Nutrition Reviews®

Volume 73, Number 8 (Suppl. 1), August 2015

YOGURT IN NUTRITION
INITIATIVE FOR A BALANCED DIET

This supplement to *Nutrition Reviews* was sponsored by Danone Institute International, Palaiseau, France. The content was peer reviewed prior to publication.

On the cover: Yogurt has been a part of the human diet for thousands of years and it remains a staple food in many countries worldwide. In order to review and evaluate the strength of current scientific knowledge regarding the health benefits of yogurt, The Second Global Summit on the Health Effects of Yogurt was organized by the Yogurt in Nutrition Initiative for a balanced diet (YINI), which aims to advance scientific knowledge on the health benefits of yogurt and broadly disseminate the findings. This initiative is a collaborative effort between the Danone Institute International, the American Society for Nutrition, and the International Osteoporosis Foundation. The summit was held April 30, 2014 at the Experimental Biology conference as a satellite scientific session of the American Society for Nutrition. Image is "The month of June: milking and cheese making" by Anonymous (15th century). Photo courtesy of Scala / Art Resource, New York.

Foreword

Introduction to the Second Global Summit on the Health Effects of Yogurt

Raanan Shamir and Sharon M. Donovan

The purpose of the Second Global Summit on the Health Effects of Yogurt was to review and evaluate the strength of current scientific knowledge regarding the health benefits of yogurt. To begin, the historical and current patterns of yogurt consumption were reviewed. Then, the evidence base for the benefits of yogurt for maintaining health throughout the life cycle, including optimal body composition, and for reducing the incidence of chronic diseases such as obesity, type 2 diabetes mellitus, and cardiovascular disease was presented. Speakers also discussed the emerging evidence for a link between gut microbiota and health, with a focus on the gut–brain axis and early programming. To conclude, the role of dairy products in a sustainable diet was presented, taking into account both nutritional and environmental factors.

On 30 April 2014, the Second Global Summit on the Health Effects of Yogurt was held as a satellite symposium to the 2014 Experimental Biology meeting. The symposium followed the successful First Global Summit that was held in Boston, Mass., in April 2013, organized by the Yogurt in Nutrition Initiative, which was established in 2012. As stated in the proceedings of that meeting, "the overall mission of the Yogurt in Nutrition Initiative is to advance scientific knowledge on the health benefits of yogurt and to broadly disseminate that information."[1] Indeed, the first and second global summits were constructed to identify and review the existing science on the health benefits of yogurt and to disseminate this knowledge.

At the 2014 summit, Dr. Fisberg[2] reviewed the history of yogurt and reminded the audience that yogurt has been a part of the human diet for thousands of years and was consumed by a diverse group of nations and ethnic groups. Yogurt consumption appeared in Turkish literature in the 11th century. In fact, Genghis Khan, the founder of the Mongol Empire, fed his army yogurt, a staple of the Mongolian diet, based on the belief that it instilled bravery in his warriors. Although yogurt has been a part of the diet of many cultures around the globe, it was not until the early 20th century that the bacteria used for milk fermentation were characterized. This led to the large-scale commercial production of yogurt and its increased availability and popularity. In recent years, the research base that supports the health benefits of yogurt has been building and includes clinical and epidemiological evidence, as well as mechanistic underpinnings.

Dr. Moreno took the audience back to childhood, where the origin of many noncommunicable diseases can be found.[3] A reminder was offered that obesity in children, as in adults, can result in hypertension, dyslipidemia, chronic inflammation, and hyperinsulinemia and that type 2 diabetes prevalence is rapidly growing in the pediatric population. The question then arose: Can dairy product consumption reduce this risk? After the available evidence in the literature was reviewed, it was concluded that "despite concerns that energy provided by dairy products may contribute to childhood obesity, the evidence overwhelmingly supports a null or inverse association between milk or dairy product intake and indicators of adiposity."[3] The results of the Healthy Lifestyle in Europe by Nutrition in Adolescence study, were also reported; this was a study that investigated the relationship between dairy consumption and cardiovascular disease risk factors in adolescents (age range, 12.5–17.5 years) in Europe. This study showed that, overall, dairy intake was the factor

Affiliation: *R. Shamir* is with the Institute for Gastroenterology, Nutrition, and Liver Diseases, Schneider Children's Medical Center, Clalit Health Services, Sackler Faculty of Medicine, Tel-Aviv University, Petach-Tikva, Israel. *S.M. Donovan* is with the Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign, Urbana-Champaign, Illinois, USA.

Correspondence: *R. Shamir*, Institute of Gastroenterology, Nutrition, and Liver Disease, Schneider Children's Medical Center of Israel, 14 Kaplan St., Petach-Tikva, 49202, Israel. E-mail: raanans@clalit.org.il. Phone: +972-3-9253673.

Key words: dairy, health, obesity.

doi: 10.1093/nutrit/nuv013

that best identified adolescents at low risk of cardiovascular disease. Higher consumption of milk and yogurt, as well as of milk- and yogurt-based beverages, was associated with lower body fat and higher cardiorespiratory fitness.

The results of the Healthy Lifestyle in Europe by Nutrition in Adolescence study were reinforced by the results of the European Prospective Investigation into Cancer (EPIC) study, as discussed by Dr. Forouhi.[4] The EPIC InterAct study showed that certain dairy products, particularly fermented dairy products including yogurt, may be relevant for the prevention of type 2 diabetes. Specifically, there was no significant association with total dairy product intake, or milk intake, but a higher combined intake of fermented dairy products (cheese, yogurt, and thick fermented milk) was inversely associated with diabetes. The EPIC InterAct study was followed by the EPIC–Norfolk study that assessed dietary dairy product intake using a real-time, 7-day food diary.[5] In that prospective study, "higher consumption of low-fat fermented dairy products was associated with a lower risk of new-onset diabetes over 11 years, compared with non-consumption."[5] The effect was mainly due to low-fat fermented dairy products, primarily yogurt. Forouhi concluded from the findings of the EPIC study (EPIC–InterAct and EPIC–Norfolk) that a focus on nutrients such as saturated fats may be wrong and that the focus should be on food items rather than specific components of these food items. The best example is meat and dairy products, as both groups are rich in total fat and saturated fat but have opposite associations with type 2 diabetes. Furthermore, the findings of the EPIC study suggest it is better to consider food-group subtypes (e.g., fermented dairy products), rather than overall food-group categories (e.g., dairy products), for their role in the prevention of chronic diseases.

Dr. Tremblay reviewed the impact of yogurt on appetite control, energy balance, and body composition.[6] His presentation highlighted the available literature that demonstrates the positive effect of yogurt consumption on weight control and weight reduction. Although this phenomenon can be explained by the substitution of yogurt for high-energy, "less healthy" foods, other explanations exist, including the demonstration that yogurt consumption is associated with effects on hunger, desire to eat, and enhanced feelings of fullness. In their literature review of the topic, Tremblay et al.[6] discuss the possibility that the high calcium and high protein contents of yogurt are responsible for yogurt's effect on weight reduction, as well as the demonstrated positive effect of milk and yogurt on levels of the appetite-reducing hormones GLP 1 and PYY in blood. The authors discuss the possibility that the matrix of yogurt or its viscosity may influence satiety, as well as the possible effects of the influence of yogurt on gut microbiota as a mediator of changes in lean and fat body mass.[6]

Dr. Bienenstock discussed the role of the intestinal microbiota on health.[7] Not only does the intestinal microbiota outnumber the amount of cells in the human body, it also affects organs remote from the intestine. In addition, there is a growing body of evidence from animal studies that supports the effect of the intestinal microbiota on the central nervous system, including effects on emotional behavior. Thus, changes in diet modulate the gut microbiota and, thus, induce changes in behavior. These effects could be mediated by changes in neurotransmitters, such as gamma amino butyric acid and in short-chain fatty acids via regulation of the immune response and induction of changes in central nervous system function. The findings from animal models are supported by evidence in humans that show possible associations between intestinal dysbiosis and psychiatric disorders, including the effect of supplementation with probiotic bacteria on anxiety. The effect of consumption of fermented milk products on activity in the brain regions that control central processing of emotion and sensation have been documented by using functional magnetic resonance imaging both before and after consumption.[8]

The importance of the gut microbiota was revisited by Dr. Goulet,[9] who reviewed the evidence that microorganisms are present in the human intestine immediately after birth and that the composition and diversity of the intestinal microbiota are influenced by infant diet. Early differences in the microbial taxa may have long-term effects on human health. Some evidence supports the concept that "metabolic programming" of obesity, allergies, and autoimmune disorders during the fetal, perinatal, and postnatal origins may well be explained by "microbial programming." Thus, it is attractive to hypothesize that active modulation of the intestinal microbiota using certain strains or modifiers of intestinal microbiota such as probiotics or yogurt may prevent or treat various diseases including irritable bowel syndrome, acute gastroenteritis, and necrotizing enterocolitis, as well as obesity, allergy, and autoimmune disorders.

Dr. Wolfe brought the discussion back to the importance of proteins in the context of the natural process of loss of lean body mass that occurs with aging (sarcopenia) and the central role of lean body mass loss in the development of many adverse health issues in the elderly.[10] Increased dietary protein intake can explain increased muscle strength and physical function, improved cardiovascular and bone health, and better weight management, which, in turn, affect long-term health outcomes. The current recommended dietary

allowance of 0.8 g protein/kg/day, as well as the average intake in the United States, which is currently about 1.2 g protein/kg/day, are below the amount recommended by expert committees of the National Academy of Sciences and the US Department of Agriculture, i.e., 46 g/day for women and 56 g/day for men. However, setting quantities of protein intake alone disregards the importance of the protein's quality. Thus, ranking proteins by their quality becomes an important issue in dietary requirements. This can be done using the "protein digestibility corrected amino acid score," which is a score that is based on the amino acid profile and the relative amounts of essential amino acids in the protein, or the more recent "digestible indispensable amino acid score," which replaces the protein digestibility corrected amino acid score and is based on the relative digestible content of the essential amino acids and the amino acid requirement pattern. Overall, protein intakes that are higher than the recommended dietary allowance promote better health outcomes in the elderly by positively affecting a wide range of body systems. Use of high-quality proteins such as milk proteins enables the elderly to achieve essential amino acid requirements with lower caloric intake, as reflected by the high-quality score of milk proteins.[10]

The final presentation was dedicated to yogurt and sustainability. Dr. van Hooijdonk[11] discussed the growing demand for dairy products, especially in emerging markets, and the major impact of dairy product consumption on the daily intake of nutrients. While milk production and processing both contribute to greenhouse gas emissions, the authors discussed the need to evaluate foods, dairy products included, from both nutritional and environmental perspectives. Such evaluations should be coupled with a shift from comparing food products in isolation to evaluating complete diets.

In summary, the presentations at the Second Global Summit on the Health Effects of Yogurt demonstrated that ongoing research continues to broaden understanding of the effects of yogurt on health and should provide stimulus for further research in this field.

Acknowledgments

The Second Global Summit on the Health Effects of Yogurt was organized by the American Society for Nutrition and Danone Institute International. The supplement coordinators are Sharon M. Donovan, University of Illinois at Urbana-Champaign, USA and Raanan Shamir, Schneider Children's Medical Center, Israel.

Funding. Writing and editorial assistance were provided by Densie Webb, PhD, RD, who was contracted and funded by Danone Institute International. R.S. and S.M.D. each received financial reimbursement for travel expenses and an honorarium from the Danone Institute International for their participation in the conference.

Declaration of interest. R.S. is president of Danone Institute International and R.S. and S.M.D. co-chair the Yogurt in Nutrition Initiative for Health Advisory Board for Danone Institute International.

REFERENCES

1. Donovan SM, Shamir R. Introduction to the Yogurt in Nutrition Initiative and the first Global Summit on the Health Effects of Yogurt. Am J Clin Nutr. 2014;99: 1209S–1211S.
2. Fisberg M, Machado R. History of yogurt and current patterns of consumption. Nutr Rev. 2015;73(Suppl):4–7.
3. Moreno LA, Bel-Serrat S, Santaliestra-Pasías A, Bueno G. Dairy products, yogurt consumption, and cardiometabolic risk in children and adolescents. Nutr Rev. 2015;73(Suppl):8–14.
4. Forouhi NG. Association between consumption of dairy products and incident type 2 diabetes—insights from the European Prospective Investigation into Cancer study. Nutr Rev. 2015;73(Suppl):15–22.
5. O'Connor LM, Lentjes MA, Luben RN, Khaw KT, Wareham NJ, Forouhi NG. Dietary dairy product intake and incident type 2 diabetes: a prospective study using dietary data from a 7-day food diary. Diabetologia. 2014;57(Suppl):909–917.
6. Tremblay A, Doyon C, Sanchez M. Impact of yogurt on appetite control, energy balance, and body composition. Nutr Rev. 2015;73:23–27.
7. Bienenstock J, Kunze W, Forsythe P. Microbiota and the gut–brain axis. Nutr Rev. 2015;73(Suppl):28–31.
8. Tillisch K, Labus J, Kilpatrick L, et al. Consumption of fermented milk product with probiotic modulates brain activity. Gastroenterology. 2013;144: 1394–1401.
9. Goulet O. Potential role of the intestinal microbiota in programming health and disease. Nutr Rev. 2015;73(Suppl):32–40.
10. Wolfe RR. Update on protein intake: importance of milk proteins for health status of the elderly. Nutr Rev. 2015;73(Suppl):41–47.
11. van Hooijdonk T, Hettinga K. Dairy in a sustainable diet: a question of balance. Nutr Rev. 2015;73(Suppl):48–54.

Supplement Article

History of yogurt and current patterns of consumption

Mauro Fisberg and Rachel Machado

*Yogurt has been a part of the human diet for several millennia and goes by many names throughout the world. The word "yogurt" is believed to have come from the Turkish word "yoğurmak," which means to thicken, coagulate, or curdle. While references to the health-promoting properties of yogurt date back to 6000 BC in Indian Ayurvedic scripts, it was not until the 20th century that Stamen Grigorov, a Bulgarian medical student, attributed the benefits to lactic acid bacteria. Today, most yogurt is fermented milk that is acidified with viable and well-defined bacteria (*Lactobacillus bulgaricus *and* Streptococcus thermophiles*). While patterns of yogurt consumption vary greatly from country to country, consumption is generally low. In the United States and Brazil, for example, only 6% of the population consume yogurt on a daily basis. Low consumption of yogurt represents a missed opportunity to contribute to a healthy lifestyle, as yogurt provides a good to excellent source of highly bioavailable protein and an excellent source of calcium as well as a source of probiotics that may provide a range of health benefits.*

HISTORY OF YOGURT

Yogurt (also spelled "yoghurt" or "yoghourt") is considered by most regulatory agencies worldwide to be a fermented milk product that provides digested lactose and specifically defined, viable bacterial strains, typically *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. It is a source of several essential nutrients, including protein, calcium, potassium, phosphorus, and vitamins B_2 and B_{12}, and serves as a vehicle for fortification.[1]

Yogurt is an ancient food that has gone by many names over the millennia: katyk (Armenia), dahi (India), zabadi (Egypt), mast (Iran), leben raib (Saudi Arabia), laban (Iraq and Lebanon), roba (Sudan), iogurte (Brazil), cuajada (Spain), coalhada (Portugal), dovga (Azerbaijan), and matsoni (Georgia, Russia, and Japan). It is believed that milk products were incorporated into the human diet around 10 000–5000 BC, with the domestication of milk-producing animals (cows, sheep, and goats, as well as yaks, horses, buffalo, and camels).[2] However, milk spoiled easily, making it difficult to use. At that time, herdsmen in the Middle East carried milk in bags made of intestinal gut. It was discovered that contact with intestinal juices caused the milk to curdle and sour, preserving it and allowing for conservation of a dairy product for extended periods of time.[3]

Indian Ayurvedic scripts, dating from about 6000 BC, refer to the health benefits of consuming fermented milk products.[4] Today, there are more than 700 yogurt and cheese products found in Indian cuisine. For millennia, making yogurt was the only known safe method for preserving milk, other than drying it. Yogurt was well known in the Greek and Roman empires, and the Greeks were the first to mention it in written references in 100 BC, noting the use of yogurt by barbarous nations. In the Bible (Book of Job), Abraham owed his longevity and fecundity to yogurt consumption, and there is reference to the "Land of Milk and Honey," which many historians have interpreted to be a reference to yogurt.[5]

Affiliation: *M. Fisberg* is with the Pediatrics Department, Escola Paulista de Medicina Federal University of São Paulo, São Paulo SP, Brazil. *R. Machado* is with the Feeding Difficulties Center, Pensi Institute, Sabara Childreńs Hospital, Brazil.

