency without anemia is increased in female athletes. Contributing to this observation is an increased iron loss through regular menstrual function as well as putative dietary factors. Given these observations, if exercise does further compromise iron status, then it would logically follow that the population of chronically exercising female athletes may be at greater risk of developing iron deficiency. A summary of surveys demonstrates that 35 percent of female athletes have a serum ferritin concentration of less than 12 mg/L; 82 percent, less than 25 mg/L; and 60 percent, less than 30 mg/L, when compared with sedentary counterparts from the nonathletic female population. Although estimations of the precise prevalence rates differ, an increased incidence of reduced serum ferritin seems to be a repeatable observation between laboratories in this population. These results may be influenced by menstrual flow, and may additionally be affected by dietary iron intake. Taken together, these investigations illustrate a statistically significant difference between female athletes and control populations. What these investigations do not demonstrate, however, is a clinically subnormal or reduced serum ferritin concentration concurrent with a demonstrated functional consequence in the absence of overt anemia.

The Recommended Dietary Allowances (RDAs) are designed for the maintenance of good nutrition of practically all healthy people, and they define intakes of iron for infants, children, men and

women, and also consider additional needs during pregnancy and lactation. From the data presented in this review, the following recommendations broadly apply to the three groups that appear to be at greatest risk of developing altered body iron given the data available. These recommendations do not assume that a deficiency will exist in these populations when consuming the RDA. However, a deficiency may be more likely, thus warranting closer dietary monitoring and regularly scheduled hematologic evaluations in these at-risk groups.

- Female athletes are advised to pay particular attention to maintaining an adequate consumption of iron in their diet.
- Distance runners should also pay attention to maintaining an adequate consumption of iron- rich foods.
- Vegetarian athletes should be particularly vigilant to include iron-rich foods in their diet.

For all three groups, monitoring of dietary intake and good nutritional counseling may preclude a negative iron balance and should be the first line of action in the prevention of iron deficiency. Indiscriminant pharmacologic intervention should be viewed as an undesirable means of achieving adequate iron intake, since in the least, it marginalizes the importance of promoting good nutritional habits in the athletic population. From the above priorities it is apparent that the female runner who consumes a vegetarian diet would likely be at greatest risk for a negative iron balance.

The use of supplements, however, must be a judicious choice based not upon the likelihood of anemia, but ideally upon hematologic evaluation. Using complex preparations may provide less iron than suspected, and warrant a careful re-examination with regard to efficacy. Clinically utilized oral iron preparations contain ferrous sulfate, hydrated or gluconated, or ferrous gluconate or ferrous fumarate. These preparations contain from 37 to 106 mg elemental iron. Supplementation is not without consequence, however; the use of high doses of supplemental iron is often associated with gastrointestinal distress and constipation, with a subsequent decline in compliance. In those genetically predisposed, hemochromatosis may develop following iron supplementation. Iron toxicity may even develop in those not genetically predisposed when ingesting dosages of 75 mg or more of supplemental iron.

In summary, it is clear that a decreased HCT and Hb will impair delivery of oxygen to tissues and lead to a reduced maximal oxygen uptake. Supplementation of individuals to normal HCT verifies the effects of hemoglobin iron on VO_2 max. However, the effects of iron supplementation upon the athletic performance of those with clinically low serum ferritin is less clear, although limited evidence seems to suggest improved endurance performance and a decreased reliance upon glucose as an oxidative substrate. Whether such adaptations are beneficial at a subclinically reduced serum ferritin has not been established.

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The Effect of Physical Activity on Thiamin, Riboflavin, and Vitamin B6 Requirements

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Two questions have frequently been asked by individuals engaged in physical activity: Does exercise increase the need for certain vitamins? and Does vitamin supplementation improve exercise performance? These are particularly relevant and timely questions with regard to thiamin, riboflavin, and vitamin B6, because these three vitamins are cofactors for many metabolic reactions that produce energy. In addition, the Recommended Dietary Allowances (RDAs) for these nutrients are dependent on energy (thiamin and riboflavin) and protein (vitamin B6) intakes. It may be logical to assume that as an individual becomes more physically active, both energy and protein intakes will increase along with the intake of these vitamins; however, this is not always true. If an individual makes poor dietary choices, these micronutrients may not increase with energy and protein intakes. Conversely, if an individual increases physical activity and restricts energy intake, the need for the vitamins may increase but the dietary intake would not.

Dietary Sources

Thiamin, riboflavin, and vitamin B6 are water-soluble B-complex vitamins found in a variety of animal and vegetable products. More specifically, thiamin is found abundantly in lean pork, yeast, legumes, and enriched cereals and breads. Riboflavin is found in eggs, lean meats, milk, milk products, broccoli, and enriched breads and cereals. Vitamin B6 is abundant in meats, especially chicken and tuna, and plant foods such as beans, cereals, and brown rice. These vitamins are also frequently added to commercially prepared foods at 25–100 percent of the RDA per serving. Thus, the consumption of fortified cereals, breakfast bars, sport bars, energy shakes and/or meal-replacement products (e.g., Ensure®, Boost®, GatorPro®) will dramatically increase total dietary intakes. These types of products are frequently used by individuals who “watch their weight” or who are engaged in physical activity. In addition, any multivitamin or vitamin/mineral supplement will usually contain 100 percent or more of the RDA for these nutrients. It is estimated that millions of Americans use dietary supplements. The estimates are as high as 50 percent for individuals engaged in physical activity. Thus, the total intake of these vitamins in the diet of active Americans may be increasing regardless of dietary choices.

Exercise-related Functions and Requirements

Thiamin, as thiamin pyrophosphate, is important for the metabolism of both carbohydrate and the branched-chain amino acids (BCAA). It is a coenzyme for the pyruvate dehydrogenase complex that catalyzes the conversion of pyruvate to acetyl CoA. Thiamin is also a coenzyme for α -ketoglutarate decarboxylase, an enzyme responsible for the formation of succinyl CoA in the tricarboxylic acid (TCA) cycle, and for branched-chain decarboxylase, an enzyme responsible for the catabolism of the BCAA. Physical activity stresses these metabolic pathways for the production of energy. Because thiamin requirements are linked to energy metabolism, the RDA for thiamin is expressed in terms of energy intake. The current U.S. recommendation is 0.5 mg/1,000 kcal, with a minimum of 1.0 mg/day required for adults. An additional 0.4 and 0.5 mg/day are recommended during pregnancy and lactation, respectively.

Riboflavin is necessary for the synthesis of two important coenzymes in the body—flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). These coenzymes are especially important in

the metabolism of glucose, fatty acids, glycerol, and amino acids for energy. Similar to thiamin, physical activity stresses the biochemical pathways that metabolize these substrates. The dietary requirement for riboflavin is also expressed in terms of energy intake. The current U.S. recommendation is 0.4 mg/1,000 kcal for people of all ages, with a minimum intake of 1.2 mg/day. Because pregnancy and lactation increase energy demands, an additional 0.3 mg/day is recommended during pregnancy and an additional 0.4–.05 mg/day is recommended during lactation.

A major function of vitamin B6 is the metabolism of proteins and amino acids. The most metabolically active form of vitamin B6 is pyridoxal 5₂-phosphate (PLP). PLP is a cofactor for transverases, transaminases, decarboxylases, and other enzymes used in the many metabolic transformations of amino acids. During exercise, the gluconeogenic process involves the breakdown of amino acids for energy in the muscle and the conversion of lactic acid to glucose in the liver. Another function of vitamin B6 directly related to energy production during exercise is the breakdown of muscle glycogen. Thus, adequate vitamin B6 must be present to release glucose-1-phosphate from muscle glycogen. Because vitamin B6 is directly involved in amino acid metabolism, the requirements for vitamin B6 are expressed in terms of protein intake. The current U.S. recommendation for adults age 25 or older is 0.032 mg of B6/g of protein. As with thiamin and riboflavin, vitamin B6 requirements increase slightly with pregnancy and lactation (0.032–0.036 mg B6/g protein).