Correspondence: *M. Fisberg*, Feeding Difficulties Center, Pensi Institute, Sabara Children' Hospital, Rua Borges Lagoa 1080 cj 603, 04038-002, São Paulo SP, Brazil. E-mail: mauro.fisberg@gmail.com. Phone: +55-11-557-53875

Key words: consumption, dairy, fermented milk, yogurt.

doi: 10.1093/nutrit/nuv020

It is believed that the word "yogurt" comes from the Turkish word "yoğurmak," which means to thicken, coagulate, or curdle.[3] The use of yogurt by medieval Turks was recorded in the books *Diwan Lughat al-Turk* by Mahmud Kashgari[6] and *Kutadgu Bilig* by K. H. Yusuf,[7] both written in the 11th century. The texts mention the word "yogurt" and describe its use by nomadic Turks. The Turks were also the first to evaluate yogurt's medicinal use for a variety of illnesses and symptoms, such as diarrhea and cramps, and to alleviate the discomfort of sunburned skin.

Genghis Khan, the founder of the Mongol Empire, is reputed to have fed his army yogurt, a staple of the Mongolian diet, based on the belief that it instilled bravery in his warriors.[3] In 1542, King Francoise I of France introduced this dairy product to Western Europe after being offered yogurt as a treatment by the country's Turkish allies for bouts of severe diarrhea. It was later mixed with a variety of ingredients, such as cinnamon, honey, fruits, and sweets, and was used as a dessert.[3]

It was not until the 20th century that researchers provided an explanation for the health benefits associated with yogurt consumption. In 1905, a Bulgarian medical student, Stamen Grigorov, was the first to discover *Bacillus bulgaricus* (now *L. bulgaricus*), a lactic acid bacteria that is still used in yogurt cultures today. Based on Grigorov's findings, in 1909, the Russian Nobel laureate, Yllia Metchnikoff, from the Pasteur Institute in Paris, suggested that lactobacilli in yogurt were associated with longevity in the Bulgarian peasant population.[3] In the beginning of the 20th century, yogurt became known for its health benefits and was sold in pharmacies as a medicine. Yogurt found commercial success when Isaac Carasso, from Barcelona, began producing yogurt with jams. After fleeing the Nazi occupation, Daniel Carasso, Isaac Carasso's son, founded Dannon (Danone in France). The first yogurt laboratory and factory were opened in France in 1932; in the United States, the first laboratory and factory were opened in 1941.[4]

YOGURT TODAY

Today, yogurt is typically milk that has been fermented and acidified with viable and well-defined bacteria, creating a thickened, often flavored, product with an extended shelf life. It contains essential nutrients and is a vehicle for fortification (added probiotics, fibers, vitamins, and minerals). It is also easily modified by sweeteners, fruits, and flavors to affect consistency and aroma. Yogurt can also be produced from rice, soy, or nuts.

Yogurt is defined by the symbiosis of 2 strains of bacteria (*S. thermophiles* and *L. bulgaricus*) in a sterile environment at a very low temperature (36°C–42°C) for 3–8 h. Both bacterial strains must remain active in the final product (with at least 10 million bacteria/g, according to CODEX 2003).[1] The process to which prepasteurized skimmed milk is submitted, before it is turned into yogurt, is responsible for changes in carbohydrates, proteins, and lipids. It yields an acidic flavor and a product with an improved appearance, taste, consistency, and digestibility. When milk lactose is used as the fermentation substrate, lactic acid and a series of other compounds are formed, contributing to its aroma. As a consequence of a decrease in pH, the development of undesirable microorganisms is delayed, the calcium and phosphorus present in milk are converted into their soluble form, and the majority of proteins, now calcium free, are better digested by proteolytic enzymes, which enhances its digestibility and overall bioavailability.[8,9]

Other bacterial strains, such as *Lactobacillus acidophilus* and *Bifidobacterium bifidus*, are often added for potential health benefits. When yogurt is consumed daily, there may be diminished growth of pathogens, which is ultimately beneficial to the human gut.[2] The protein content of some yogurts, such as Greek yogurt, is modified by concentrating or adding protein to provide twice the amount present in regular yogurt products. Calcium and vitamin D are also added to some products, adding nutritional value for populations with a high incidence of lactose intolerance or a low intake of dairy foods.

The types of yogurt consumed today are influenced by local traditions or correspond to certain lifestyles. In Eastern Europe and Asia, people consume milk that has undergone alcoholic fermentation by combining bacteria and yeasts (e.g., Kefir, Koumis); in Germany and Spain, yogurt is typically heat-treated to kill the bacteria; and in other countries, various probiotics and/or prebiotics are added to the mix.[1]

LOOKING AHEAD: CONSUMPTION OPPORTUNITIES

The majority of populations worldwide do not consume enough dairy products to meet several nutrient needs, particularly calcium. Three common barriers to consuming enough dairy include an allergy to cow's milk, lactose intolerance, and lack of accessibility. Of 16 European Union nations that provide data on dairy intake, the mean intake of dairy was 266 g/day. Denmark and Finland are 2 countries with population calcium intakes at or near 1000 mg/day, which is higher than the majority of the rest of the world. In the United States, 90%–95% of adult females and 75%–90% of adult males fall short of the recommended 3 servings of dairy per day.[10] In Brazil, low calcium intakes are far worse; 99% of adults in Brazil do not reach the minimum amount

of recommended calcium intake. Among Brazilian children, 99% consume only 500–600 mg of calcium per day.[11]

Patterns of yogurt consumption also vary greatly from country to country, but consumption is generally low. In the United States, where consumption of dairy products is broadly encouraged through nutrition education efforts, yogurt consumption is very low, with only about 6% of the population consuming yogurt on a daily basis. Contrast that to consumption levels in France, where the majority of the population consumes at least 1 serving per day and more than 1/3 of the population consumes at least 5 servings each week. Research in 15 countries also shows that those who consume the largest amounts of yogurt live in the Netherlands, France, Turkey, Spain, and Germany, while those who consume the smallest amounts live in Egypt, Colombia, Russia, Romania, and South Africa (Euromonitor 2013 data collected by the A.C. Nielsen Center for Market Research at the University of Wisconsin, Madison).[12]

In developing countries, yogurt consumption is often an indicator of economic change taking place. In Brazil, for example, though yogurt consumption is low, it increased more than 7-fold between 1974 and 2003.[13] However, while 40% of the Brazilian population consumes dairy products, only 6% consumes yogurt.[11]

In general, yogurt consumption is more common among healthier, leaner, more highly educated individuals from higher socioeconomic levels and is most common among women. In a survey of the population in São Paulo, Brazil (G. Possa, R. Fisberg, and M. Fisberg, unpublished data), it was found that most consumers were younger, white, female, nondiabetic, nonhypertensive, more educated, nonsmokers, and from higher socioeconomic levels. This has also been found among American and French populations.[14,15] This new pattern of consumption leads to the assumption that consumers may be interested mostly in the health aspects of yogurt, which opens a window of opportunity to introduce new forms of preparation and presentation that could reach populations with the lowest rates of yogurt intake.

In addition to helping to meet nutritional needs, research has demonstrated that yogurt can have positive effects on the gut microbiota and is associated with a reduced risk for gastrointestinal disease and improvement of lactose intolerance (especially among children),[16,17] cardiovascular disease,[2,18,19] metabolic syndrome[2,20] and type 2 diabetes,[2,21] allergies and respiratory diseases,[19] as well as improved dental and bone health[2,22–24] and pregnancy outcomes.[18,25–27] Yogurt can thus be an appealing dairy alternative for increasing nutrient intakes, as well as enhancing health and helping to prevent diseases among populations.

CONCLUSION

Yogurt is an ancient food that has been a part of the human diet for thousands of years and has been promoted as a healthy food for much of that time. Low consumption of yogurt represents a missed opportunity to contribute to a healthy lifestyle, as yogurt provides a good to excellent source of highly bioavailable protein and an excellent source of calcium as well as a source of probiotics that may provide a range of health benefits. Yogurt is not considered a snack or a sweet but rather as a dairy food that can be consumed with any meal. It is rich in calcium and potassium, which is especially important for Asian, African American, and American Indian populations in which lactose intolerance dominates and is a deterrent to consumption of dairy foods.

Acknowledgments

The authors thank Esteban Carmuega and Ricardo Weill for their contributions and insights.

The content of this article was presented as part of the Second Global Summit on the Health Benefits of Yogurt, held as a satellite to the Experimental Biology meeting in San Diego, California, on 30 April 2014. The conference was organized by the American Society for Nutrition and Danone Institute International. The supplement coordinators are Sharon M. Donovan, University of Illinois at Urbana-Champaign, USA, and Raanan Shamir, Schneider Children's Medical Center, Israel.

Funding. Writing and editorial assistance were provided by Densie Webb, PhD, RD, who was contracted and funded by Danone Institute International. M.F. received financial reimbursement for travel expenses and an honorarium from the Danone Institute International for his participation in the conference.

Declaration of interest. M.F. has presented at conferences for private and governmental organizations and nongovernmental organizations; has received grants for research from Abbott, Danone Research, Nestle, Pfizer, and Mondelez; and is a member of the Board of Danone Institute International and Global Stevia Institute.

M.F. also serves on the Yogurt in Nutrition Initiative for Health Advisory Board for the Danone Institute International. R.M. has no disclosures.

REFERENCES

1. Bodot V, Soustre Y, Reverend B. Best of 2013: Yogurt Special. French National Dairy Council (CNIEL): Scientific and Technical Affairs Division; 2013. http://www.idfdairynutrition.org/Files/media/FactSheetsHP/EXE-EN_BofYogurt.pdf. Accessed October 11, 2014.

2. Moreno Aznar LA, Cervera Ral P, Ortega Anta RM, et al. [Scientific evidence about the role of yogurt and other fermented milks in the healthy diet for the Spanish population (Spanish)]. Nutr Hosp. 2013;28:2039–2089.
3. McGee H. Fresh fermented milks and creams. In: P Dorfman, J Greene, A McGee, eds. Food and Cooking: The Science and Lore of the Kitchen. New York: Scribner; 2004; 44–51.
4. Brothwell D, Brothwell P. Food in antiquity: a survey of the diet of early peoples. Baltimore: Johns Hopkins University Press; 1997.
5. Batmanglij N. A Taste of Persia: An Introduction to Persian Cooking. Washington, DC: Mage Publishers; 2007.
6. Kashgari M. Divan-Lugat at-Turk. Tranlated by R. Dankoff with J. Kelley as A Compendium of Turkish Dialects. Vol 2. Cambridge, MA: Cambridge University Press; 1984.
7. Yusuf KH. Wisdom of Royal Glory (Kutadgu Bilig): A Turko-Islamic Mirror for Princes, translated with an introduction and notes by Robert Dankoff. Chicago: University of Chicago Press; 1983.
8. Cirone K, Huberman Y, Morsella C, et al. Growth of *Mycobacterium avium* subsp. paratuberculosis, *Escherichia coli*, and *Salmonella enteritidis* during preparation and storage of yogurt. Microbiol. Dec. 16;2013:247018.
9. Atamian S, Olabi A, Kebbe Baghdadi O, et al. The characterization of the physicochemical and sensory properties of full-fat, reduced-fat and low-fat bovine, caprine, and ovine Greek yogurt (Labneh). Food Sci Nutr. 2014;2:164–173.
10. International Dairy Federation (IDF). Global Dairy Plaform: Sustainable Dairy Nutrients Are Essential to Human Health. 2012. www.fil-idf.org. Accessed October 5, 2014.
11. Instituo Brasileiro de Geografia e Estatistica. Pesquisa de orçamentos familiares 2008–2009: Antropometria e estado nutricional de crianças adolescentes e adultos no Brasil Ministerio da Saude. Ministerio do Planejamento, Orcamento e Gestao, Rio de Janeiro; 2010.
12. Danone Nutricia Research. Global Yoghurt Consumption Per Capita and Per Year. The NutriJournal Web site. December 30, 2013. http://nutrijournal.danone.com/en/articles/stories/global-yoghurt-consumption-per-capita-and-per-year. Accessed October 5, 2014.
13. Schlindwein MM, Kassouf AL. Mudancas o padrao de consumo de alimentos temp-intensivos e de alimentos poupadores de temp, por regiao do Brasil: Gasto e consumo das familias brasileiras contemporaneas. Brasilia: IPEA; 2007.
14. Wang H, Troy LM, Rogers GT, et al. Longitudinal association between dairy consumption and changes of body weight and waist circumference: the Framingham Heart Study. Int J Obes. 2014;38:299–305.
15. Samara A, Herbeth B, Ndiaye NC, et al. Dairy product consumption, calcium intakes, and metabolic syndrome-related factors over 5 years in the STANISLAS study. Nutrition. 2013;29:519–524.
16. Thum C, Cookson AL, Otter DE, et al. Can nutritional modulation of maternal intestinal microbiota influence the development of the infant gastrointestinal tract? J Nutr. 2012;142:1921–1928.
17. Canani RB, Di Costanzo M. Gut microbiota as potential therapeutic target for the treatment of cow's milk allergy. Nutrients. 2013;5:651–662.
18. Kai SH, Bongard V, Simon C, et al. Low-fat and high-fat dairy products are differently related to blood lipids and cardiovascular risk score. Eur J Prev Cardiol. 2014;21:1557–1567.
19. Ralston RA, Lee JH, Truby H, et al. A systematic review and meta-analysis of elevated blood pressure and consumption of dairy foods. J Hum Hypertens. 2012;26:3–13.
20. Beydoun MA, Gary TL, Caballero BH, et al. Ethnic differences in dairy and related nutrient consumption among US adults and their association with obesity, central obesity, and the metabolic syndrome. Am J Clin Nutr. 2008;87:1914–1925.
21. Tong X, Dong JY, Wu ZW, et al. Dairy consumption and risk of type 2 diabetes mellitus: a meta-analysis of cohort studies. Eur J Clin Nutr. 2011;65:1027–1031.
22. Li H, Zou Y, Ding G. Dietary factors associated with dental erosion: a meta-analysis. PLoS One. 2012;7:e42626.
23. Twetman S, Keller MK. Probiotics for caries prevention and control. Adv Dent Res. 2012;24:98–102.
24. Wu L, Chang R, Mu Y, et al. Association between obesity and dental caries in Chinese children. Caries Res. 2013;47:171–176.
25. Rushing J, Neu J. Probiotics for pregnant women and preterm neonates. Am J Clin Nutr. 2011;93:3–4.
26. Sanz Y. Gut microbiota and probiotics in maternal and infant health. Am J Clin Nutr. 2011;94(6 Suppl):2000S–2005S.
27. Brantsaeter AL, Myhre R, Haugen M, et al. Intake of probiotic food and risk of preeclampsia in primiparous women: the Norwegian Mother and Child Cohort Study. Am J Epidemiol. 2011;174:807–815.

Supplement Article

Dairy products, yogurt consumption, and cardiometabolic risk in children and adolescents

Luis A. Moreno, Silvia Bel-Serrat, Alba Santaliestra-Pasías, and Gloria Bueno

The high prevalence of obesity in children is a global health issue. Obesity in children and adolescents can result in hypertension, dyslipidemia, chronic inflammation, and hyperinsulinemia, increasing the risk of death, as children grow into adulthood, and raising public health concerns. Type 2 diabetes in children and adolescents is a cardiovascular disease (CVD) risk factor. Dairy consumption may have a protective effect against the development of CVD, but there is scarce evidence of this in children and adolescents. Within the Healthy Lifestyle in Europe by Nutrition in Adolescence, the objective of this study was to investigate the relationship between dairy consumption and CVD risk factors in a sample of adolescents (aged 12.5–17.5 years) from 8 European cities. Overall, dairy products emerged as the food group that best identified adolescents at low CVD risk. Higher consumption of milk and yogurt and of milk- and yogurt-based beverages was associated with lower body fat, lower risk for CVD, and higher cardiorespiratory fitness.

INTRODUCTION

Obesity is an excess of body fat. In practice, methods to define or measure excess body fat in children and adolescents have limitations.[1] Children grow at different rates at different times, making obesity in children and adolescents difficult to define.[2] In addition, the definitions of overweight and obesity in children differ among epidemiological studies, hindering comparisons with cross-sectional prevalence data.[1] Despite its limitations, the body mass index (BMI) is the most widely used index to assess excess body fat in epidemiological studies. In developed countries, obesity prevalence steadily increased until around the year 2000; currently, it appears to have stabilized or even decreased in some countries.[3,4] A growing number of developing countries are affected by the double burden of malnutrition in which undernutrition and overnutrition (overweight and obesity) coexist in the same communities and families.[5]

As with adults, obesity in children and adolescents can result in hypertension, dyslipidemia, chronic inflammation, and hyperinsulinemia, increasing the risk of death, as children grow into adulthood, and raising public health concerns.[6] This clustering of cardiovascular disease (CVD) risk factors, known as the insulin resistance syndrome (metabolic syndrome), has been recognized in early life.[7] A decade ago, type 2 diabetes accounted for <3% of all new cases of diabetes in adolescents; today, type 2 diabetes accounts for approximately 45% of new cases.[8] Some studies have suggested that dairy consumption and its contribution to calcium intake may have a protective effect against the development of CVD.[9] However, dairy fat is often also portrayed as a negative component of milk and dairy products,[10] and some research has

Affiliation: *L.A. Moreno, S. Bel-Serrat, A. Santaliestra-Pasías*, and *G. Bueno* are with the Growth, Exercise, Nutrition and Development Research Group, Universidad de Zaragoza, Zaragoza, Spain. *L.A. Moreno, S. Bel-Serrat, and A. Santaliestra-Pasías* are with the Facultad de Ciencias de la Salud, Universidad de Zaragoza, Zaragoza, Spain. *G. Bueno* is with the Departamento de Pediatría, Universidad de Zaragoza, Zaragoza, Spain.

Correspondence: *L.A. Moreno*, Facultad de Ciencias de la Salud, Universidad de Zaragoza, C/Domingo Miral s/n, 50009, Zaragoza, Spain. E-mail: lmoreno@unizar.es. Phone: +34-976761000 (ext. 4457).

Key words: adolescents, cardiovascular disease, children, dairy, diabetes, milk, obesity, HELENA, yogurt.

doi: 10.1093/nutrit/nuv014

suggested an increased risk of obesity with frequent dairy consumption.[11] There is a lack of consistent evidence of the effect that dairy consumption has on obesity and CVD risk in adults. The consumption of yogurt and other dairy products in observational studies is associated with a reduced risk of weight gain and obesity, as well as of CVD, and these findings are, in part, supported by randomized trials.[12,13] There is an even greater lack of data to address this possible association in children and adolescents. The aim of the present review is to address the available information on the association between dairy products intake, especially yogurt intake, and cardiometabolic risk factors in children and adolescents, focusing on results from adolescents who participated in the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) study.[14]

DAIRY CONSUMPTION, OBESITY, AND CARDIOMETABOLIC RISK IN CHILDREN AND ADOLESCENTS

The Committee on Nutrition of the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition reviewed the literature and, in 2011, concluded that "available evidence does not allow recommendations on the role of calcium or dairy products in the development of obesity."[15] In a more recent review that focused specifically on children and adolescents,[16] it was found that 34 of 35 observational and intervention studies reported null or inverse associations between dairy intake and BMI, body fat, or energy balance. Four of 5 randomized control trials (RCTs) that were included in the review showed no positive associations between dairy intake and measures of adiposity, while 1 trial showed an inverse association. Twenty-three of the 35 studies included in the review analyzed data collected in the United States. The authors concluded that despite concerns that energy provided by dairy products may contribute to childhood obesity, the evidence overwhelmingly supports a null or inverse association between milk or dairy product intake and indicators of adiposity.[16]

Dairy products have also been shown to have antihypertensive effects as the result of the unique proteins and peptides they contain (angiotensin-converting enzyme [ACE] inhibitory peptides).[17,18] Two prospective cohort studies found that children who consume more dairy products early in life (age range, 18–59 months) have lower blood pressure in middle childhood and early adolescence.[19,20] Milk fat increases high-density lipoproteins,[21] which are thought to be protective against CVDs, and some of the saturated fats present in milk fat have a neutral effect on low-density lipoproteins,[22] which constitute a recognized risk factor.

FINDINGS OF THE HEALTHY LIFESTYLE IN EUROPE BY NUTRITION IN ADOLESCENTS STUDY

Within the HELENA study, the current analysis of data was undertaken to investigate the relationship between dairy consumption and CVD risk factors in a sample of adolescents (age range, 12.5–17.5 years) from 8 European cities (Athens, Greece; Dortmund, Germany; Ghent, Belgium; Lille, France; Rome, Italy; Stockholm, Sweden; Vienna, Austria; and Zaragoza, Spain). The cross-sectional HELENA study was conducted between 2006 and 2007.[14] Measurements were obtained for diet, waist circumference, skin-fold thicknesses (biceps, triceps, subscapular, suprailiac), systolic blood pressure, insulin resistance, triglycerides, total cholesterol/high-density lipoprotein ratio, and cardiorespiratory fitness for a subset (511) of 3528 adolescents. Approximately half of the subset was composed of males. Due to the lack of appropriate criteria to define the metabolic syndrome in children and adolescents, it has been suggested that clustering CVD risk factors may be an adequate measure of cardiovascular health. Individual, sex-specific *z*-scores of CVD risk factors, considering systolic blood pressure, sum of 4 skin-fold thicknesses (bicipital, tricipital, subscapular, and suprailiac), serum triglyceride concentrations, total cholesterol to HDL-cholesterol ratio, Homeostatic Model Assessment index, and cardiorespiratory fitness, were summed to compute sex-specific clustered CVD risk scores.[23] Cardiorespiratory fitness was multiplied by −1 to indicate higher CVD risk with increasing value. The lower the score, the better the overall CVD risk factor profile. Because this was a multicenter study, both intra- and interobserver reliability of anthropometric measurements were assessed and found to be 95% and 90%, respectively.[24]

Dietary intakes were assessed using the validated HELENA dietary assessment tool (DIAT),[25] which includes two 24-hour recalls (1 weekday and 1 weekend day). The participants' usual consumption of food groups was estimated using the multiple source method.[26] The adolescents completed the 24-hour recall twice (time-span of 2 weeks) during school time; at both times, trained staff, including a dietitian, were present. The HELENA-DIAT incorporated special techniques to support and enhance respondents' memory, which allowed a more detailed description and quantification of the dishes/foods consumed. HELENA-DIAT was validated in European adolescents[25] ($r_s = 0.86–0.91$, for all nutrients and energy intake). The "milk" food group included both milk and buttermilk, and the "yogurt and milk- and yogurt-based beverages" group

included yogurt, yogurt- and milk-based beverages such as chocolate milk and probiotic beverages, and "fromage blanc."[14] Cheese and milk-based desserts were considered as 2 separate food groups due to their differing nutrient composition. No distinctions regarding the fat content in any of the 2 food groups was made.

Concerning confounding variables, in brief, socioeconomic status was estimated by means of the family affluence scale, which is based on the concept of material conditions in the family including car ownership, bedroom occupancy, home computers, and internet access. The average time engaged in 2 sedentary behaviors (TV viewing and playing video games) was estimated by means of a self-administered questionnaire. Uniaxial accelerometers (Actigraph MTI, model GT1M, Manufacturing Technology Inc., Fort Walton Beach, FL, USA) were used to objectively measure physical activity. At least 3 days of recording, with a minimum of 8 hours of registration per day, was set as an inclusion criterion. Time spent at moderate-to-vigorous physical activity (>3 metabolic equivalents) was calculated through the following cutoff point: 2000 counts per minute for moderate-to-vigorous physical activity.

In comparison to the rest of the food groups, milk, yogurt, and milk- and yogurt-based beverages accounted for greater variability for most CVD risk factors in both male and female adolescents.[14] Multiple linear regressions were performed to examine the association of individual CVD risk factors and CVD risk score (dependent variables) with dairy consumption (independent variables). Confounders adjusted for in the analyses included the following: socioeconomic status, pubertal maturity, moderate-to-vigorous physical activity, sedentary behavior, and daily energy intakes. In agreement with data from other studies that reported an inverse association between dairy consumption and waist circumference in adolescents,[27,28] waist circumference for both adolescent boys and adolescent girls was significantly greater among those in tertile 1 of total dairy consumption compared with those in tertile 3. In adolescent girls, greater consumption of yogurt was associated with a lower *z*-score for waist circumference. The association was greater among adolescent girls for both waist circumference and skin-fold thicknesses when the milk group was added to yogurt and yogurt-based beverages. A positive association between consumption of foods in the yogurt group and cardiorespiratory fitness was also observed among adolescent girls. Among adolescent boys, there was an inverse association between overall dairy consumption and both the sum of skin-fold thickness measurements and cardiorespiratory fitness.

In addition to previously published findings,[8] additional analyses were performed on 1422 adolescents (44.95% male) aged 12.5–17.5 years, focusing on body composition, with complete measurements for waist circumference, skin-fold thicknesses, and two 24-hour dietary recalls (S. Bel-Serrat and L. A. Moreno, unpublished data). Greater overall dairy consumption was associated with lower BMI, reduced skin-fold thicknesses, and smaller waist circumference (Figures 1–3). Pubertal maturity, study center, socioeconomic status, sedentary behavior, physical activity, and daily energy intake were confounders that were corrected for in the analyses.

Overall, dairy emerged as the food group that best identified adolescents at low CVD risk. Higher consumption of milk, yogurt, and milk- and yogurt-based beverages was associated with lower body fat and higher cardiorespiratory fitness. Despite its contributions to nutrient intake and evidence suggesting that intake may help reduce the risk of overweight and obesity along with other CVD risk factors, consumption of milk and dairy products by children and adolescents has decreased in many countries in recent decades.[16] A substantial proportion of children and adolescents now fail to meet even minimum recommendations for intake of dairy foods, and consumption tends to decrease with age through childhood and early adolescence.

Discrepant results among studies[10] could be due to differences in dietary assessment methods and the food items/groups considered in every study. In a systematic review of the association between dairy intake and adiposity in children and adolescents, 36 relevant studies were identified.[13] Sufficient data for effect size estimation and inclusion in the metaanalyses were obtained from 22 studies. In the reviewed studies that contained data on adolescents, 9 were cross-sectional, 4 were longitudinal, and 2 were RCTs. To assess dietary intake in the cross-sectional studies, 6 used the 24-hour recall method, 2 used a food frequency questionnaire, and 1 used dietary records; 3 longitudinal studies used a food frequency questionnaire and 1 used dietary records. Concerning the exposure, 8 considered milk alone and the remaining 7 considered dairy products in general.

Possible mechanisms

Several potential mechanisms have been suggested to explain the association between dairy consumption and indicators of body composition and reduced risk of CVD in humans. One mechanism may be the shift in food consumption patterns observed in children in recent decades. The Bogalusa Heart Study[29] revealed a significant decrease in the amount of milk consumed by US children, whereas the amounts of beverages and fruit/fruit juices consumed increased significantly. The decrease in milk consumption, concomitant with the increased consumption of sugar-sweetened beverages,

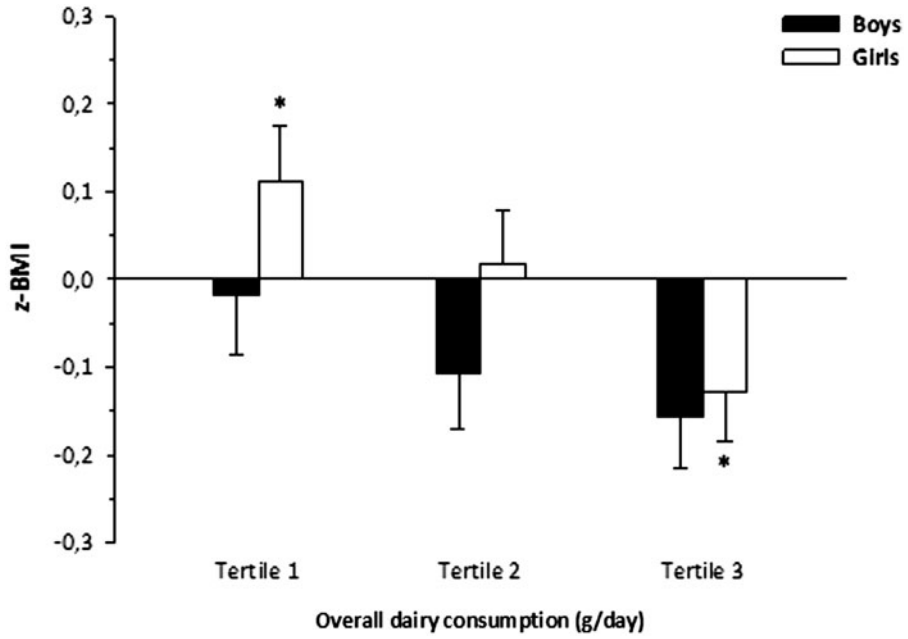

Figure 1 **Relationship between body mass index *z*-score and dairy consumption (g/day) in adolescent (12.5–17.5 years) boys and girls from 8 European cities participating in the Healthy Lifestyle in Europe by Nutrition in Adolescence Study.** Dairy consumption was divided into tertiles of consumption. Data are expressed as mean ± standard error. Asterisk indicates differences between tertiles of intake at $P < 0.05$. *Abbreviation*: BMI, body mass index.