Rationale for Increased Need

Because exercise stresses metabolic pathways that use thiamin, riboflavin, and vitamin B6, it has been suggested that the requirements for these vitamins are increased in athletes and active individuals. Theoretically, exercise could increase the need for these nutrients in the following ways: increase the turnover, metabolism, or loss of the nutrient; increase the enzymes that require the nutrient; or increase the need for the nutrient for tissue maintenance and repair. Although biochemical evidence of vitamin deficiencies in some active individuals has been reported for these nutrients, studies examining these issues are limited and equivocal. Reasons for these inconsistencies may be related to a number of factors: dietary control, type and intensity of exercise, status indices measured, level of regular physical activity, type of subjects, or lack of a control group.

We do know that exercise increases both energy and protein needs and, thus, also increases the total daily needs of thiamin, riboflavin, and vitamin B6 in active individuals. However, the RDAs for these nutrients are already linked to energy and protein intakes. Thus, ideally dietary intakes of these vitamins by active individuals should be adequate unless dietary food choices are poor or energy intake is restricted. No extensive research has been conducted on whether nutrient requirements are higher in active individuals with chronic diseases, energy restriction, musculoskeletal injuries, or individuals under high stress.

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Does Dietary Creatine Supplementation Have a Role to Play in Exercise Metabolism?

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The Biosynthesis and Distribution of Creatine

Creatine (Cr), or methyl guanidine-acetic acid, is a naturally occurring compound. In healthy individuals, the total body Cr pool is approximately 120 g, 95 percent of which is found in skeletal muscle. This pool is replenished at a rate of approximately $2 \text{ g} \cdot \text{d}^{-1}$ by endogenous Cr synthesis and/or dietary Cr intake, e.g., meat and fish. Oral ingestion of Cr has been demonstrated to suppress biosynthesis, but this response is abolished upon the cessation of supplementation.¹

The precursors of Cr synthesis were first determined by labeling nitrogenous compounds with ^{15}N and isolating subsequent creatine formed.^{2,3} Synthesis proceeds via two successive reactions involving two enzymes. The first reaction is catalyzed by glycine transaminidase and results in an amidine group being reversibly transferred from arginine to glycine, forming guanidinoacetic acid. The second reaction involves the irreversible transfer of a methyl group from S-adenosylmethionine (SAM) catalyzed by guanidinoacetate methyltransferase, resulting in the methylation of guanidinoacetate and the formation of Cr. The distribution of the two enzymes across tissues varies between mammalian species. In man, the liver is the major site of de novo Cr synthesis; however, the pancreas is also known to have some capability. As little Cr is found in the major sites of synthesis, it is logical to assume that transport of Cr from its sites of synthesis to storage must occur, thus allowing a separation of biosynthesis from utilization.¹

Muscle Cr uptake occurs actively against a concentration gradient, possibly involving Cr interacting with a specific membrane site that recognizes the amidine group.⁴ Two mechanisms have been proposed to explain the very high Cr concentration within skeletal muscle. The first involves the transport of Cr into muscle by a specific saturable entry process. In this respect, a specific Cr transporter has recently been identified in skeletal muscle, heart, and brain.⁵ The second mechanism necessitates the intracellular trapping of Cr. Approximately 60 percent of muscle total Cr store exists in the form of phosphocreatine (PCr), which, due to its phosphorylated state, is unable to pass through membranes, thus trapping Cr. This entrapment results in the generation of a concentration gradient. However, phosphorylation is not the sole mechanism of cellular Cr retention. For example, the binding of Cr to intracellular components and the existence of restrictive cellular membranes have been proposed as mechanisms that facilitate muscle Cr retention.¹

Creatinine has been established as the sole end product of Cr degradation being formed non-enzymatically in an irreversible reaction.^{3,6} As skeletal muscle is the major store of the body creatine pool, this is the major site of creatinine production. The daily renal creatinine excretion is relatively constant in an individual, but can vary between individuals, depending on the total muscle mass in healthy individuals.^{3,6}

The Role of Creatine in Muscle Energy Metabolism and Fatigue

In human skeletal muscle, Cr is present at a concentration of about $125 \text{ mmol} \cdot \text{kg}^{-1}$ dry muscle (dm), of which approximately 60 percent is in the form of PCr at rest. A reversible equilibrium exists between Cr and PCr ($\text{PCr} + \text{ADP} + \text{H}^+ \rightleftharpoons \text{ATP} + \text{Cr}$), and together they function to maintain intracellular adenosine triphosphate (ATP) availability, modulate metabolism, and buffer hydrogen ion accumulation

during contraction. The availability of PCr has been proposed as one of the most likely limitations to muscle performance during intense, fatiguing, short-lasting exercise, i.e., where the anaerobic ATP demand is very high.⁷ This conclusion has been drawn from studies involving short bouts of maximal electrically evoked muscle contraction and/or voluntary dynamic exercise, and from animal studies in which the muscle Cr store has been depleted, prior to maximal electrical stimulation, using the Cr analog β -guanidinopropionate (β -GPA). More recent studies have taken the complexity of investigation one step further by implicating the availability of PCr specifically in type II muscle fibers as being of critical importance to the maintenance of performance during maximal short-lasting exercise.⁷ The availability of free Cr has also been ascribed a central role in the control of phosphocreatine resynthesis, its function in the regulation of mitochondrial ATP resynthesis, and thereby PCr resynthesis, having been the subject of much debate.^{8,9}

The Effect of Creatine Ingestion on Muscle Creatine Concentration in Humans

Studies earlier this century demonstrated that Cr administration resulted in a small increase in urinary creatinine excretion.^{2,3,6} However, there was no increase in excretion until a significant amount of the administered Cr had been retained. It was also noted that Cr retention was greatest during the initial stages of administration. In general, urinary creatinine excretion rose slowly during prolonged Cr administration and, upon cessation, approximately 5 weeks elapsed before a significant fall in creatinine excretion was observed.

These studies invariably involved periods of chronic Cr ingestion. However, with the application of the muscle biopsy technique, it has become apparent that the ingestion of 20 g of Cr each day in solution for 5 days (4 x 5 g doses) can lead to an average increase in muscle total Cr concentration of about 20 percent, of which approximately 30 percent is in the form of PCr.^{10,11} In agreement with earlier urinary excretion studies, it appears that the majority of muscle Cr retention occurs during the initial days of supplementation; e.g., about 30 percent of the administered dose is retained during the initial 2 days of supplementation, compared with 15 percent from days 2–4.¹⁰ The natural time-course of muscle Cr decay following 5 days of 20 g day^{-1} ingestion occurs over the course of several weeks rather than days.¹²

As might be expected, lower dose Cr supplementation (e.g., 3 g day^{-1} for 2 weeks) is less effective at raising muscle Cr concentration than is a 5-day regimen of 20 g day^{-1} .¹² However, following 4 weeks of supplementation at this lower dose, muscle Cr accumulation is no different when regimens are compared.¹² It also appears that muscle Cr stores remain elevated for several weeks when the supplementation regimen of 20 g day^{-1} for 5 days is followed by lower dose supplementation (2 g day^{-1}),¹² which seems to agree with the earlier suggestion that Cr is ‘trapped’ within skeletal muscle once absorbed and that muscle Cr degradation to creatinine occurs at a rate of about 2 g day^{-1} .