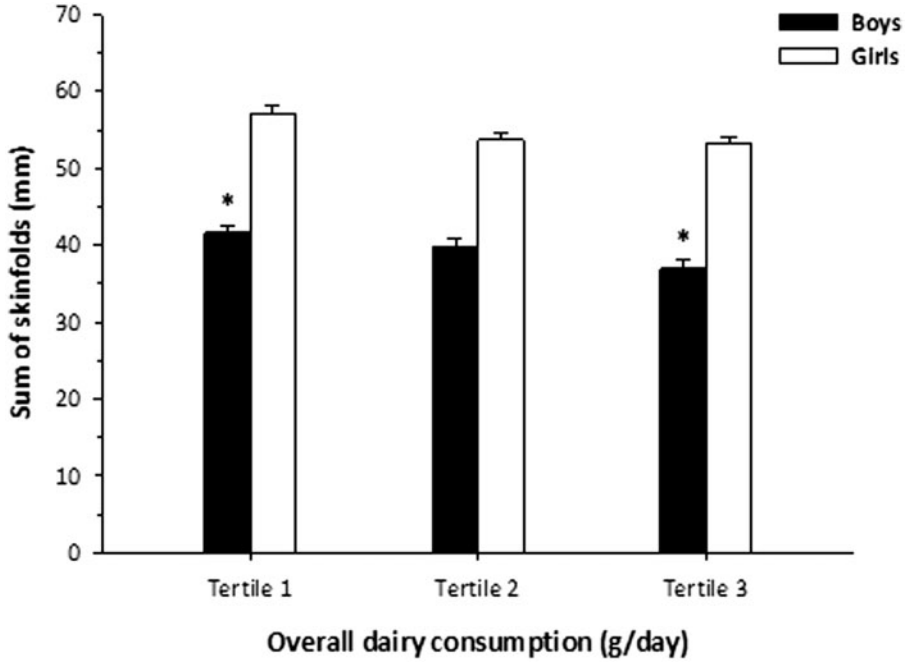

Figure 2 **Relationship between sum of skin-fold thicknesses (mm) and dairy consumption (g/day) in adolescent (12.5–17.5 years) boys and girls from 8 European cities participating in the Healthy Lifestyle in Europe by Nutrition in Adolescence Study.** Dairy consumption was divided into tertiles of consumption. Data are expressed as mean ± standard error. Asterisk indicates differences between tertiles of consumption at $P < 0.05$.

may be responsible, in part, for the hypothesized inverse relationship between dairy intake and obesity in both children and adolescents.[30] According to Huang et al.,[30] 3 energy-dependent effects have been hypothesized to occur when milk is replaced with sugar-sweetened beverages: 1) increased energy intake by consuming more calories per common serving and due to the typically larger serving sizes of these beverages compared with

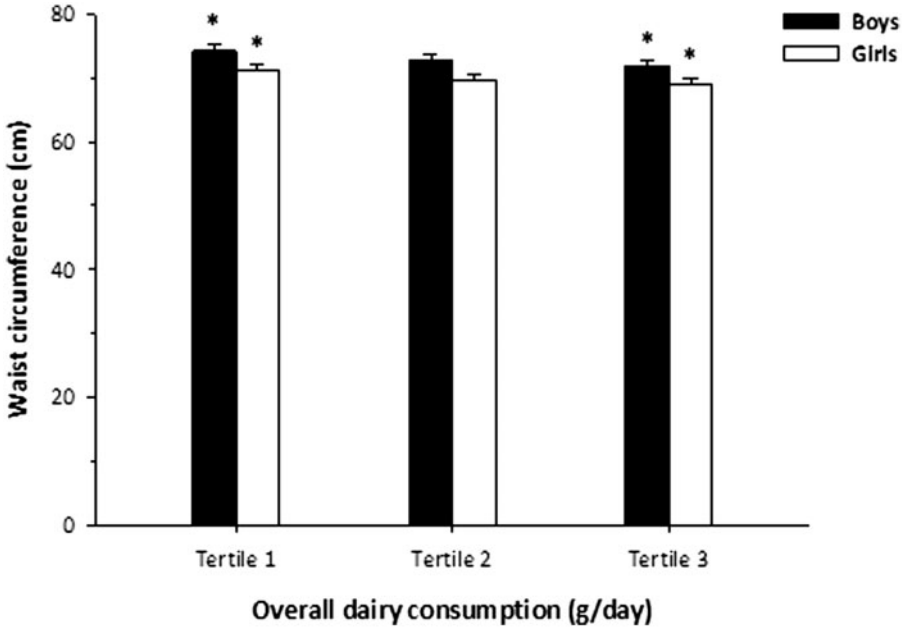

Figure 3 **Relationship between waist circumference (cm) and dairy consumption (g/d) in adolescent (12.5–17.5 years) boys and girls from 8 European cities participating in the Healthy Lifestyle in Europe by Nutrition in Adolescence Study**. Dairy consumption was divided into tertiles of consumption. Data are expressed as mean ± standard error. Asterisk indicates differences between tertiles of consumption at $P < 0.05$.

milk; 2) decreased satiety with consumption of high-sugar beverages, resulting in a higher intake of other foods and energy; or 3) lower energy expenditure linked to the consumption of high-sugar beverages. In support of hypothesis 2, Huang and McCrory[30] referred to a study in adolescents which found that compared with caffeinated or noncaffeinated sodas, low-fat milk intake yielded lower postprandial glucose, insulin, and free fatty acid responses, as well as lower scores of hunger and desire to eat.[31] Recently, it was observed that frequent yogurt consumption was associated with a high-quality diet, leading also to a healthier insulin profile in children.[32]

In addition, it has been suggested that several components naturally present in dairy foods, such as calcium, play a protective role in weight management. Numerous researchers have investigated the hypothesis of an inverse relationship between calcium intake and body weight, weight gain, and/or percent fat.[33] Several mechanisms have been proposed to explain the influence of calcium intake on body weight and/or body fat. While some mechanisms are related to whole-body energy balance, i.e., fecal fat excretion and appetite control, others seem to be linked to cellular processes such as fat mobilization and oxidation.[33] Dietary calcium may contribute to a negative energy balance through its ability to decrease intestinal fat absorption and elevate the amount of fatty acids eliminated in the feces, which consequently has a beneficial effect on circulating lipids.[33] That effect is attributable to the formation of insoluble calcium–fatty acids soaps, which then pass unabsorbed through the intestinal track.[33] Moreover, dietary calcium could also modulate body weight and/or body fat through an influence on appetite control. However, the satiating effect of calcium and/or dairy supplementation has not been confirmed in all of the studies conducted in this regard, and it is not clear whether that effect is due to calcium or to the food matrix present in dairy foods.[33] In terms of cellular mechanisms, Zemel[34] proposed that high-calcium diets attenuate body fat accumulation and weight gain by mediating circulating calcitriol, a regulator of adipocyte intracellular Ca^{2+}. Increased intracellular Ca^{2+} stimulates lipogenic gene expression and lipogenesis and suppresses lipolysis, which results in adipocyte lipid filling and increased adiposity. The increased calcitriol produced in response to low-calcium diets stimulates adipocyte Ca^{2+} influx-promoting adiposity, while higher calcium intakes inhibit lipogenesis; promote lipolysis, lipid oxidation, and thermogenesis; and inhibit diet-induced obesity.[34] An effect of dietary calcium on increased energy expenditure and thermogenesis has also been investigated; however, all studies have failed to confirm this hypothesis.[35] An inverse association between frequency of dairy consumption and serum inflammatory markers such as C-reactive protein, interleukin-6, and tumor necrosis factor-α was found in healthy individuals; however, not all studies support that hypothesis.[33]

Furthermore, it has been shown that dairy sources of calcium exert greater effects in accelerating fat loss

compared with other food sources. This could be explained, in part, by several bioactive compounds present in the whey fraction of dairy, such as ACE inhibitors and branched chain amino acids (BCAAs), e.g., leucine, that act synergistically with calcium to attenuate weight and fat gain.[34] Whey proteins account for 20% of milk proteins, and lactoglobulin comprises about half of the total protein present in whey from cow's milk.[36] Whey proteins, specifically α-lactorphin and β-lactorphin, derived from α-lactoglobulin and β-lactoglobulin, respectively, together with albutensin, appear to have ACE inhibitory activity. These peptides are considered to be potent ACE inhibitory peptides because they are absorbed intact from the intestine to reach their target organ.[37] Casein-derived peptides, known as casokinins, have also been shown to have hypotensive effects.[36] Taking these findings into consideration, food-derived peptides would represent a safe option for decreasing high blood pressure.[37]

Dairy proteins seem to support better muscle protein synthesis than plant proteins.[37] Dairy foods, mainly whey proteins, contain the highest concentration of BCAAs, especially leucine, of all dietary proteins.[38] BCAAs have been found to be mostly available for protein synthesis, and, among them, leucine has been recognized as a potent stimulator of muscle protein synthesis.[38] This enhanced anabolism caused by milk proteins could potentially increase energy expenditure, but no conclusion can be drawn yet in this regard.[38] Additionally, leucine may also play a role in the repartitioning of dietary energy from adipose tissue to skeletal muscle, promoting fat loss.[39]

Conjugated linoleic acid (CLA) is present in dairy foods derived from ruminant sources. Many studies have shown a role of CLA in modulating body composition, especially by reducing the accumulation of adipose tissue.[39] Studies conducted in humans revealed that supplementation with CLA for short periods of time, i.e., no longer than 12 weeks, reduced body weight and body fat.[40] Available data suggest that CLA enhances sympathetic nervous activity, leading to increased energy metabolism and reduced adipose tissue mass.[40] Additionally, CLA's ability to reduce adipose tissue mass has been linked with induction of adipocyte apoptosis and/or differentiation and reduction of triglyceride accumulation in adipocytes.[40]

The consumption of yogurt may ensure changes in the balance and metabolic activities of the indigenous microbiota.[41] It has been observed that the intestinal microbiota in children who are overweight/obese is different from those with a BMI within normal ranges[42] or in lean children.[43] It has also been suggested that abnormal development of gut microbiota could contribute to the development of obesity during childhood.[44] Also in children and adolescents, consumption of a synbiotic that includes *Lactobacillus* spp. and *Bifidobacterium* sp. was shown to have a beneficial effect on weight control and cardiometabolic risk.[45] Mechanisms to explain the effect of probiotics in weight control are not yet clear; however, it seems they could be related to an interaction with the gut microbiota, thus affecting the metabolic pathways implicated in fat metabolism.[46] In humans, yogurt consumption may lead to changes in the equilibrium and metabolic activity of gut microbiota.[47,48]

CONCLUSION

In the HELENA study of adolescents in Europe, an inverse association was observed between consumption of yogurt and of milk- and yogurt-based beverages and some CVD risk factors, especially total and abdominal excess body fat. The association was stronger when milk intake was added to dairy product intake. To date, most of the data on dairy intake and health outcomes has been obtained from observational studies performed in the United States. More studies are needed in which yogurt is considered as an individual food category, which typically has not been the case. RCTs are also needed to provide evidence to support the HELENA findings and to further understand the mechanisms underlying the associations between dairy (especially yogurt) intake and obesity, diabetes, and other CVD risk factors.

Acknowledgments

The content of this article was presented as part of the Second Global Summit on the Health Benefits of Yogurt, held as a satellite to the Experimental Biology meeting in San Diego, California, on 30 April 2014. The conference was organized by the American Society for Nutrition and Danone Institute International. The supplement coordinators are Sharon M. Donovan, University of Illinois at Urbana-Champaign, USA and Raanan Shamir, Schneider Children's Medical Center, Israel.

Funding. Writing and editorial assistance were provided by Densie Webb, PhD, RD, who was contracted and funded by Danone Institute International. The HELENA study was conducted with the financial support of the European Community Sixth Research, Technological development, and Demonstration (RTD) Framework Program (FOOD-CT-2005-007034). L.A.M. received financial reimbursement for travel expenses and an honorarium from the Danone Institute International for his participation in the conference; he also serves on the Yogurt in Nutrition Initiative for

Health Advisory Board for the Danone Institute International.

Declaration of interest. The authors have no relevant interests to declare.