The Effect of Creatine Ingestion on Exercise Performance

As stated previously, a reversible equilibrium exists between Cr and PCr, and the development of fatigue during maximal short-duration exercise has been associated with the depletion of muscle PCr stores.⁷ Creatine in its free and phosphorylated forms therefore occupies a pivotal role in the regulation and homeostasis of skeletal muscle energy metabolism and fatigue. However, despite these important roles for Cr during muscle contraction, little has been published relating to Cr ingestion and exercise performance. In 1981, Sipila et al.¹³ reported that, in a group of patients receiving 1 g of creatine day^{-1} as a treatment for gyrate atrophy, there was a comment from some of a sensation of strength gain following a 1-year period of supplementation. Indeed, Cr ingestion was shown to reverse the type II muscle fiber atrophy associated with this disease, and one athlete in the group of patients improved his personal

best record for the 100 meter sprint by 2 seconds.

Based on more recently published work, it would appear that the ingestion of 4 x 5 g of Cr \cdot day⁻¹ for 5 days will significantly increase exercise performance in healthy male volunteers during repeated bouts of fatiguing, short-lasting maximal exercise, e.g., maximal isokinetic knee extensor exercise, maximal dynamic cycling exercise, maximal isokinetic cycling exercise, and controlled “track” running experiments.¹⁴⁻¹⁷ The consistent finding from these studies is that Cr ingestion significantly increased exercise performance by 5–7 percent by sustaining force or work output during exercise. Some studies have also reported an increase in maximal strength or torque at the onset of contraction.¹⁵ More recently, however, results from our laboratory indicate that approximately 30 percent of subjects who have ingested Cr in an attempt to increase muscle Cr concentration have failed to retain substantial quantities of Cr.^{10,18} These results have also shown that any improvement in exercise performance as a consequence of Cr supplementation is clearly associated with the extent of muscle Cr retention during supplementation; i.e., those individuals who experienced the highest muscle Cr accumulation during supplementation were those who demonstrated the better improvements in exercise performance.¹⁸ It would appear therefore that the extent of muscle Cr retention during feeding is critical to subsequent exercise performance.

In support of this conclusion, further results from our laboratory have shown that those individuals who experienced more than a 25-percent increase in total Cr muscle concentration following 5 days of Cr ingestion also showed an accelerated rate of PCr resynthesis during 2 minutes of recovery from intense, fatiguing muscular contraction. Conversely, those individuals who experienced no or little Cr accumulation during ingestion (on average an 8-percent increase) showed no measurable change in PCr resynthesis during recovery.¹⁰

Creatine's Mechanism of Action

The exact mechanism by which Cr ingestion improves performance during maximal exercise is not yet clear. The available data indicate that it may be related to the stimulatory effect that Cr has upon pre-exercise PCr availability¹⁸ and on PCr resynthesis during exercise and recovery.¹⁰ Given that PCr availability is generally thought to limit exercise performance during maximal exercise, both of these effects would increase muscle contractile capability by maintaining ATP turnover during exercise. This suggestion is supported by reports showing that the accumulation of plasma ammonia and hypoxanthine (accepted markers of skeletal muscle adenine nucleotide loss) are reduced during maximal exercise following Cr ingestion, despite a higher work output being achieved.^{14,16} More convincing evidence comes from a recent study showing that Cr supplementation can reduce the extent of muscle ATP degradation by 25 percent during maximal isokinetic cycling exercise, while, at the same time, increasing work output.¹⁸

Future Work

The precise biochemical route by which an increase in muscle Cr concentration produces an improvement in muscle contractile function during maximal exercise remains to be clearly demonstrated and is currently the subject of intense debate. This area of research should be a focus of future in vivo and in vitro work.

Research has shown that the extent of muscle Cr accumulation during supplementation varies greatly between individuals. Given that improvements in maximal exercise performance and PCr resynthesis during recovery from exercise appear to be critically dependent on the extent of muscle Cr accumulation during Cr supplementation, future work should focus on elucidating the principal factors regulating

muscle Cr transport in man. It would also be of interest to determine the mechanism by which muscle Cr transport is upregulated in vegetarians and downregulated during daily Cr feeding. This may offer some insight into the mechanisms regulating the exaggerated loss of muscle Cr during fasting and disease. Finally, the consequences of long-term Cr supplementation on health and lifestyle in normal and diseased states requires investigation.

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Supplemental Carnitine and Exercise

Eric P. Brass, M.D., Ph.D.

Introduction

Carnitine (L-3-hydroxytrimethylammoniumbutanoate) is an endogenous molecule with several established roles in mammalian cellular metabolism.¹ All of carnitine's known biochemical functions are mediated via the reversible transfer of activated carboxylic acids ("acyl" moieties) from coenzyme A to carnitine. In this way, the formation of acylcarnitines is critical for the mitochondrial oxidation of long-chain fatty acids, and formation of short-chain acylcarnitines protects the cell from potentially toxic acyl-CoA accretion.² Thus, cells contain both carnitine and acylcarnitines, with the acylcarnitines composed of a spectrum of specific acylcarnitines (i.e., acetylcarnitine). Total carnitine refers to carnitine plus all acylcarnitines.

In humans, carnitine is derived from a variety of dietary sources, particularly dairy and meat products. Carnitine can also be synthesized in humans with lysine providing the carbon backbone of the molecule. Available evidence indicates that the biosynthetic capacity of carnitine is adequate to meet the needs of most people, and dietary intake is not required. In contrast, pathophysiologic conditions exist in which carnitine supplementation is of clear therapeutic value.

Carnitine metabolism is highly compartmentalized in humans. Tissues contain widely varied amounts of total carnitine, ranging from 60 mmol/L in plasma to 4,000 mmol/kg in skeletal muscle. Additionally, the distribution of the carnitine pool between carnitine and acylcarnitines can vary among tissues,³ and tissues have distinct turnover rates for carnitine.⁴

Carnitine Metabolism During Exercise

The bioenergetics of exercise require an enormous increase in muscle adenosine triphosphate (ATP) production and related changes in substrate fluxes. Carnitine, because of its intimate relationship with the coenzyme A pool, reflects many of these metabolic changes. As the ATP demands increase with increasing exercise workloads, a metabolic transition workload, termed the lactate threshold, is reached.⁵ At workloads below the lactate threshold, lactate does not accumulate in plasma or muscle, the respiratory exchange ratio (RER) remains approximately 0.80, and the exercise can be sustained. At workloads above the lactate threshold, lactate accumulates, the RER reaches 1.00, and performance at the workload cannot be sustained. At rest, the skeletal muscle carnitine pool contains a distribution of 80–90 percent carnitine and 10–20 percent acylcarnitines (primarily short-chain acylcarnitines). At workloads below the lactate threshold, the distribution of the muscle carnitine pool is not altered. However, as workloads exceed the lactate threshold, the muscle carnitine pool is redistributed into acylcarnitines so that 50–70 percent of the pool is in the form of acylcarnitines.^{6,7} This redistribution is not normalized 60 minutes after a 30-minute exercise session above the lactate threshold. It is now clear that this increase in acylcarnitine content is the result of acetyl-CoA accumulation, leading to acetylcarnitine generation.⁸ Importantly, the dramatic changes in the muscle carnitine pool during exercise are not reflected in the plasma or urine compartments.

Rationale for use of Carnitine to Enhance Exercise Performance

The relationship of the carnitine pool to the critical metabolic processes of bioenergetics has led to much speculation about the potential benefits in normal humans of supraphysiologic carnitine levels. The possible mechanisms by which carnitine could have an effect are varied and include the following:

- Enhancing oxidation of fatty acid, a critical substrate, during exercise
- Preserving muscle glycogen during exercise, a factor potentially related to fatigue resistance
- Shifting fuel substrate use toward glucose, thereby decreasing the oxygen requirement for the workload-defined ATP requirement
- Replacing the carnitine depleted by acetylcarnitine formation
- Enhancing acetylcarnitine formation, lowering acetyl-CoA content, and, thus, activating pyruvate dehydrogenase
- Improving muscle fatigue resistance
- Replacing carnitine losses that occur during aerobic training
- Increasing oxidative capacity in skeletal muscle.