REFERENCES

1. Rodriguez G, Pietrobelli A, Wang Y, et al. Methodological aspects for childhood and adolescence obesity epidemiology. In: LA Moreno, I Pigeot, W Ahrens, eds. Epidemiology of Obesity in Children and Adolescents. New York: Springer; 2011: 21–40.
2. Moreno LA, Bel-Serrat S, Santaliestra-Pasias AM, et al. Obesity prevention in children. World Rev Nutr Diet. 2013;106:119–126.
3. Rokholm B, Baker JL, Sorensen TI. The levelling off of the obesity epidemic since the year 1999—a review of evidence and perspectives. Obes Rev. 2010;11: 835–846.
4. Food and Agriculture Organization of the United Nations. Milk and Dairy Products in Human Nutrition. Rome: Food and Agriculture Organization of the United Nations; 2013.
5. Tzioumis E, Adair LS. Childhood dual burden of under- and overnutrition in low- and middle-income countries: a critical review. Food Nutr Bull. 2014;35:230–243.
6. Ebbeling CB, Pawlak DB, Ludwig DS. Childhood obesity: public-health crisis, common sense cure. Lancet. 2002;360:473–482.
7. Olza J, Gil-Campos M, Leis R, et al. Presence of the metabolic syndrome in obese children at prepubertal age. Ann Nutr Metab. 2011;58:343–350.
8. D'Adamo E, Caprio S. Type 2 diabetes in youth: epidemiology and pathophysiology. Diabetes Care. 2011;34 (Suppl 2):S161–S165.
9. Yerlikaya O, Acu M, Kinik O. Importance of dairy products in cardiovascular diseases and type 2 diabetes. Crit Rev Food Sci Nutr. 2013;53:902–908.
10. Kratz M, Baars T, Guyenet S. The relationship between high-fat dairy consumption and obesity, cardiovascular, and metabolic disease. Eur J Nutr. 2013;52:1–24.
11. Berkey CS, Rockett HR, Willett WC, et al. Milk, dairy fat, dietary calcium, and weight gain: a longitudinal study of adolescents. Arch Pediatr Adolesc Med. 2005; 159:543–550.
12. Astrup A. Yogurt and dairy product consumption to prevent cardiometabolic diseases: epidemiologic and experimental studies. Am J Clin Nutr. 2014;99 (5 Suppl): 1235S–1242S.
13. Dror DK. Dairy consumption and pre-school, school-age and adolescent obesity in developed countries: a systematic review and meta-analysis. Obes Rev. 2014;15: 516–527.
14. Bel-Serrat S, Mouratidou T, Jimenez-Pavon D, et al. Is dairy consumption associated with low cardiovascular disease risk in European adolescents? Results from the HELENA study. Pediatr Obes. 2014;9:401–410.
15. ESPGHAN Committee on Nutrition, Agostoni C, Braegger C, et al. Role of dietary factors and food habits in the development of childhood obesity: a commentary by the ESPGHAN Committee on Nutrition. J Pediatr Gastroenterol Nutr. 2011;52: 662–669.
16. Dror DK, Allen LH. Dairy product intake in children and adolescents in developed countries: trends, nutritional contribution, and a review of association with health outcomes. Nutr Rev. 2014;72:68–81.
17. FitzGerald RJ, Murray BA, Walsh DJ. Hypotensive peptides from milk proteins. J Nutr. 2004;134:980S–988S.
18. Maes W, Van Camp J, Vermeirssen V, et al. Influence of the lactokinin Ala-Leu-Pro-Met-His-Ile-Arg (ALPMHIR) on the release of endothelin-1 by endothelial cells. Regul Pept. 2004;118:105–109.
19. Rangan AM, Flood VL, Denyer G, et al. The effect of dairy consumption on blood pressure in mid-childhood: CAPS cohort study. Eur J Clin Nutr. 2012;66: 652–657.
20. Moore LL, Singer MR, Bradlee ML, et al. Intake of fruits, vegetables, and dairy products in early childhood and subsequent blood pressure change. Epidemiology. 2005;16:4–11.
21. Mensink RP, Zock PL, Kester AD, et al. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. Am J Clin Nutr. 2003;77: 1146–1155.
22. Yu S, Derr J, Etherton TD, Kris-Etherton P. Plasma cholesterol-predictive equations demonstrate that stearic acid is neutral and monounsaturated fatty acids are hypocholesterolemic. Am J Clin Nutr. 1995;61:1129–1139.
23. Andersen LB, Harro M, Sardinha LB, et al. Physical activity and clustered cardiovascular risk in children: a cross-sectional study (the European Youth Heart Study). Lancet. 2006;368:299–304.
24. Nagy E, Vicente-Rodriguez G, Manios Y, et al. Harmonization process and reliability assessment of anthropometric measurements in a multicenter study in adolescents. Int J Obes 2008;32 (Suppl 5):S58–S65.
25. Vereecken CA, Covents M, Sichert-Hellert W, et al. Development and evaluation of a self-administered computerized 24-h dietary recall method for adolescents in Europe. Int J Obes 2008;32 (Suppl 5):S26–S34.
26. Harttig U, Haubrock J, Knuppel S, et al. The MSM program: web-based statistics package for estimating usual dietary intake using the Multiple Source Method. Eur J Clin Nutr. 2011;65 (Suppl 1):S87–S91.
27. Abreu S, Santos R, Moreira C, et al. Association between dairy product intake and abdominal obesity in Azorean adolescents. Eur J Clin Nutr. 2012;66:830–835.
28. Bradlee ML, Singer MR, Qureshi MM, et al. Food group intake and central obesity among children and adolescents in the Third National Health and Nutrition Examination Survey (NHANES III). Public Health Nutr. 2010;13:797–805.
29. Nicklas TA, Demory-Luce D, Yang SJ, et al. Children's food consumption patterns have changed over two decades (1973–1994): the Bogalusa Heart Study. J Am Diet Assoc. 2004;104:1127–1140.
30. Huang TT, McCrory MA. Dairy intake, obesity, and metabolic health in children and adolescents: knowledge and gaps. Nutr Rev. 2005;63:71–80.
31. Hajduk CL, Gupta N, McCrory MA, Roberts SB. Effects of milk versus soda on short-term hunger and energy intake in children. FASEB J. 2003;17:A809.
32. Zhu Y, Wang H, Hollis JH, Jacques PF. The associations between yogurt consumption, diet quality, and metabolic profiles in children in the USA. Eur J Nutr. 2014 July 18. [Epub ahead of print]. doi: 10.1007/s00394-014-0735-7.
33. Villarroel P, Villalobos E, Reyes M, et al. Calcium, obesity, and the role of the calcium-sensing receptor. Nutr Rev. 2014;72:627–637.
34. Zemel MB. The role of dairy foods in weight management. J Am Coll Nutr. 2005; 24 (6 Suppl):537S–546S.
35. Van Loan M. The role of dairy foods and dietary calcium in weight management. J Am Coll Nutr. 2009;28 (Suppl 1):120S–129S.
36. Shah NP. Effects of milk-derived bioactives: an overview. Br J Nutr. 2000;84 (Suppl 1):S3–S10.
37. Pihlanto-Leppala A, Koskinen P, Piilola K, et al. Angiotensin I-converting enzyme inhibitory properties of whey protein digests: concentration and characterization of active peptides. J Dairy Res. 2000;67:53–64.
38. Gilbert JA, Bendsen NT, Tremblay A, et al. Effect of proteins from different sources on body composition. Nutr Metab Cardiovasc Dis. 2011;21 (Suppl 2):B16–B31.
39. Layman DK. Role of leucine in protein metabolism during exercise and recovery. Can J Appl Physiol. 2002;27:646–663.
40. Belury MA. Dietary conjugated linoleic acid in health: physiological effects and mechanisms of action. Annu Rev Nutr. 2002;22:505–531.
41. Marette A, Picard-Deland E. Yogurt consumption and impact on health: focus on children and cardiometabolic risk. Am J Clin Nutr. 2014;99 (5 Suppl):1243S–1247S.
42. Karlsson CL, Onnerfalt J, Xu J, et al. The microbiota of the gut in preschool children with normal and excessive body weight. Obesity. 2012;20:2257–2261.
43. Bervoets L, Van Hoorenbeeck K, Kortleven I, et al. Differences in gut microbiota composition between obese and lean children: a cross-sectional study. Gut Pathog. 2013;5:10.
44. Kalliomaki M, Collado MC, Salminen S, et al. Early differences in fecal microbiota composition in children may predict overweight. Am J Clin Nutr. 2008;87: 534–538.
45. Safavi M, Farajian S, Kelishadi R, et al. The effects of synbiotic supplementation on some cardio-metabolic risk factors in overweight and obese children: a randomized triple-masked controlled trial. Int J Food Sci Nutr. 2013;64:687–693.
46. Arora T, Singh S, Sharma RK. Probiotics: interaction with gut microbiome and anti-obesity potential. Nutrition. 2013;29:591–596.
47. Alvaro E, Andrieux C, Rochet V, et al. Composition and metabolism of the intestinal microbiota in consumers and non-consumers of yogurt. Br J Nutr. 2007;97: 126–133.
48. Garcia-Albiach R, Pozuelo de Felipe MJ, Angulo S, et al. Molecular analysis of yogurt containing *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* in human intestinal microbiota. Am J Clin Nutr. 2008;87:91–96.

Supplement Article

Association between consumption of dairy products and incident type 2 diabetes—insights from the European Prospective Investigation into Cancer study

Nita G. Forouhi

The public health burden of type 2 diabetes has risen unabated over the past decades, fueled by obesity and lifestyle influences, including diet quality. Epidemiological evidence is accumulating for an inverse association between dairy product intake and type 2 diabetes risk; this is somewhat counterintuitive to the saturated fat and cardiometabolic disease paradigm. The present report reviews the contribution that the findings of the European Prospective Investigation into Cancer (EPIC) study have made to this debate, noting that types of dairy products, particularly fermented dairy products including yogurt, may be more relevant than overall dairy intake for the prevention of type 2 diabetes. The EPIC study has contributed evidence through complementary approaches of a large prospective study across 8 European countries with heterogeneous dietary intakes assessed using food-frequency questionnaires (EPIC-InterAct study) and through a more detailed examination of diet assessed using a 7-day food diary (EPIC-Norfolk study). The implications of these findings are placed in the wider context, including the use of individual fatty acid blood biomarkers in the EPIC-InterAct study and an appraisal of current research gaps and suggestions for future research directions.

INTRODUCTION

The global burden of diabetes mellitus is high, and increasing, with the latest estimates from the International Diabetes Federation suggesting that 382 million people had diabetes in 2013; that number is projected to increase to 592 million by 2035.[1] The multiple serious consequences of diabetes, including macrovascular and microvascular complications that lead to premature morbidity and mortality, pose a major threat to public health. For type 2 diabetes, the most common form of diabetes, there is high-quality evidence from clinical trials in diverse settings that lifestyle interventions are effective for it's primary prevention.[2–6] However, in day-to-day practice in real-world settings outside of clinical trials, uncertainty remains about the specific dietary factors that relate to diabetes risk and the optimal dietary advice for individuals and populations.

There is increasing interest in the potential role that dairy products might play in diabetes etiology, though research evidence has been mixed as to whether different types of dairy products have a beneficial, detrimental, or null association with type 2 diabetes.[7–10] The focus within dietary guidelines to reduce the consumption of saturated fat for the prevention of cardiovascular

Affiliation: *N.G. Forouhi* is with the Medical Research Council Epidemiology Unit, University of Cambridge School of Clinical Medicine, Institute of Metabolic Science, Cambridge Biomedical Campus, Cambridge, UK.

Correspondence: *N.G. Forouhi*, Medical Research Council Epidemiology Unit, University of Cambridge School of Clinical Medicine, Institute of Metabolic Science, Cambridge Biomedical Campus, Cambridge, CB2 0QQ, UK. E-mail: nita.forouhi@mrc-epid.cam.ac.uk. Phone: +44-1223-330315

Key words: dairy, EPIC study, saturated fatty acids, type 2 diabetes, yogurt.

doi: 10.1093/nutrit/nuv018

disease[11] has generally supported the view that dairy products should be consumed for bone health. However, as they are typically high in saturated fat content, dairy products should be consumed in moderate amounts and as low-fat varieties. A specific example of caution against dairy products is the "reverse coding" within algorithms for estimating adherence to a Mediterranean diet pattern, whereby dairy foods are given a "reverse" coding with a score of 0 for high (at or above median) and 1 for low (below median) dairy product consumption, while the converse is the case for perceived "healthy" foods such as fruits, vegetables, legumes, cereals, and fish.[12] In a previous study, a similar principle of assigning a value of 0, 1, or 2 to intakes of first, second, and third tertiles of intakes of the "beneficial" components of the Mediterranean diet was applied, but a reverse coding system was used for dairy product intake.[13] Thus, uncertainty remains about the role of dairy products in chronic disease outcomes. On the one hand, dairy products are presumed to be beneficial, as they are nutrient dense with large amounts of calcium, magnesium, vitamin A, vitamin D (when fortified), and high-quality protein.[14] On the other hand, their contribution to saturated fat intake is seen as potentially detrimental to health outcomes.

Contrary to expectations and based on the strong focus on saturated fat as a risk factor for cardiometabolic disease, recent appraisal of the evidence has not been convincing for the effects of saturated fatty acids on such outcomes.[15,16] Simultaneously, a dialogue has begun on whether the focus of dietary advice should move away from nutrients to a food-based approach.[17] In light of these developments, it has become of great interest to investigate the potential role the intake of dairy products could have on cardiometabolic health. The focus of the present report is on the European Prospective Investigation into Cancer (EPIC) study's contribution to furthering understanding of the association between the amount and type of dairy product consumption and the risk of developing incident type 2 diabetes.

In particular, EPIC investigators addressed 2 interlinked objectives in order to advance this field of inquiry. The first objective was to investigate the association between consumption of different amounts and types of dairy products and the development of incident type 2 diabetes; this was done using the heterogeneity of dietary exposures measured with food-frequency questionnaires (FFQs) across 8 European countries in the EPIC-InterAct study.[18] The second objective was to investigate the association between dairy intake and diabetes using more detailed dietary information obtained from a prospective 7-day food diary in the UK–based EPIC Norfolk study.[19]

RATIONALE, METHODS, AND FINDINGS FROM THE EPIC STUDY

EPIC-InterAct study: intake of dairy products across 8 European countries

At the time the InterAct project was conducted,[20] little research evidence was available from Europe on the association between dairy products and incidence of diabetes; only 3 studies had been published that, together, included fewer than 600 incident cases of type 2 diabetes, and 2 of those studies were restricted to men.[21–23] The majority of past research had been conducted in the United States and Asia, where the intake of dairy products is generally lower than in Europe.[24] In addition, there are differences in the nutritional composition of dairy products by location. Although the large variation across Europe in the intake levels of different types of dairy products had previously been described,[25] an appraisal of the association between different types of dairy products with diabetes risk had not been undertaken in this population. Thus, it was timely and appropriate to undertake this analysis within InterAct.

Described in detail previously,[18,20] the EPIC-InterAct study was a case-cohort study nested within 8 of the 10 countries participating in the EPIC study. A total of 340,234 EPIC participants were followed up between 1991 and 2007 for 3.99 million person-years; among them, the InterAct consortium partners ascertained and verified 12,403 incident cases of type 2 diabetes and randomly selected a subcohort of 16,835 individuals. After exclusions, the sample eligible for analysis included 10,694 diabetes cases and 13,780 subcohort participants, with 673 diabetes cases present in the subcohort, as per the design of the case-cohort study, which allowed for a small number of future incident cases to be included randomly within the subcohort. The statistical analysis took this design characteristic into account.[26] Dietary intake was assessed by locally developed and validated semiquantitative FFQs.[27] Intake of total dairy products was calculated as the sum of all dairy subtypes reported in the dietary questionnaires, with the exception of butter, which was not included. For the analysis of dairy subtypes, the study included intakes of milk, yogurt, thick fermented milk and cheese; a combined category of fermented dairy products comprised the sum of cheese, yogurt, and thick fermented milk.[18] Statistical analysis used a modified Cox regression suitable for the case-cohort design.[26] Country-specific hazard ratios (HRs) and 95% confidence intervals (CIs) for associations of quintiles of dairy products and dairy subtypes with incident type 2 diabetes were calculated, and a random-effects metaanalysis was performed to calculate a pooled HR. A series

of statistical models was constructed that accounted for several relevant potential confounding factors including study center, age, and sex (model 1); plus body mass index (BMI), education level, smoking status, physical activity level, alcohol intake (model 2); plus total energy intake and energy-adjusted intakes of fruits, vegetables, red meat, processed meat, sugar-sweetened soft drinks, coffee, cereals, and cereal products (model 3). A number of sensitivity analyses and tests for interaction were prespecified.

Analyses found no significant association between diabetes and total dairy product intake or milk intake, but a higher combined intake of fermented dairy products (cheese, yogurt, and thick fermented milk) was inversely associated with diabetes (HR, 0.88; 95% CI, 0.78–0.99; *P* trend, 0.02) in adjusted analyses comparing extreme intake quintiles. In separate analyses for yogurt and thick fermented milk intake, there was an inverse association with diabetes incidence in model 1, which was rendered nonsignificant after further adjustment. For cheese intake, there was a significant inverse association with type 2 diabetes in model 1 (HR, 0.84; 95% CI, 0.74–0.95), but this was attenuated and became nonsignificant upon further adjustment for confounders, though an inverse trend remained across increasing quintiles of cheese intake (*P* linear trend, 0.01). These findings were robust to a range of sensitivity analyses, and there were no interactions between dairy product intake and each of sex, BMI, physical activity, or smoking habit and the risk of type 2 diabetes.

EPIC-Norfolk study: intake of dairy products assessed using a prospective food diary

Though the EPIC-InterAct study provided the first large-scale evidence across Europe for the relationships of total and subtypes of dairy products and diabetes and contributed meaningfully to the research area, there were further unresolved issues. The issue of distinguishing between low-fat and high-fat dairy products could not be addressed in InterAct, while other studies that addressed this relied largely on participants' self-report of preselected food items, indicating low-fat varieties with a variable degree of comprehensiveness.[8,10] This was due to the fact that research thus far had predominantly used the FFQ, which is a commonly used dietary assessment tool in nutritional research because of its comparative ease of dietary data collection and its relatively lower cost to administer and analyze in large studies. Well-known limitations, however, include the restrictive preselected list of food items as well as the issues of errors due to misreporting based on the need to recall dietary information over the prior year. The EPIC-Norfolk study provided a unique opportunity to assess dietary intake of dairy products with a real-time 7-day food diary. This offered the advantages of being able to capture the intake of all food items consumed by participants, including dairy products as main ingredients in composite dishes. Food weights were estimated using photographs that represented portion sizes, household measures, and standard units.[28] It also enabled the categorization of reported dairy product intakes into high- (or full-fat) and low-fat using 3.9% fat as a cutoff point, representing the fat content of whole milk in the United Kingdom. High-fat dairy included whole milk; all hard, processed, and soft cheese; full-fat, unripened cheese; cream; sour cream; crème fraiche; and butter. Low-fat dairy included all yogurt, semi-skimmed and skimmed milk, and low-fat unripened cheeses such as fromage frais and cottage cheese.[19]

The EPIC-Norfolk study is a population-based cohort study in Norfolk, United Kingdom, which recruited 25,639 men and women aged 40–79 years from lists of family physicians at baseline in 1993–1997.[29] Participants have been followed up for incident events. The case ascertainment and verification exercise used multiple sources of information with record linkage to medical records and yielded 892 incident cases of type 2 diabetes through July 2006. The investigators assembled a nested case-cohort design. This included 4000 subcohort participants selected at random from the entire cohort. Due to the random nature of the subcohort, 143 of the future 892 type 2 diabetes cases were included within the subcohort, which the case-cohort design allows for in the analysis (as described above for the design of the EPIC-InterAct study). After exclusions, the final sample included 4127 participants (753 diabetes cases and 3502 subcohort participants, including 128 cases in the subcohort).[19] With analyses that accounted for the case-cohort design,[26] modified Cox regression models were used with comprehensive adjustments for confounding factors. Model 1 included age and sex; model 2 additionally included BMI, family history, smoking, alcohol intake, physical activity, social class, and education; and model 3 additionally included dietary factors (total energy intake and intake of fruits, vegetables, red meat, processed meat, fiber, and coffee).[19] A number of sensitivity analyses and tests for interaction were included to test the robustness of the findings.

The higher consumption of low-fat fermented dairy products was associated with a lower risk of new-onset diabetes over 11 years compared with nonconsumption. Low-fat fermented dairy products consisted largely (87%) of yogurt but also included low-fat, unripened cheeses, e.g., fromage frais. In adjusted analyses, the HR for the association of low-fat, fermented dairy (highest compared with lowest tertile of intake) with incident diabetes was 0.76 (95% CI, 0.60–0.99; *P* trend, 0.049). For

yogurt intake, the corresponding HR was 0.72 (95% CI, 0.55–0.95; *P* trend, 0.017). Other subtypes of dairy and total dairy, whether high fat or low fat, were not significantly associated with type 2 diabetes risk.

Interpretation of findings from the EPIC-InterAct and EPIC-Norfolk studies

Despite the differences in the detail of dietary assessment used, with country-/center-specific FFQs in the EPIC-InterAct study and the 7-day food diary in the EPIC-Norfolk study, the overall findings were remarkably consistent. These findings suggested that the consumption of dairy subtypes, particularly of the fermented variety, rather than all dairy, may be beneficial for the prevention of diabetes, highlighting the relevance of food group subtypes for public health messages.

Overall, observational evidence for the connection between dairy intake and diabetes has been summarized to date in 4 metaanalyses.[7–10] Two of them included the results from the EPIC-InterAct study,[9,10] but none included the findings from the EPIC-Norfolk study, as these were unavailable at the time the metaanalyses were published. Within the metaanalyses, the pooled analyses showed an inverse association with total dairy intake, a finding not observed in the EPIC-based analyses, which may be due to the association with some, but not all, dairy subtypes.

While the analyses from the EPIC-InterAct and EPIC-Norfolk studies had several strengths, including the large sample size and number of cases included, the prospective study design, and the comprehensive adjustment for several relevant confounding factors, some of the limitations of nutritional epidemiology remain. The issue of misreporting based on recalled dietary intake with the FFQ was minimized by the use of the 7-day food diary (which records intake in real time) in the EPIC-Norfolk study. However, without repeat dietary assessment, both studies could not account for change in dietary habits over time. The issue of potential residual confounding is a possibility in both studies as the confounding factors may be measured with error or unknown confounders may remain unaccounted for.

A cause-and-effect relationship, for which a randomized trial would provide the highest form of evidence, cannot be established. However, in reality, such a trial is unlikely to be feasible for a dietary intervention for a "hard" endpoint such as type 2 diabetes, which would require participants to adhere to particular diets for several years in order to allow enough time for onset of the disease. Alternatively, criteria such as those proposed by Hill[30] can be used to appraise the likelihood of causal inference, including strength of association, consistency, repeatability, specificity, temporality, dose response, and biological plausibility. Regarding the final point, though the mechanisms of association between intakes of subtypes of dairy products and incidence of type 2 diabetes are not well understood, several possibilities exist. Potential mechanisms through which dairy products may generally exert beneficial effects include the many vitamins and minerals included in these products, such as calcium, vitamin D (in fortified dairy), and magnesium as well as high-quality protein. More specifically, fermented dairy products may have additional benefits through probiotic bacteria and menaquinones, as previously discussed.[18,19] Whether individual saturated fatty acids from dairy products also play a role in the etiology of type 2 diabetes is of interest but has been little researched. The EPIC-InterAct study provided an opportunity to investigate this issue.

EPIC-InterAct study: rationale and findings for objectively measured saturated fatty acids

Fatty acids are the building blocks of fat, and reducing the consumption of saturated fatty acids (SFAs) to below 10% or even below 7% of total energy intake has been deeply embedded in dietary guidelines.[11,31] The focus on dietary SFA reduction was based on cardioprotection related to the direct association between SFA intake and total- and LDL-cholesterol levels, the latter being an established risk factor for coronary heart disease. SFA intake has also been considered a risk factor for insulin resistance and diabetes.[32,33] However, a recent appraisal of the evidence highlighted the equivocal nature of the previous conclusions about SFA intake for both cardiovascular disease and diabetes.[15] For diabetes, neither observational evidence nor trial evidence supported an adverse effect of high SFA intake on risk of type 2 diabetes.[15] Indeed, the Women's Health Initiative Diet Modification trial suggested no benefit of a reduction in SFA intake on the incidence of type 2 diabetes.[34] A further issue is that within the SFA/metabolic disease paradigm there is accumulating incongruous evidence that dairy products, which are typically high in SFA content, are inversely associated with incident type 2 diabetes.[7–10,18,19]

In identifying the reasons for some of the observed discrepancies, it is important to acknowledge that previous research on SFA and diabetes focused on total SFA intake, without distinguishing between SFAs of different carbon chain lengths, which can have important differences in biological action. This, in turn, has been the result of past nutritional research that relied on dietary assessment based on self-report from questionnaires, which did not readily permit the examination of SFA of

different carbon chain lengths. In contrast, the objective measurement of SFAs of different carbon chain lengths in blood factions enables the assessment of individual SFAs.[35] While there are complexities of uncertainty about the extent to which circulating individual SFAs represent diet vs endogenous processes, it is of great interest to investigate the association between individual SFAs of different carbon chain lengths and incident type 2 diabetes in order to inform this field of inquiry. Past evidence is restricted to a handful of studies limited in sample size, number of diabetes cases, and the varying number of SFAs assessed using different methods.[36–42]

Thus, the aim was to investigate the prospective association between objectively measured individual SFAs in the plasma phospholipid fraction and incident type 2 diabetes using the advantages of the EPIC-InterAct study, including variation in SFA levels across 8 European countries.[43]

Described in detail previously,[43] for this investigation, a profile of 37 fatty acids in the plasma phospholipid fraction was measured using gas chromatography.[44] Each fatty acid was expressed in relative units as the percentage of total phospholipid fatty acids (mol%). Nine SFAs of different carbon chain lengths and with relative concentrations higher than 0.05% were included in the analyses, of which 15:0 and 17:0 were the 2 SFAs considered derived from dietary dairy fat.[35,45,46] In analyses that accounted for a range of potential confounders such as sociodemographics, obesity, and lifestyle factors, including diet and energy intake, these odd-chain SFAs were associated inversely with incident diabetes. Per 1 standard deviation difference in SFA, the HR for 15:0 (pentadecanoic acid) was 0.79 (95% CI, 0.73–0.85), and the HR for 17:0 (heptadecanoic acid) was 0.67 (95% CI, 0.63–0.71). In contrast, the even-chain SFAs were positively associated (14:0 [myristic acid] HR 1.15 [95% CI, 1.09–1.22], 16:0 [palmitic acid] HR 1.26 [95% CI, 1.15–1.37], and 18:0 [stearic acid] 1.06 [95% CI, 1.00–1.13]). When comparing quintiles of the SFA distribution for the odd-chain SFAs, the adjusted HR comparing the top with the bottom quintile of 15:0 was 0.46 (95% CI, 0.37–0.56; P trend, < 0.0001) and for 17:0 it was 0.24 (95% CI, 0.20–0.30; P trend, < 0.0001). Conversely, for the even-chain SFAs, the corresponding HRs were 1.64 (95% CI, 1.47–1.83) for 14:0, 1.75 (95% CI, 1.35–2.27) for 16:0, and 1.75 (95% CI, 1.46–2.09) for 18:0 (P trend across quintiles was < 0.0001 for all even-chain SFAs). Interpretation of the findings for even-chain SFAs is complex because these circulating SFAs are mainly derived from hepatic endogenous synthesis (de novo lipogenesis), stimulated by intakes of carbohydrates and alcohol.[35,47–49] However, the findings for odd-chain SFAs (15:0 and 17:0) can be understood in terms of their exogenous source from dairy fat.[35,45,46]

Overall contribution from the EPIC study and its implications

With the EPIC-InterAct study across 8 European countries and the EPIC-Norfolk study in the United Kingdom, large-scale and robust evidence has been generated among European populations on the association between the consumption of dairy products and the incidence of type 2 diabetes. There was a remarkable consistency of findings for fermented dairy products being inversely associated with diabetes across the 2 dietary instruments (FFQ in the EPIC-InterAct study and the 7-day food diary in the EPIC-Norfolk study). The EPIC-Norfolk study allowed greater differentiation by fat content status, i.e., low-fat fermented dairy products (including yogurt, where all yogurt was low-fat by virtue of $<3.9\%$ fat content) were inversely associated with diabetes risk. Moreover, the measurement of individual circulating SFAs in the InterAct study enabled the world's largest appraisal of the association of SFAs of different carbon chain lengths with the risk of type 2 diabetes. This is an important step toward recognizing that SFAs are not a single homogenous group and that differences exist between the differential health effects of subtypes of blood SFAs. The question of whether 15:0 and 17:0, presumed derived from dairy fat, have direct physiological effects on the development of diabetes or whether they are markers of other components in dairy is currently unclear and should be the subject of further research, together with gaining a better understanding of the extent to which the content of these odd-chain SFAs varies by type of dairy product. The implication, however, of the EPIC-InterAct study's blood fatty acid biomarker findings is that it informs the recognition that it is not enough to provide public health messages about overall saturated fat intake, but that more nuanced messages acknowledging the food sources of different types of SFAs are required.

Taken together, the findings from the EPIC study (EPIC-InterAct and EPIC-Norfolk) indicate that a public health focus solely on nutrients (e.g., SFAs) may be misplaced, and what is required is consideration of the food sources associated with those nutrients. For instance, both meat and dairy products are rich in total fat and SFAs, but their association with type 2 diabetes is in opposite directions: a positive association has been observed between red and processed meat intake and diabetes risk,[50–54] while there is now consistent evidence from EPIC[18,19] and elsewhere[7–10] for an inverse association between the consumption of specific types of dairy products and incident diabetes.

Box 1 **Sample of unanswered research questions regarding dairy products and health.**

- If there is a cause–effect relationship between dairy product consumption and type 2 diabetes, does dairy intake exert direct effects on insulin sensitivity or are the effects on diabetes risk exerted through changes in weight or obesity?
- Which components of dairy products exert the health effects, including specific fatty acids, protein, minerals, vitamins, and constituents associated with fermentation? What are the roles of milk sugars in dairy products? What are the mechanisms by which the components of dairy products exert health effects?
- To what extent are there differences in composition (e.g., of SFA content) and health effects by the type of dairy product, such as full-fat, low-fat or reduced-fat or fermented/nonfermented dairy, and are these dependent on cattle-feed? More specifically, how do different types of dairy products vary in their content of 15:0 and 17:0?
- What is the net effect on cardiometabolic risk of consuming reduced-fat dairy products that have added sugars?
- As sources of animal-derived protein and fat, how do dairy and meat products compare in their relative effects on cardiometabolic health, and is there a difference in meat products from ruminants vs other animals?
- Do the odd-chain SFAs such as 15:0 and 17:0 have direct physiological benefits or are they merely correlates of other beneficial substances in dairy products?
- What is the effect of fatty acids in dairy products (other than SFAs) on cardiometabolic risk?
- Are there healthy and less healthy dairy options? Should butter be included within the definition of "dairy" or kept separate?
- To what extent is the consumption of dairy products a marker of overall (healthier or unhealthier) diets and (healthier or unhealthier) lifestyles?
- What are the facilitators and barriers to dairy product consumption?

Findings in context and future directions

Considerable progress has been made through the EPIC study and other studies that have advanced understanding of the relationship between dairy consumption and development of type 2 diabetes. However, it is important to note that dairy products should be consumed within an overall healthy diet. Moreover, healthy diets should be complemented with other healthy lifestyle factors, such as taking part in regular physical activity, maintaining a healthy weight, and not smoking, to provide greater potential for the prevention of type 2 diabetes and other chronic diseases.

More research on dairy products and health is warranted because there are still unanswered questions. A nonexhaustive list of currently unresolved issues is outlined in Box 1. Research continues in order to address some of these unresolved issues, and a concerted effort by the scientific community will be needed to tie together the different strands of evidence that range from observational to experimental. A recent review has summarized much of the evidence thus far, including that from randomized clinical trials showing the effects of dairy intake on intermediate markers of cardiometabolic risk,[55] but much more research is still needed. Greater collaboration amongst different disciplines is also required to undertake collaborative research that spans nutritional epidemiology and dietary public health, as well as the study of physiological processes and biological mechanisms that underpin associations between dairy consumption and health outcomes.