Each of these concepts can be justified on theoretical grounds and has been partially validated through in vitro or limited animal studies. However, equally persuasive arguments can be constructed as to why they would not apply to exercise in normal subjects receiving carnitine supplementation. Thus, experimental evidence in humans is required to address this issue before conclusions can be reached.

Considerations in Carnitine Dosing in Normal Humans

Carnitine is available for use in intravenous or oral preparations. In pharmacological doses, the bioavailability of oral carnitine is 5–15 percent. Elevations in plasma carnitine content after carnitine dosing lead to rapid increases in urinary carnitine elimination. Further, the muscle compartment reaches steady state with respect to the plasma carnitine pool only slowly and is quantitatively much larger. Thus, use of carnitine supplementation to perturb the muscle carnitine pool is problematic, given the body's handling of the compound.⁹

Carnitine is well tolerated in humans, and administration of the pure L-isomer has been associated with no serious side effects. Gastrointestinal symptoms, including diarrhea and cramping, are the most frequently noted adverse effects of the compound.

Effect of Carnitine Supplementation on Exercise Performance in Normal Humans

Clinical studies of carnitine's effect on exercise performance or metabolism during exercise have usually involved small numbers of patients; lacked comprehensive evaluation of the patients' carnitine,

metabolic and performance status after receiving carnitine; and have used varied populations (i.e., athletes versus nonathletes). Given these considerations, it is difficult to draw generalized, definitive conclusions concerning carnitine supplementation. Several conclusions do seem justified based on the available data in normal humans:

- Acute or chronic carnitine administration can increase plasma carnitine concentrations.
- Acute carnitine administration, or daily administration for a period of 1–2 weeks, does not increase skeletal muscle total carnitine content or the response of the muscle carnitine pool to exercise.
- Administration of carnitine for 4–6 months to athletes engaged in training prevents a training-associated decrease in muscle total carnitine content, and may increase total carnitine content compared with baseline measurements.¹⁰
- Carnitine, whether administered intravenously or orally, acutely or chronically, has no consistent effects on key metabolic parameters during exercise or exercise performance.^{16–18}
- Carnitine administration for months may modify mitochondrial enzyme expression during training of athletes.¹¹
- No well-designed clinical trials are available demonstrating that carnitine enhances exercise performance in normal subjects.

These conclusions do not justify the use of carnitine to improve exercise performance in normal humans. Multiple studies exist, with results of which could be interpreted as at least partially positive, particularly in athletes,^{10–15} but additional, comprehensive clinical trials are needed before general use can be supported. Such trials should include sufficient metabolic studies to provide insights into which of carnitine's multiple potential effects is responsible for any benefits seen.

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Effects of Choline on Athletic Performance and Fatigue

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Certain neurotransmitters, including acetylcholine, catecholamines, and serotonin, are formed from dietary constituents such as choline, tyrosine, and tryptophan. Changing the consumption of choline can alter the synthesis and release of its respective neurotransmitter product acetylcholine.¹ Consumption of supplemental choline can also increase the release of acetylcholine from nerve endings, including those that cause skeletal muscle to contract.²⁻⁵ Choline is also incorporated into cell membranes⁴ that can serve as an alternative choline source for acetylcholine synthesis when there is a deficiency in circulating choline.

It has been shown that increasing the concentration of choline in skeletal⁵ and cardiac⁶ muscle increases acetylcholine release.⁷ My colleagues and I,⁸ like others,^{9,10} demonstrated a significant reduction in plasma choline levels following exercise. The reduction in plasma choline levels associated with strenuous exercise (e.g., long distance running or extended swimming) may reduce acetylcholine content, and thus its release, and could thereby affect endurance and performance. We hypothesized that replacement of choline lost during exercise or prevention of that loss could influence neuronal release of acetylcholine and, subsequently, affect measures of athletic performance and fatigue.

The running and swimming exercise paradigms used in a series of experiments produced similar depletions in plasma choline levels following that particular exercise.^{9,10} Running 20 miles or swimming for 2 hours led to a significant fall in plasma choline levels (40–50 percent). Spector et al.¹¹ failed to show a fall in plasma choline levels after either a brief (approximately 2 minutes duration), but highly intensive and a longer (approximately 73 minutes duration) submaximal exercise on a stationary bicycle. Apparently, the duration and type of exercise are important determinants of whether plasma choline levels will fall postexercise.

Providing 2 g of free choline prior to exercise prevented a fall in choline levels (25–40 percent) and raised choline levels above baseline values for up to 2 hours postexercise. The bitartrate or citrate salt forms of choline were equally effective. Randomized placebo-controlled crossover studies found improvements in running times and a timed, swim test and suggested that performance in these activities is sensitive to changes in choline levels. In one study, long-distance runners improved running times by an average of 5 minutes over a 20-mile course when compared with those taking a placebo. In a second study, a higher percentage of swimmers who took choline prior to their swim experienced an improved performance on a timed swim test than when they consumed a placebo.

Findings of degree of fatigue and vigor levels were consistent across all postexercise paradigms. The level of fatigue was lower and the level of vigor was increased in long-distance running, swimming, and a 2-hour basketball workout. It is interesting to note that in two multiple-dosing studies, when choline was given daily for 5–7 days, pre-exercise fatigue was also reduced. This finding suggests that daily administration of choline during strenuous daily exercise periods may be of benefit prior to beginning another bout of exercise.

The data suggest that choline supplementation prior to strenuous exercise may improve performance in certain athletic paradigms as well as reduce fatigue and increase vigor.

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Selected Herbals and Exercise Performance

Luke R. Bucci, Ph.D.

Despite a long tradition of use by physically active people, the scientific study of effects of herbs on physical performance has been mostly ignored. Difficulties with belief systems, taxonomic classification, identification and consistency of herbal components, dose–response, accessibility of foreign research, and lack of funding have prevented more than cursory research on single herbs or combinations. Precedent for an ergogenic effect of herbs on exercise performance is evidenced by the ban on certain herbal components by sports regulatory agencies, such as excess caffeine or ephedrine and related alkaloids. These substances have been purified and studied outside their herbal context and shown to enhance certain aspects of exercise performance.¹

Herbs that have been studied the most include the various types of ginseng (*Panax spp.* and *Eleutherococcus senticosus*).^{1,2} In general, animal studies frequently showed enhancements of exercise performance, whereas human studies showed mixed results.^{1–4} A dose–response effect is apparent, since studies using the highest doses produced enhancements of physical or mental performance. Mechanisms of action are vague.

Other herbs with a tradition of popular use as a tonic or for enhancement of physical performance include *Cordyceps sinensis* (a fungus cultivated on caterpillars), *Schizandra chinensis* (a Manchurian berry), *Panax pseudoginseng* (similar to Chinese ginseng), *Astragalus membranaceus* root, *Lyceum spp.* fruit, *Rhodiola spp.* (an herb similar to ginseng), *Mummio* (Shilajit) (aged juniper berry exudate from Himalayan rock crevices), *Tribulus spp.*, *Smilax officinalis* (for steroid saponin content), and *Ashwagandha*, an Ayurvedic tonic herb.

An overlooked aspect of herbs and exercise is the extensively documented, systemic antioxidant action of some herbs: (1) green tea [*Camellia sinensis*] (polyphenols, e.g., catechins and gallate esters); (2) milk thistle seed [*Silybum marianum*] (silymarins); (3) *Ginkgo biloba* [ginkgolides]; (4) curcumin from *Curcuma longa* root (turmeric); (5) proanthocyanidins from red wine, grape seeds, bilberries, blueberries, cranberries, and maritime pine tree bark; (6) rosemary, sage and thyme; and (7) bioflavonoids from citrus fruit extracts or buckwheat.

The area of herbs and physical performance remains largely unstudied. Combinations frequently used in traditional settings are almost completely unexplored scientifically. Effects of herbs on mental functions, mood, or behavior are also poorly tested.

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Antioxidants: What are They and What Role Do They Play in Physical Activity and Health?

Priscilla M. Clarkson, Ph.D.

Are antioxidant supplements necessary for those who exercise regularly? Should antioxidant supplements be part of the “nutritional game plan” of athletes? These are common questions directed to fitness leaders, athletic trainers, and other health professionals who are consulted about the role of antioxidants in a healthy, active lifestyle.

The reason for this interest in antioxidants is the finding that certain highly reactive chemical species called free radicals increase during exercise. Free radicals contain one or more unpaired electrons in their outer orbit that allows them to attack cellular components. During oxidative metabolism, most of the consumed oxygen ends up bound to hydrogen, forming water. Because this process is not 100 percent effective, 4–5 percent of the oxygen is not completely reduced and forms free radicals which, in turn, lead to other harmful oxidation products. When free radicals attack cellular membranes, a chain of reactions called lipid peroxidation produces additional damage. Thus, as oxygen consumption is increased during exercise, there will be a concomitant increase in free radicals and lipid peroxidation in skeletal muscle cells.

Exercise can also generate free radicals by other means including (1) increased intake of oxygen which itself is a diradical, (2) increased amounts of epinephrine and other catecholamines that can produce oxygen radicals when they are metabolically inactivated, (3) production of lactic acid that can convert a weakly damaging free radical (superoxide) into a strongly damaging one (hydroxyl), and (4) response to muscle damage as a consequence of overexertion, which can lead to lipid peroxidation of membranes and an increase in macrophages and white blood cells in damaged muscle.

The body contains an elaborate antioxidant defense system that depends on dietary intake of antioxidant vitamins and minerals and the endogenous production of antioxidant compounds such as glutathione. Vitamins C, E, and beta carotene are the primary vitamin antioxidants. In addition to glutathione, there are numerous enzymes involved in the quenching or removal of free radicals.

Whether the body’s natural antioxidant defense system is sufficient to counteract the increase in free radicals with exercise or whether additional supplements are needed is not fully known. Those who engage in chronic physical activity, placing a constant oxidative stress on the muscles and other cells, may require additional antioxidants to help them recover from exercise. However, physical training may enhance the antioxidant system to counteract the barrage of free radicals produced during exercise. Antioxidant supplementation may benefit the “weekend athlete” whose defenses may not be prepared to handle a sudden increase in oxidative stress. Although it has been suggested that antioxidant supplements will enhance physical performance, the data are equivocal.

Exercise and Oxidative Stress: How is it Detected?

Because there is no way to directly detect free radical production in humans, indirect methods have been developed. Basically these methods rely on the breakdown products of lipid peroxidation, such as conjugated diene, malondialdehyde (MDA), and hydrocarbons. Measurement of malondialdehyde and conjugated dienes in the blood or urine and the assessment of hydrocarbon production by measurement of expired pentane provide evidence of lipid peroxidation. Malondialdehyde is most commonly measured

by its reaction with thiobarbituric acid, which generates thiobarbituric acid reactive substances (TBARS). These methods have been criticized for not representing an accurate measure of lipid peroxidation. Because lipid peroxidation can occur in all tissues, blood levels of peroxidation products or expired pentane can provide no information on where lipid peroxidation is occurring. Furthermore, expired pentane can reflect flushing of hydrocarbons from adipose tissue, and many natural compounds other than lipids can produce TBARS.

Measurements of the body's antioxidant defense system, such as glutathione levels and activities of glutathione peroxidase, superoxide dismutases, catalase, and glutathione reductase, have been used to assess changes in antioxidant status. Blood levels of vitamins E (tocopherol), C, and A have also been assessed.

Because no one of these measures provides an accurate assessment of lipid peroxidation or antioxidant status, studies have incorporated several assessments. However, until more valid techniques are developed, our knowledge of free radical generation, oxidative stress, and antioxidant status is limited.

Effects of Exercise, Training, and Antioxidant Supplementation

In general, there is indication that acute bouts of strenuous exercise can increase lipid peroxidation and that regular participation in physical training can enhance antioxidant status. Studies have shown that exercise increases expired pentane and plasma MDA and conjugated dienes levels. Glutathione concentration and glutathione reductase activity are also altered by exercise. Training appears to augment the antioxidant defense system. For example, highly trained runners have elevated levels of erythrocyte vitamin E, glutathione, and catalase activity.

There is limited information on the effects of antioxidant supplementation on exercise oxidative stress. Vitamin E supplementation has been shown to result in a significant reduction in the increase of expired pentane and MDA during exercise. Selenium supplementation has also been found to reduce the MDA response to exercise. A combination of vitamin C and glutathione supplementation reduced resting levels of expired pentane and MDA. Increased ingestion of vitamin C has been associated with a low incidence of respiratory tract infection in ultra marathon runners.

Because oxidation causes damage to muscle fibers, it has been suggested that oxidation could also result in exercise-induced muscle soreness. Several studies have examined whether antioxidant supplements would reduce soreness. Although vitamin E supplements have not been shown to be effective, vitamin C has been found to reduce soreness. These results are interesting, but further studies with a larger sample size are needed to fully document whether antioxidants will reduce muscle soreness or the damage associated with soreness. Furthermore, there is no clear documentation that oxidative stress contributes to soreness.

Vitamin Supplementation and Exercise Performance

Antioxidant supplements have been touted by manufacturers as allowing individuals to recover more quickly and fully from vigorous exercise and/or allowing them to train more strenuously. However, the theoretical basis for why antioxidants should enhance performance is not clear.

Several studies have investigated the effects of vitamin E on exercise performance and have found no beneficial effects on measures of endurance or aerobic capacity. The only study to find that vitamin E supplementation enhanced performance was done at high altitude. In one study where diets

poor in vitamin E were administered to subjects, there was no effect on workload and no reports of muscle weakness.

The results regarding vitamin C supplementation are equivocal, but most well-controlled studies report no beneficial effect on either endurance or strength performance. Likewise, studies of vitamin C restriction showed that a marginal vitamin C deficiency did not affect performance.

Antioxidant Status of Athletes

Studies have assessed vitamin status and dietary intakes of athletes to determine whether chronic participation in exercise results in compromised antioxidant status. Most athletes ingest adequate amounts of antioxidants as compared with the RDA, with the exception of those maintaining low body weights, such as dancers, gymnasts, and wrestlers. The studies that have assessed the vitamin A, C, or E status of athletes by analyzing blood samples have found no evidence of a deficiency. Most athletes had adequate or above adequate blood levels of these vitamins. However, there are no data available for athletes who may be at risk of inadequate antioxidant intake and compromised status because of a restricted caloric intake to maintain low body weights. Those on very low fat diets may have compromised status because fat ingestion is important to vitamin E intake.

On the other hand, it has also been suggested that the “weekend athletes,” those who only exercise strenuously on occasion, should be sure that their antioxidant levels are adequate. The reason for this is that physical training enhances the body’s antioxidant defense system so that athletes who overexert themselves are prepared to deal with tissue oxidative damage. Weekend athletes would not have this augmented defense system and may be more susceptible to tissue damage. Further studies are needed to determine the levels of antioxidants that are necessary for an optimally functioning defense system.