CONCLUSION

Efforts to understand the mechanisms of association and to investigate potential cause–effect relationships between dairy consumption and health outcomes are ongoing, but the collective epidemiological findings thus far suggest that specific types of dairy products, particularly fermented dairy products including yogurt, may help prevent type 2 diabetes within overall healthy lifestyles. Such findings highlight the importance of considering food group subtypes (e.g., fermented dairy products such as yogurt), rather than overall food group categories (e.g., dairy products), when examining the role of diet in the prevention of chronic diseases.

Acknowledgments

The content of this article was presented as part of the Second Global Summit on the Health Benefits of Yogurt, held as a satellite to the Experimental Biology meeting in San Diego, California, on 30 April 2014.

The conference was organized by the American Society for Nutrition and Danone Institute International. The supplement coordinators are Sharon M. Donovan, University of Illinois at Urbana-Champaign, USA, and Raanan Shamir, Schneider Children's Medical Center, Israel.

Funding. N.G.F. received reimbursement for travel expenses from the American Society for Nutrition. She declined an honorarium for participation in the conference.
The InterAct project was funded by the European Union (Integrated Project LSHM-CT-2006-037197 in the Framework Programme 6 of the European Commission). The EPIC-Norfolk study is supported by program grants from the Medical Research Council UK and Cancer Research UK. The funders did not participate in the study design, data collection and analysis, interpretation, or manuscript preparation. N.G.F. is supported by the Medical Research Council Epidemiology Unit (MC_UU_12015/5).

Declaration of interest. The author has no relevant interests to declare.

REFERENCES

1. International Diabetes Federation. IDF Diabetes Atlas: 6th Editon. http://www.idf.org/diabetesatlas. Brussels, Belgium; 2013. Accessed 2 October 2014.
2. Gillies CL, Abrams KR, Lambert PC, et al. Pharmacological and lifestyle interventions to prevent or delay type 2 diabetes in people with impaired glucose tolerance: systematic review and meta-analysis. BMJ. 2007;334:299–307.
3. Knowler WC, Barrett-Connor E, Fowler SE, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med. 2002;346:393–403.
4. Ramachandran A, Snehalatha C, Mary S, et al. The Indian Diabetes Prevention Programme shows that lifestyle modification and metformin prevent type 2 diabetes in Asian Indian subjects with impaired glucose tolerance (IDPP-1). Diabetologia. 2006;49:289–297.
5. Tuomilehto J, Lindstrom J, Eriksson JG, et al. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N Engl J Med. 2001;344:1343–1350.
6. Li G, Zhang P, Wang J, et al. The long-term effect of lifestyle interventions to prevent diabetes in the China Da Qing Diabetes Prevention study: a 20-year follow-up study. Lancet. 2008;371:1783–1789.
7. Elwood PC, Pickering JE, Givens DI, Gallacher JE. The consumption of milk and dairy foods and the incidence of vascular disease and diabetes: an overview of the evidence. Lipids. 2010;45:925–939.
8. Tong X, Dong JY, Wu ZW, et al. Dairy consumption and risk of type 2 diabetes mellitus: a meta-analysis of cohort studies. Eur J Clin Nutr. 2011;65:1027–1031.
9. Gao D, Ning N, Wang C, et al. Dairy products consumption and risk of type 2 diabetes: systematic review and dose-response meta-analysis. PLoS ONE. 2013;8:e73965.
10. Aune D, Norat T, Romundstad P, et al. Dairy products and the risk of type 2 diabetes: a systematic review and dose-response meta-analysis of cohort studies. Am J Clin Nutr. 2013;98:1066–1083.
11. US Department of Agriculture, US Department of Health and Human Services. Dietary Guidelines for Americans, 2010. Washington DC; 2010. U.S. Government Printing Office.
12. Trichopoulou A, Orfanos P, Norat T, et al. Modified Mediterranean diet and survival: EPIC-elderly prospective cohort study. BMJ. 2005;330:991.
13. InterAct Consortium, Romaguera D, Guevara M, et al.; on behalf of the InterAct Consortium. Mediterranean diet and type 2 diabetes risk in the European Prospective Investigation into Cancer and Nutrition (EPIC) study: the InterAct project. Diabetes Care. 2011;34:1913–1918.
14. Weaver CM. How sound is the science behind the dietary recommendations for dairy? *Am J Clin Nutr*. 2014;99(5 Suppl):1217S–1222S.
15. Micha R, Mozaffarian D. Saturated fat and cardiometabolic risk factors, coronary heart disease, stroke, and diabetes: a fresh look at the evidence. Lipids. 2010;45:893–905.
16. Chowdhury R, Warnakula S, Kunutsor S, et al. Association of dietary, circulating, and supplement fatty acids with coronary risk: a systematic review and meta-analysis. Ann Intern Med. 2014;160:398–406.
17. Mozaffarian D, Ludwig DS. Dietary guidelines in the 21st century—a time for food. JAMA. 2010;304:681–682.
18. Sluijs I, Forouhi NG, Beulens JW, et al. The amount and type of dairy product intake and incident type 2 diabetes: results from the EPIC-InterAct study. Am J Clin Nutr. 2012;96:382–390.
19. O'Connor LM, Lentjes MA, Luben RN, et al. Dietary dairy product intake and incident type 2 diabetes: a prospective study using dietary data from a 7-day food diary. Diabetologia. 2014;57:909–917.
20. Langenberg C, Sharp S, Forouhi NG, et al. Design and cohort description of the InterAct Project: an examination of the interaction of genetic and lifestyle factors on the incidence of type 2 diabetes in the EPIC study. Diabetologia. 2011;54:2272–2282.
21. Montonen J, Jarvinen R, Heliovaara M, et al. Food consumption and the incidence of type II diabetes mellitus. Eur J Clin Nutr. 2005;59:441–448.
22. Lecomte P, Vol S, Caces E, et al. Five-year predictive factors of type 2 diabetes in men with impaired fasting glucose. Diabetes Metab. 2007;33:140–147.
23. Elwood PC, Pickering JE, Fehily AM. Milk and dairy consumption, diabetes and the metabolic syndrome: the Caerphilly prospective study. J Epidemiol Community Health. 2007;61:695–698.
24. International Dairy Federation. Bulletin: The World Dairy Situation. 2007. Brussels, Belgium.
25. Hjartaker A, Lagiou A, Slimani N, et al. Consumption of dairy products in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort: data from 35c955 24-hour dietary recalls in 10 European countries. Public Health Nutr. 2002;5:1259–1271.
26. Barlow WE, Ichikawa L, Rosner D, et al. Analysis of case-cohort designs. J Clin Epidemiol. 1999;52:1165–1172.
27. Margetts BM, Pietinen P. European Prospective Investigation into Cancer and Nutrition: validity studies on dietary assessment methods. Int J Epidemiol. 1997;26(Suppl 1):S1–S5.
28. Bingham SA, Welch AA, McTaggart A, et al. Nutritional methods in the European Prospective Investigation of Cancer in Norfolk. Public Health Nutr. 2001;4:847–858.
29. Day N, Oakes S, Luben R, et al. EPIC-Norfolk: study design and characteristics of the cohort. European Prospective Investigation of Cancer. Br J Cancer. 1999;80(Suppl 1):95–103.
30. Hill AB. The environment and disease: association or causation? Proc R Soc Med. 1965;58:295–300.
31. Lichtenstein AH, Appel LJ, Brands M, et al. Diet and lifestyle recommendations revision 2006: a scientific statement from the American Heart Association Nutrition Committee. Circulation. 2006;114:82–96.
32. Eyre H, Kahn R, Robertson RM, et al. Preventing cancer, cardiovascular disease, and diabetes: a common agenda for the American Cancer Society, the American Diabetes Association, and the American Heart Association. Circulation. 2004;109:3244–3255.
33. Riserus U, Willett WC, Hu FB. Dietary fats and prevention of type 2 diabetes. Prog Lipid Res. 2009;48:44–51.
34. Tinker LF, Bonds DE, Margolis KL, et al. Low-fat dietary pattern and risk of treated diabetes mellitus in postmenopausal women: the Women's Health Initiative randomized controlled dietary modification trial. Arch Intern Med. 2008;168:1500–1511.
35. Hodson L, Skeaff CM, Fielding BA. Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake. Prog Lipid Res. 2008;47:348–380.
36. Patel PS, Sharp SJ, Jansen E, et al. Fatty acids measured in plasma and erythrocyte-membrane phospholipids and derived by food-frequency questionnaire and the risk of new-onset type 2 diabetes: a pilot study in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk cohort. Am J Clin Nutr. 2010;92:1214–1222.
37. Krachler B, Norberg M, Eriksson JW, et al. Fatty acid profile of the erythrocyte membrane preceding development of type 2 diabetes mellitus. Nutr Metab Cardiovasc Dis. 2008;18:503–510.
38. Hodge AM, English DR, O'Dea K, et al. Plasma phospholipid and dietary fatty acids as predictors of type 2 diabetes: interpreting the role of linoleic acid. Am J Clin Nutr. 2007;86:189–197.
39. Kroger J, Zietemann V, Enzenbach C, et al. Erythrocyte membrane phospholipid fatty acids, desaturase activity, and dietary fatty acids in relation to risk of type 2 diabetes in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study. Am J Clin Nutr. 2011;93:127–142.
40. Wang L, Folsom AR, Zheng ZJ, et al. Plasma fatty acid composition and incidence of diabetes in middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) study. Am J Clin Nutr. 2003;78:91–98.

41. Laaksonen DE, Lakka TA, Lakka HM, et al. Serum fatty acid composition predicts development of impaired fasting glycaemia and diabetes in middle-aged men. Diabet Med. 2002;19:456–464.
42. Mozaffarian D, de Oliveira Otto MC, Lemaitre RN, et al. Trans-palmitoleic acid, other dairy fat biomarkers, and incident diabetes: the Multi-Ethnic Study of Atherosclerosis (MESA). Am J Clin Nutr. 2013;97:854–861.
43. Forouhi NG, Koulman A, Sharp SJ, et al. Differences in the prospective association between individual plasma phospholipid saturated fatty acids and incident type 2 diabetes: the EPIC-InterAct case-cohort study. Lancet. Diabetes Endocrinol. 2014;2:810–818.
44. Wang LY, Summerhill K, Rodriguez-Canas C, et al. Development and validation of a robust automated analysis of plasma phospholipid fatty acids for metabolic phenotyping of large epidemiological studies. Genome Med. 2013;5:39.
45. Smedman AE, Gustafsson IB, Berglund LG, et al. Pentadecanoic acid in serum as a marker for intake of milk fat: relations between intake of milk fat and metabolic risk factors. Am J Clin Nutr. 1999;69:22–29.
46. Wolk A, Vessby B, Ljung H, et al. Evaluation of a biological marker of dairy fat intake. Am J Clin Nutr. 1998;68:291–295.
47. Hudgins LC, Hellerstein M, Seidman C, et al. Human fatty acid synthesis is stimulated by a eucaloric low fat, high carbohydrate diet. J Clin Invest. 1996;97:2081–2091.
48. King IB, Lemaitre RN, Kestin M. Effect of a low-fat diet on fatty acid composition in red cells, plasma phospholipids, and cholesterol esters: investigation of a biomarker of total fat intake. Am J Clin Nutr. 2006;83:227–236.
49. Siler SQ, Neese RA, Hellerstein MK. De novo lipogenesis, lipid kinetics, and whole-body lipid balances in humans after acute alcohol consumption. Am J Clin Nutr. 1999;70:928–936.
50. Micha R, Wallace SK, Mozaffarian D. Red and processed meat consumption and risk of incident coronary heart disease, stroke, and diabetes mellitus: a systematic review and meta-analysis. Circulation. 2010;121:2271–2283.
51. Aune D, Ursin G, Veierod MB. Meat consumption and the risk of type 2 diabetes: a systematic review and meta-analysis of cohort studies. Diabetologia. 2009;52:2277–2287.
52. Pan A, Sun Q, Bernstein AM, et al. Red meat consumption and risk of type 2 diabetes: 3 cohorts of US adults and an updated meta-analysis. Am J Clin Nutr. 2011;94:1088–1096.
53. Bendinelli B, on behalf of the InterAct Consortium. Association between dietary meat consumption and incident type 2 diabetes: the EPIC-InterAct study. Diabetologia. 2012;56:47–59.
54. Pan A, Sun Q, Bernstein AM, et al. Changes in red meat consumption and subsequent risk of type 2 diabetes mellitus: three cohorts of US men and women. JAMA Intern Med. 2013;173:1328–1335.
55. Astrup A. Yogurt and dairy product consumption to prevent cardiometabolic diseases: epidemiologic and experimental studies. Am J Clin Nutr. 2014;99(5 Suppl):1235S–1242S.

Supplement Article

Impact of yogurt on appetite control, energy balance, and body composition

Angelo Tremblay, Caroline Doyon, and Marina Sanchez

Recent data support the idea that regular yogurt consumption promotes body weight stability. The simplest explanation is that regular consumption of healthful foods such as yogurt results in decreased intake of less healthful foods containing high amounts of fat and/or sugar. There is also evidence to suggest that the high calcium and protein contents of yogurt and other dairy foods influence appetite and energy intake. The existence of a calcium-specific appetite control mechanism has been proposed. Milk proteins differ in terms of absorption rate and post-absorptive responses, which can influence their satiating properties. Studies in humans have shown that consumption of milk and yogurt increases the circulating concentration of the anorectic peptides glucagon-like peptide (GLP)-1 and peptide YY (PYY). The food matrix can also affect appetite and satiety. Yogurt is a fermented milk that contains bacteria that enrich the microbiota of the host. It appears that lean vs obese humans differ in the composition of their gut microbiota. The available relevant literature suggests that yogurt is a food that facilitates the regulation of energy balance.

INTRODUCTION

In the context of the current obesity epidemic, the growing preoccupation with healthy eating has stimulated interest in research that documents the effects of specific foods on appetite and energy intake. Yogurt is a good candidate for a food with the potential to help manage appetite and body weight under free-living conditions. Indeed, yogurt is a nutrient-dense food that generally has a low energy density. It also offers flexibility in that it can be consumed daily at any meal or as a snack. As described here, yogurt is a satiating food that may favorably influence energy balance and body composition.

YOGURT AND BODY WEIGHT MANAGEMENT

The most consistent evidence to demonstrate that yogurt may favorably influence weight management has been reported by Zemel et al.[1] In a 1-year intervention, during which 1 portion of yogurt was consumed daily, African-American participants displayed a mean body fat loss of 4.9 kg.[1] In a subsequent clinical trial, yogurt supplementation was found to increase total and abdominal fat loss in obese individuals.[2] This research team reported on additional clinical trials that demonstrated that dairy[3,4] increased body weight loss in obese, low-calcium consumers.

Recent population data add evidence to support the idea that regular yogurt consumption promotes body weight stability. A study of large cohorts of participants revealed that yogurt and nuts were the food groups for which consumption was associated with the greatest weight loss over a 4-year follow-up period.[5] This is in agreement with another cohort analysis that highlighted the ability of regular yogurt consumers to maintain their body weight over time.[6] The ability of dairy foods such as yogurt to influence body weight can be

Affiliation: *A. Tremblay*, *C. Doyon*, and *M. Sanchez* are with the Department of Kinesiology, Pavillon de l'éducation physique et des sports (PEPS), Laval University, Quebec City, Quebec, Canada.