Recommendations for Antioxidant Use by Athletes

Although there are data documenting that antioxidants will reduce lipid peroxidation, it is still uncertain exactly what amounts are needed for a beneficial effect. Popular belief is that high doses of vitamins C, E, and beta carotene are not harmful. However, in recent years concern has arisen over the long-term use of megadoses of selected nutrients. When one considers that the body operates on a finely tuned homeostasis, it would appear that megadoses of any nutrient could upset the delicate balance. Moreover, it may take a long time before the resulting negative effects are evident.

Many sports nutritionists recommend that adequate amounts of antioxidants be obtained from the diet, and that athletes should make intelligent food choices. As an “insurance policy,” athletes could be encouraged to take a multivitamin/mineral supplement containing no more than the recommended dietary allowance (RDA). In contrast, other experts believe that there is sufficient information to suggest that athletes supplement their diets with antioxidants in excess of the RDA. Some have suggested that it borders on malpractice not to recommend antioxidant supplements to athletes.

Although the issue of whether to supplement with antioxidants and how much to supplement remains unresolved, what is clear is the importance of ingesting foods rich in antioxidants for those who exercise regularly as well as those who exercise on occasion.

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Exercise and Oxidative Stress: Effect of Vitamin E and Aging

William J. Evans, Ph.D.

Previous research conducted by my colleagues and I has demonstrated that unaccustomed exercise may result in delayed onset muscle soreness resulting from microscopic tears in the muscle. We first observed this in marathon runners, who have remarkable delayed onset soreness and damage that takes up to 3 months after a marathon to fully repair. The damage results from what is called “eccentric” exercise, also known as negatives by weight lifters, and involves the muscle lengthening while it produces force. Apparently, the downhill portion of the marathon tends to cause the damage. Eccentric exercise is a natural component of all forms of exercise. Performing tasks with a high eccentric component (e.g., downhill skiing or weightlifting) may result in severe muscle damage and soreness the first or second time it is performed.

Because of this damage, my colleagues and I became interested in the ongoing repair process. Our experimental protocol involves having our subjects perform eccentric exercise and then examining the responses. We have seen that exercise results in muscle damage and repair and that muscle continues to show signs of damage for days after the exercise. This increasing amount of damage is likely due to an increase in the production of oxygen free radicals within the muscles.

We therefore examined the effects of vitamin E supplementation of muscle damage and repair in young and older men. Our hypothesis was that 2 months of supplementation (800 IU vitamin E/day) would make the muscle tissue less likely to exhibit damage following eccentric exercise. Although our study showed minimal effects on the responses of the young subjects, vitamin E had a substantial effect on the responses of the older men.

The adaptive response to damaging exercise involves an “acute phase response,” similar to that which people exhibit to infection, stress, or trauma, and involves the immune system and its many effects. It appears that young people have a robust response to the exercise, whereas the elderly do not. Our research has demonstrated that vitamin E supplementation in the older subjects restored their response to that which was seen in the young subjects. This involved the mobilization of immune cells to invade the damaged muscle and ultimately to stimulate repair. In addition, we demonstrated that vitamin E supplementation greatly decreased the production of oxygen free radicals in both groups of subjects. Taken together, these studies reveal that vitamin E, and perhaps other antioxidant vitamins (C and beta carotene), will help to accelerate the adaptive response to exercise in the elderly and that in both young and older subjects, antioxidant supplementation may help to reduce the continued damaging effects of oxygen radicals.

Although there is compelling evidence that vitamin E enhances the adaptive response to exercise, there is little evidence that vitamin E serves as an ergogenic aid during exercise. Vitamin E supplementation has not been demonstrated to enhance endurance, anaerobic, or strength exercise performance, nor is there any scientific rationale to believe that it has any ergogenic effects.

Selenium and Other Antioxidant Issues

Carl L. Keen, Ph.D.

Recently an increasing amount of attention has been given to the concept that oxidative and reactive oxygen-initiated processes may play key roles in the initiation and/or progression of several chronic health disorders, including atherosclerosis, certain cancers, cataractogenesis, and rheumatoid arthritis. Indirect support for this concept is robust and evidenced by an extensive epidemiological literature demonstrating inverse correlations of varying magnitudes between the consumption of foods rich in antioxidant composition and certain chronic diseases.

Numerous conditions are thought to induce increased levels of oxidative stress in humans, including excessive exposure to sunlight, heavy metals, cigarette smoke, alcohol, and air pollution. Assays measuring putative biomarkers of oxidative damage in cell cultures, experimental animals, and humans support the concept that these conditions can result in increased oxidative stress. Even lifestyle habits that are viewed as “positive,” such as exercise, have been shown to be associated with an increased risk for oxidative stress and tissue damage. For many of the disease states described above, there is evidence that antioxidant supplements may provide some protection with respect to disease progression. However, to a significant extent, the antioxidant supplements in these cases may often be viewed as a means of correcting disease-induced deficiencies of select nutrients. Less clear is whether the provision of antioxidant supplements will also provide protection to healthy well-nourished subjects engaged in activities such as modest exercise. In this talk, the effect of select antioxidant supplements on exercise-induced tissue oxidative damage is reviewed. Evaluation of the putative value of selenium and other essential mineral supplements is also emphasized.

During the last decade there has been increasing interest in the idea that individuals engaged in strenuous exercise may have an increased need for several essential minerals. This concept has resulted in the widespread perception that mineral supplements may be advantageous to this population group. The concept is based on two perceptions: (1) Individuals engaged in strenuous exercise have a higher requirement for some minerals compared with sedentary individuals because of increased rates of urinary and sweat losses of select minerals and (2) the perceived inadequate intake of these minerals results in a reduced performance of the individual and/or an impairment/delay in his/her ability to recover from injury.

With respect to selenium, a limited literature suggests that chronic exercise can influence selenium metabolism. In an early study, Consolazio et al. reported that sweat losses of selenium could exceed 300 μ g/day in individuals who exercised rigorously.¹ However, given the fact that typical dietary selenium intake is often less than 100 μ g/day, this value for sweat loss seems excessive. Singh reported that plasma selenium concentration decreased in men who were engaged in a 5-day rigorous training program, despite “adequate” dietary selenium intake.² Singh and her colleagues suggested that this decrease in plasma selenium is in part due to a shift in selenium from the plasma pool to tissues which required it for antioxidant protection—a suggestion that would be consistent with the increased oxidative stress that can be associated with exercise.³⁻⁶ Consistent with their suggestion, several investigators have reported a rise in muscle and/or blood glutathione peroxidase (GSHPx) activity following exercise training.^{7,8}

Based on the above idea, a number of investigators have tested the hypothesis that selenium supplements may be beneficial in the attenuation of exercise-induced oxidative stress. Olinescu et al.⁹ and Tessier et al.¹⁰⁻¹¹ have reported that selenium supplementation can be associated with a reduction in

oxidative stress in athletes. Both groups suggested that the reduction in oxidative stress was secondary to a supplement-induced increase in blood and muscle GSHPx activity. Although the above studies are intriguing, it is important to note that other investigators have not been able to document an increased need for selenium in athletes.¹²

It is significant to note that the increase in GSHPx activity noted following selenium supplementation was not observed by Rokitzki et al.,¹³ when they provided vitamin E and C supplementation to trained athletes. Thus, the rise in GSHPx activity reported in the selenium supplementation studies may not occur if antioxidant supplements lacking selenium are provided.

Similar to the case for selenium, several investigators have suggested that supplementation of zinc, copper, and/or magnesium may be of benefit to the athlete with respect to the attenuation of exercise-induced tissue oxidative damage.¹⁴ These theories will be briefly reviewed.

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Physical Exercise and Thiol Homeostasis: Possible Implications

Chandan K. Sen, Ph.D.

Oxygen Toxicity in Exercise

Oxygen, as a metabolic fuel, allows an attractive yield of energy-rich phosphates per unit substrate, and oxidative metabolism avoids the formation of lactic acid—a significant factor in the development of muscle fatigue during exercise. Recent advances in free radical biochemistry have made it clear that not all of the oxygen consumed by live cells is completely (tetravalently) reduced. An estimated 2–8 percent of the total oxygen consumed may escape from the metabolic path after being partly reduced. Such incompletely reduced forms of oxygen and their byproducts, collectively referred to as reactive oxygen species, are implicated in a wide variety of reactions that may adversely affect our state of health and longevity. During physical exercise O_2 flux through the body is remarkably enhanced. The rate of oxygen uptake by the body increases by tenfold to fifteenfold and is accompanied by more than a hundredfold increase in O_2 flux in the active skeletal muscles.

A considerable body of experimental evidence has accumulated in recent years suggesting that physical exercise, under certain circumstances, induces oxidative stress.^{1,2} This is an area of critical concern because exercising is not only a recreational activity but also has well-established therapeutic value. For patients suffering from disorders that are known to have an oxidative stress-related etiology, exercise-induced oxidative stress should be treated with particular concern. For example, oxidative modification of low density lipoprotein is known to play a major role in the pathogenesis of atherosclerosis. Recently, Shern et al.³ reported that the susceptibility of low density lipoprotein to oxidation was higher in exercising humans ($n = 22$ per group) than in their relatively sedentary counterparts. This suggests that we should consider the exercise-induced oxidative stress factor when designing exercise regimes for such patient groups.

Physical training enhances tissue antioxidant defenses, but such added protection may not be sufficient to defend against oxidative stress during long, strenuous exercise. Habitual physical exercise is a precious therapeutic tool in preventive medicine. A thorough understanding of the complications associated with exercise-induced oxidative stress is necessary for effective handling of this undesired effect. Such knowledge will help in designing exercise and nutrition protocols that will better serve our interests.

Defense Against Oxidative Stress

In biological systems, an imbalance in the pro- and anti-oxidant forces in favor of the former is referred to as oxidative stress. Processing of the one-electron reduction product of molecular oxygen, superoxides, to hydrogen peroxide is catalyzed by the enzyme family superoxide dismutases. Decomposition of tissue hydrogen peroxide can be catalyzed by the enzymes catalase or glutathione peroxidase. Glutathione peroxidase activity requires glutathione as a substrate and selenium as a cofactor. Vitamin E serves as a major lipid phase antioxidant that protects against oxidative lipid damage. Vitamin C, in the absence of free transition metal ions, may serve as an effective antioxidant.

Oxidant-antioxidant interaction involves electron transfer from the antioxidant to the electron-seeking oxygen-free radical. As a result, during such radical neutralization interaction, antioxidants are transformed to an oxidized state that is no longer able to detoxify reactive oxygen. In this situation, it

becomes necessary to recycle this oxidized antioxidant to its potent reduced form. In tissues, several antioxidants may act in concert to let such recycling happen. Thus, the strength of antioxidant defense is not dependent on a single antioxidant but on the efficacy with which several antioxidants cooperate to let the antioxidant chain reaction function.^{2,4}

Thiols Play a Central Role in Antioxidant Recycling

Functional sulfhydryl (SH) residue-rich protein and nonprotein agents in the biological system, commonly referred to as biothiols, have multifaceted functions including protein synthesis, detoxification, cell division, and regulation of intracellular signal transduction. In addition to its other major functions, the abundant nonprotein thiol glutathione has proved to be a master physiological antioxidant. Glutathione itself, and other pro-glutathione agents such as α -lipoic acid,⁵ play a crucial role in the antioxidant chain reaction by helping to maintain favorable redox states of other major antioxidants such as vitamins C and E.²

Physical Activity Influences Tissue Glutathione Level

Studies from several laboratories have suggested that physical training may upregulate the activities of glutathione-dependent antioxidant enzymes in some tissues. Higher glutathione content in skeletal muscle of exercise-trained animals also have been reported from our laboratory and others. Somani has reported that physical training may improve the redox state of glutathione in different regions of the brain.¹ In our experiments with beagle dogs that were endurance-trained on a treadmill for 55 weeks or activity-restricted by fiber-cast immobilization of one of the pelvic limbs, we observed that the state of physical activity is an important determinant of skeletal muscle glutathione content. Endurance training increased and activity restriction decreased red gastrocnemius muscle glutathione content.

N-Acetylcysteine Spared Blood Glutathione Oxidation in Humans

Physical exercise is followed by rapid oxidation of blood glutathione, mainly in the erythrocytes. Exercise-induced tissue glutathione oxidation has been consistently observed in our laboratory as well as in several others. In a recent study of young healthy men, we observed that blood glutathione oxidation induced by maximal bicycle ergometric test (mean duration = 14 minutes) could be spared in subjects who took 200 mg of N-acetylcysteine four times daily for 2 days and an additional 800 mg 2 hours before the exercise test. N-acetylcysteine is a common mucolytic drug that has been safely used in the clinic over a long period of time. A few months after our report was published, Reid et al.⁶ reported that N-acetylcysteine inhibits muscle fatigue in humans. In a later rat study, we observed that N-acetylcysteine supplementation may also spare exercise-induced glutathione oxidation in the lung—an organ in which this drug is known to be bioavailable.

Enhancing Tissue Glutathione by use of Nutritional Supplements

When ingested, antioxidants such as vitamins C and E are bioavailable to a certain extent. However, enhancing tissue glutathione level by nutritional supplementation is a challenging task. A few reports have claimed that an oral or intraperitoneal supply of glutathione may remarkably enhance endurance during exercise. These studies were brief and did not follow any biochemical parameter to

explain the results. We conducted a more thorough study to evaluate the significance of endogenous and exogenously supplied glutathione with respect to exercise-induced oxidative stress. Glutathione-deficient rats in which tissue glutathione synthesis was arrested had a remarkably lower endurance (treadmill run) before exhaustion. This suggested that endogenous glutathione is an important factor in exercise performance. This is understandable because glutathione not only functions as an antioxidant but also has several other critical functions, including delivery of cysteine for protein synthesis. When a lower exercise intensity was selected for mice, Ji and associates did not observe any effect of glutathione deficiency on endurance. In our study, exogenously supplied (intraperitoneal) glutathione was poorly available to most tissues and did not influence endurance.

Availability of cysteine, in its reduced form, in the cell is a limiting step in intracellular glutathione synthesis. Several agents have been tested for their efficacy in this respect. Among the ones that hold clinical promise are N-acetylcysteine and alpha-lipoate.⁵ Both agents have proved to be safe for human use. Alpha-lipoate has been recently introduced in the United States as a dietary supplement. It has been used in Germany for a long time for the treatment of diabetic polyneuropathies. Recently the laboratory of Klip has reported that alpha-lipoate also stimulates glucose uptake by cultured skeletal muscle cells.

Thiols As Critical Determinants of Cell Function and Response to Stress

To develop a better understanding of the exact mechanisms that underlie reactive oxygen species-dependent disorders in biological systems, recent studies have focused on the regulation of gene expression by oxidants, antioxidants, and other determinants of the intracellular reduction-oxidation (redox) state.⁷⁻⁹ At least two well-defined transcription factors, nuclear factor (NF)- κ B and activator protein (AP)-1 are regulated by the intracellular redox state. One major clinical significance of NF- κ B activation is that it enhances HIV gene expression. The long terminal repeat of HIV-1 has been shown to contain two NF- κ B binding sites that may be crucial in regulating AIDS latency. AP-1 is an important mediator of tumor promotion, and is thus a focal point in cancer research.

Certain intracellular protein and nonprotein thiols are known to act as “redox sensors” that signal for much of the activity of the aforementioned transcription factors.⁷ Under conditions of oxidative stress, certain thiols such as glutathione and thioredoxin are transformed from a reduced sulfhydryl (-SH) state to an oxidized disulfide (-S-S-) state. This change serves as a signal for redox-sensitive transactivation to start. In the nucleus, these transcription factors are known to require a reducing atmosphere to be able to bind with the consensus DNA sites and initiate transactivation. Again, certain protein thiols in the nucleus regulate this DNA binding. In brief, subtle changes in intracellular thiol-disulfide status have an important bearing on the molecular events associated with cellular response to the stress. For example, it is suggested that elevated GSSG/GSH in the cytosol may be implicated in NF- κ B activation.^{7,10} A number of studies have shown that physical exercise may increase the tissue GSSG/GSH ratio, but does this lead to NF- κ B activation? We are currently conducting a pilot human study to address this issue. If indeed physical exercise induces NF- κ B activation, does this mean that the rate of progression of AIDS may be accelerated in strenuously exercising HIV-positive individuals? This is one of many exercise-induced oxidative stress-related issues that deserve careful attention. Activation of NF- κ B may also upregulate the expression of adhesion and other molecules that are known to be implicated in the etiology of atherosclerotic and diabetic complications.

So, is exercising bad? Certainly not. A physically active lifestyle coupled with well-balanced nutrition is of great help. However, antioxidant defenses of active tissues can be overwhelmed by excess reactive oxygen generated during exercise. A vivid understanding of the possible mechanisms that may contribute to exercise-induced oxygen toxicity, associated physiological response, and the design of appropriate measures to circumvent or minimize such toxicity is fundamental to (1) enhancing the effectiveness of physical exercise as a preventive and therapeutic tool in clinical practice and (2) controlling exercise-induced oxygen toxicity-dependent tissue damage and augmentation of other possible health risks.

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Antioxidant Methodology: A Critique

Robert R. Jenkins, Ph.D.

Background

It has been estimated that some 3×10^9 years ago, blue-green algae appeared and there went the environment, so to speak. Up to that time it is likely that oxygen existed only in oxides. We now live in an atmosphere consisting of a 21 percent oxygen radical or diradical, to be correct. Since the time of Lavoisier, Priestly, and Scheele, the pioneers in oxygen research, a vast literature related to oxygen has emerged. For instance, there have been nearly 131,000 papers on oxygen published since 1968. That was the year that McCord and Fridovich¹ demonstrated that certain enzymes liberated the superoxide radical. Within the next decade, McCord² demonstrated that radicals induce damage and scavenging enzymes protect against such damage; Chance, Sies and Boveris³ published a seminal review on hydroperoxide metabolism; and Vladimirov et al.⁴ summarized their work on radical-induced membrane peroxidation. Such breakthroughs attracted new workers from a wide array of disciplines to the emerging topic of oxidative stress and antioxidant protection. The enormous amount of subsequent research caused Forster and Estabrook⁵ to suggest that it is proper to conceive of oxygen as an essential nutrient that presents us with problems of malnutrition and overnutrition.

Analysis Methods

The principal difficulty in trying to study free radical events in biological systems rests in the fleetingly short life times of radicals and reactive species. Chance et al.³ quote Warburg as saying, "Wieland has processed whole dogs and not found one drop of H_2O_2 !" The problem was not the inability to detect H_2O_2 but the failure to realize how rapidly it was degraded in biological systems. It is essential to keep that point in mind when designing research related to oxidative stress.

Radicals themselves exist for only a brief time, and their observation and characterization are limited to various techniques involving a special spectrometer capable of determining electron spin resonance (ESR), also known as electron paramagnetic resonance. The application of ESR to biological samples is fraught with complications. For instance, aqueous solutions have a high dielectric absorption of microwave energy. Investigators have resorted to low-temperature techniques or the use of compounds known as spin traps in an attempt to overcome this problem. Furthermore, tissue preparation itself can generate radicals. These problems coupled with the method's inapplicability to in vivo systems has resulted in its infrequent use in exercise-related radical studies.

Lacking a suitable method for direct observation of radicals, most investigators have resorted to various "fingerprint" approaches to establishing that oxidative stress reactions have occurred. One of the most frequently employed techniques involves reacting samples with thiobarbituric acid (TBA) in fluorometric or spectrophotometric assays to establish what are often called TBARS for thiobarbituric reaction products. Some investigators persist in referring to this reaction as a measure of malondialdehyde (MDA), a reaction product of lipid peroxidation. However, it is well known that TBA will react with molecules other than MDA. MDA can be assayed directly by high-pressure liquid chromatography

(HPLC)⁶ or by gas chromatography.⁷ Often, investigators have failed to detect a rise in plasma thiobarbituric acid reactive substances (TBARS) subsequent to exercise. Such an observation does not necessarily indicate that oxidative stress has not occurred. We have shown that TBARS is rapidly cleared from electrically stimulated muscle and that rats fatigued by running showed a significant increase in urinary TBARS.⁸

The use of a hydrocarbon breath test has been underutilized in exercise research. The peroxidation of n-3 and n-6 fatty acid families results in the production of the volatile alkanes ethane and pentane. The compounding problems with this approach include the fact that the atmosphere breathed may be contaminated by hydrocarbons and our gastrointestinal tract bacteria also make these compounds. Snider et al.⁹ have found that the contamination problems can be overcome by including a wash out period, during which the subjects breathed hydrocarbon-free air prior to the experimental period.

During radical reactions, there is an electron jump from ground state to an excited state. When the electron falls back to ground state, a low level of light energy is emitted; this is called chemiluminescence. We employ a single photon counting system similar to that described by Boveris et al.¹⁰ to determine a tissue's ability to defend itself against oxidative stress. The tissue can be challenged by various organic or inorganic hydroperoxides. When a variety of antioxidants are systematically added to the incubation mixture, the procedure may yield evidence to implicate the radical species that may be involved.

Recently we have begun to couple assays of mitochondrial oxygen consumption and the chemiluminescence analysis, with the intent of developing a model system for the study of oxidative stress. Robinson and co-workers¹¹ have demonstrated that an increased electron flux capacity is necessary to achieve the increased peak oxygen consumption derived by training. Furthermore, Kehrer and Lund¹² have provided an excellent review on the relationship between cellular reducing equivalents and oxidative stress. We employ myxothiazol, a tight inhibitor of complex III, in mitochondrial suspensions. This results in an inhibition of state III respiration and a subsequent increase in chemiluminescence. Although still in the preliminary stages of development, this approach offers some promise, since reducing equivalents and antioxidants can easily be added to the system.

Obviously, *in vivo* studies involving antioxidant supplementation should be accompanied by marker assays to demonstrate that the dosage regimen has altered the antioxidant concentration. The *Methods in Enzymology* series¹³⁻¹⁶ and Evans et al.¹⁷ text provide a convenient source of reliable assays. Since antioxidants often are involved in an interrelated chemistry, it is useful to attempt to understand how the protocol of interest has influenced the total milieu. Cao and co-workers have developed a technique suitable for that purpose.¹⁸

Needed Research

To date, the preponderance of studies related to exercise and oxidative stress have centered on determining evidence of stress and the influence of one antioxidant at a time. Literature related to a multiple supplement approach is sparse. While youth sport leagues in the United States expose children to high aerobic doses, there are virtually no oxidative stress-related data available on children younger than age 18. Furthermore, just as some members of the general population are apt to receive orthopedic

trauma from certain types of intense aerobic activity, we might assume that some genetic sets are unable to cope with oxidative stress. Oxidative stress research is needed to detect aberrant responses to exercise rather than to focus on group means. Finally, we are beginning to understand that radicals play many vital roles in our biochemistry. We must question whether massive doses of antioxidants are violating that beneficial chemistry.

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