Correspondence: *A. Tremblay*, Department of Kinesiology, PEPS, Room 0234, 2300, rue de la Terrasse, Laval University, Quebec, Canada G1V 0A6. E-mail: angelo.tremblay@kin.ulaval.ca. Phone: +418-656-7294.

Key words: appetite, body composition, body weight, satiety, yogurt.

doi: 10.1093/nutrit/nuv015

explained by a variety of biological and circumstantial factors.

USE OF YOGURT TO REPLACE LESS HEALTHFUL FOODS

The simplest explanation of the proposed effect of dairy consumption on energy intake is that regular consumption of healthful foods such as yogurt results in a decreased intake of less healthful foods, which contain large amounts of fat and/or sugar. This concept is supported by the observation that a high intake of calcium, most of which is provided by dairy foods, was found to be negatively related to the consumption of carbonated and other sweetened beverages.[7] This study also demonstrated the existence of a significant association between calcium intake and variations in body fat, i.e., higher calcium intakes were associated with lower body fat. More recently, Chapelot and Payen[8] examined the effects of isocaloric portions of liquid yogurt and chocolate bars on appetite sensations. Their results showed that yogurt consumption resulted in a more pronounced effect on hunger, the desire to eat, and feelings of fullness. However, these beneficial effects of yogurt on appetite sensations were not accompanied by significant delays in requesting the next meal or a reduction in *ad libitum* energy intake at the subsequent meal.

SPECIFIC EFFECTS OF NUTRIENTS

Variations in the need for and in the metabolism, stores, and/or intakes of some nutrients are known to affect appetite control. These effects are related to carbohydrate, lipid, protein, and energy metabolism and have led to the formulation of classic theories of appetite control. More recently, Tordoff[9] proposed the existence of a calcium-specific appetite control mechanism by referring to the numerous physiological functions of calcium in vertebrates and to the fact that calcium deficiency promotes a preferential calcium intake in animals when the opportunity is given. This is in agreement with clinical experience in obese, female, very-low–calcium consumers who were tested in the context of a 15-week, diet-based, weight-reduction program with or without calcium and vitamin D supplementation.[10] Indeed, as shown in Table 1, mean body weight and fat loss were approximately 4 times greater in the supplement-receiving participants than in the nonsupplement-receiving controls, even when each group received the same dietary guidance. The table also shows that calcium (600 mg) + vitamin D (5 µg) supplementation twice daily induced a decrease in fat intake in a buffet-type meal test that was significantly different from the increased intake in the control group. Accordingly, a highly significant correlation was found between low fat intakes associated with the supplements and reductions in body weight and body fat during the program.

Table 1 **Mean change in body weight, fat mass, and *ad libitum* lipid intake (test meal) in obese very-low–calcium consumers in response to dietary restriction with or without calcium + vitamin D supplementation**

Measure	Calcium + vitamin D group	Control group
Body weight (kg)	−5.8	−1.4*
Fat mass (kg)	−4.7	−1.2*
Lipid intake (g)	−18.2	7.5*

*Significant difference between groups, $P < 0.05$.
Adapted from Major et al. (2009).[10]

Beyond the potential impact of low calcium intake on appetite control, other biological mechanisms may explain the influence of calcium on energy balance. According to Zemel et al.,[1] low calcium intake is related to an increase in intra-adipocyte calcium content, which promotes a switch from fat cell lipolysis toward lipogenesis. The resulting decrease in fat mobilization reduces fat utilization, which is concordant with the findings of Melanson et al.,[11,12] who showed that a low calcium intake favors a decrease in daily fat oxidation. Furthermore, calcium, particularly of dairy origin, promotes the formation of insoluble calcium soaps with fatty acids, thereby accentuating fecal fat loss by about 50–75 kcal/day.[13,14] To date, no link has been established between this gut-related effect of calcium and a calcium-specific appetite control.

Although the role of calcium in the regulation of energy and fat balance has been demonstrated consistently, it is relevant to emphasize that many clinical trials have not shown a body weight-reducing effect of dairy or calcium intake.[15] In one cohort of obese females participating in a weight-reduction program, the group receiving calcium–vitamin D supplementation displayed the same weight loss as the placebo-receiving controls[16]; however, when results were further analyzed to compare very-low–calcium consumers to those consuming a borderline or adequate amount of calcium, differences in body weight/fat loss were observed.[10] These differences, shown in Table 1, indicate that the impact of calcium–vitamin D supplementation on energy balance in very-low–calcium consumers was quantitatively important; the energy equivalent of the 3.5-kg between-group difference in fat loss was about 300 kcal/day. Taken together, these observations suggest that calcium supplementation is effective for augmenting weight loss in obese individuals whose consumption of the mineral is inadequate. This is concordant with the clinical trials of Zemel et al.,[2–4] who reported significant effects of calcium on weight/fat loss in obese, low- to very-low–calcium consumers.

Vitamin D is another dairy nutrient that has been considered for its potential independent effects on body weight. However, Soares et al.[17] recently reported a detailed literature survey that led to the observation that "the data on vitamin D supplementation during weight loss were too few to make firm conclusions."

Proteins are another nutrient in dairy foods that can induce a satiating effect. In milk, casein and whey protein represent the main protein components. They differ in terms of absorption rates and post-absorptive responses, which can influence their satiating properties. Whey protein is known to be more readily absorbed than casein,[18] which may explain its short-term effect on reducing appetite sensations and energy intake. For instance, in the study by Akhavan et al.,[19] 10–40 g of whey protein with water as a preload 30 minutes before a meal significantly reduced subsequent energy intake. Casein forms a clot via gastric acid action[20,21] that slows gastric emptying and mediates a sustained release of amino acids.[18] Thus, the rapid and sustained effect of dairy products on satiety and energy intake is likely attributable, in part, to the combined effects of its 2 main protein fractions.

Douglas et al.[22] recently investigated the effects of several yogurts on appetite and energy intake using the preload paradigm that is commonly used to measure the satiating effects of nutrients and different food vehicles. Specifically, they evaluated the impact of yogurts that contained different levels of protein on appetite markers and energy intake. They found that in healthy women, an afternoon snack of Greek yogurt with a high protein content (24 g) reduced hunger, increased fullness, and delayed subsequent eating compared with lower protein snacks or no snacks. Although the energy consumed at dinner was lower following the 160-kcal energy content of the yogurt snack vs no snack, energy intake was not fully compensated. This can be explained, in part, by the fact that the request for a dinner test meal was presented to participants who were served a high-protein snack almost 1 hour later than it was presented to those in a "no snack" control condition.

To summarize, evidence suggests that the high calcium and protein contents of yogurt and other dairy foods may explain, at least in part, the ability of yogurt to influence appetite and energy intake. This is corroborated by results of a recent clinical trial that demonstrated that milk supplementation facilitates appetite control during weight loss compared with an isocaloric, calcium-free, reduced-protein soy milk supplementation.[23]

IMPACT OF HORMONES

Dairy intake influences gastrointestinal hormones in a manner that is compatible with a hunger-reducing effect. Human studies have shown that milk and yogurt intake increase the circulating concentration of the anorectic peptides Glucagon-like peptide (GLP)-1 and Peptide YY (PYY).[24] This effect has also been demonstrated in obese individuals on a weight-loss regimen. Jones et al.[25] studied overweight and obese individuals on a calorie-restricted diet for 2 weeks, during which they received either a small amount of dairy (1 serving per day) or a large amount of dairy (3–4 servings per day). The use of a meal test before and after each condition demonstrated that a high dairy intake resulted in greater levels of PYY for several hours after the meal was ingested.

Plasma ghrelin concentrations also have been measured in obese, low-calcium consumers exposed to a diet-based, weight-reducing program that was supplemented with milk or an isoenergetic placebo.[23] Changes in ghrelin concentration predicted the desire to eat. In agreement with the above evidence, those in the supplemented group experienced decreases in the orexigenic hormone and a smaller increase in hunger and the desire to eat compared with those in the placebo groups.

EFFECTS OF FOOD STRUCTURE

The concept of the food matrix refers to differences in food structure that can modify properties of a food, independent of its nutrient content. The structure of yogurt lends itself to accommodate changes that affect appetite and food intake, such as the addition of fiber. Recently, Lluch et al.[26] reported on a study in which protein supplementation was tested along with a change in the food structure. Specifically, a control yogurt was supplemented with protein and the food matrix was modified to accommodate a fiber supplement. These modifications in the food matrix resulted in significant decreases in both subjective appetite and subsequent energy intake. Interestingly, these modifications did not affect palatability. The study was then expanded to test the effects of variations in the protein structure of yogurt and the addition of fibers to the food matrix. Variations in protein structure were tested based on the findings of preliminary laboratory work that allowed the comparison of isocaloric-, isovolumetric-, and isoproteinemic yogurts in which the whey protein-to-casein ratios were doubled. The main hypothesis was that the increase in the relative content of whey protein in a yogurt served at snack time would exert a more pronounced effect on the reduction of *ad libitum* energy intake at lunchtime. Preliminary results showed that mean energy intake at lunch was significantly decreased following consumption of yogurt high in whey and protein, which may be explained by the fact that this protein is more rapidly digested than casein.[27] Also of

interest is the fact that subsequent compensation in energy intake was substantially greater than the energy content of the yogurt preload.

A food's viscosity can also influence satiety. Tsuchiya et al.[28] compared the effects of 2 yogurts (semi-solid and liquid) on hunger and fullness and found that both resulted in lower hunger and higher fullness ratings compared with a fruit drink or a dairy-fruit drink.

YOGURT AS A VEHICLE FOR MICROORGANISMS

Yogurt contains bacteria that enrich the host's microbiota. This is relevant in the study of obesity, as it appears there are differences between lean and obese humans in the composition of their gut microbiota.[29] A decrease in *Bacteroidetes* and an increase in the *Firmicutes-to-Bacteroidetes* ratio in obese participants compared with lean participants have been observed.[30] Furthermore, the gut microbiota has been proposed to modulate energy intake and appetite in humans through its fermentation activity and the regulation of gut peptide secretion.[31]

Recent research offers unique perspectives regarding the use of probiotics in the management of obesity. Yogurt represents an ideal food vehicle for the incorporation of probiotics that can reinforce the microbiota and favorably modify its composition. For instance, Kadooka et al.[32] showed that supplementation of fermented milk with *Lactobacillus gasseri* SBT2055 over 12 weeks induced significant weight loss and a decrease in abdominal fat in overweight men and women. Recently, a placebo-controlled, double-blind, crossover clinical study reported that consumption of 2 servings per day of yogurt supplemented with *Lactobacillus amylovorus* (10^9 colony-forming units per serving of yogurt during 43 days) led to a decrease in total body fat mass.[33] Furthermore, Ilmonen et al.[34] showed that nutritional counseling combined with probiotic supplementation (*Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* Bb12) in pregnant women reduced the risk of central adiposity and improved control of glycemia at 6 months postpartum.[34]

A clinical trial was recently completed in which obese men and women adhered to a diet-based, 12-week weight loss program followed by 12 weeks of weight maintenance.[35] In both groups (men and women), participants were randomly assigned to supplementation of *L. rhamnosus* CGMCC1.3724 (3.24×10^8 colony-forming units) or a placebo during the 2 phases of the program. In men, changes in body weight and fat were comparable in the weight-loss and the weight-maintenance phases of the program. This contrasts with results obtained in women in whom the probiotic supplementation accentuated body weight and fat loss in the 2 phases. Accordingly, the data indicated that energy intake tended to be reduced more in women given the probiotic supplements.[35] As shown in Table 2, the between-group difference in body composition suggests that the estimated global energy deficit over 24 weeks was about 50% greater in the supplemented participants. Furthermore, results demonstrated that the abundance of *Lachnospiraceae*, a strain of the Firmicutes family, was significantly decreased in the women who were supplemented compared with the women in the control group.

Table 2 **Mean reductions in body weight, fat, and energy in obese women subjected to 12-week dietary restriction with or without probiotic supplementation with *Lactobacillus rhamnosus* CGMCC1.3724**

Measure	Supplementation group	Placebo group
Body weight (kg)	−4.4*	−2.6
Fat mass (kg)	−3.75*	−2.55
Body energy (kcal/d)	−421*	−283

*Significant difference between groups, $P < 0.05$.
Adapted from Sanchez et al. (2014).[35]

CONCLUSION

The available relevant literature suggests that yogurt facilitates the regulation of energy balance. This can be explained by the fact that yogurt consumption may reduce the intake of energy-dense foods that favor hyperphagia. Some studies have also emphasized the potential of yogurt nutrients such as calcium and proteins to favorably influence appetite control. In addition, the flexibility of yogurt's structure enables it to accommodate supplementation of ingredients, e.g., fibers and bacteria that also have the potential to promote negative energy balance. These effects are likely the main determinants of the observed weight loss of several kilograms documented in yogurt consumers tested in clinical interventions and observational studies.

Acknowledgments

The content of this article was presented as part of the Second Global Summit on the Health Benefits of Yogurt, held as a satellite to the Experimental Biology meeting in San Diego, California, on 30 April 2014. The conference was organized by the American Society for Nutrition and Danone Institute International. The supplement coordinators are Sharon M. Donovan, University of Illinois at Urbana-Champaign, USA, and Raanan Shamir, Schneider Children's Medical Center, Israel.

Funding. Writing and editorial assistance were provided by Densie Webb, PhD, RD, who was contracted and funded by Danone Institute International. A.T. received

financial reimbursement for travel expenses and an honorarium from the Danone Institute International for his participation in the conference.

Declaration of interest. A.T. serves on the Yogurt in Nutrition Initiative for Health Advisory Board for the Danone Institute International. His research has been funded, in part, by Dairy Farmers of Canada, the Dairy Research Institute of the United States, Wyeth Consumer Healthcare, and Nestlé. The other authors have no relevant interests to declare.

REFERENCES

1. Zemel MB, Shi H, Greer B, et al. Regulation of adiposity by dietary calcium. FASEB J. 2000;14:1132–1138.
2. Zemel MB, Richards J, Mathis S, et al. Dairy augmentation of total and central fat loss in obese subjects. Int J Obes. 2005;29:391–397.
3. Zemel MB, Richards J, Milstead A, et al. Effects of calcium and dairy on body composition and weight loss in African-American adults. Obes Res. 2005;13: 1218–1225.
4. Zemel MB, Thompson W, Milstead A, et al. Calcium and dairy acceleration of weight and fat loss during energy restriction in obese adults. Obes Res. 2004;12: 582–590.
5. Mozaffarian D, Hao T, Rimm EB, et al. Changes in diet and lifestyle and long-term weight gain in women and men. N Engl J Med. 2011;364:2392–2404.
6. Wang H, Troy LM, Rogers GT, et al. Longitudinal association between dairy consumption and changes of body weight and waist circumference: the Framingham Heart Study. Int J Obes. 2014;38:299–305.
7. Skinner JD, Bounds W, Carruth BR, et al. Longitudinal calcium intake is negatively related to children's body fat indexes. J Am Diet Assoc. 2003;103:1626–1631.
8. Chapelot D, Payen F. Comparison of the effects of a liquid yogurt and chocolate bars on satiety: a multidimensional approach. Br J Nutr. 2010;103:760–767.
9. Tordoff MG. Calcium: taste, intake, and appetite. Physiol Rev. 2001;81:1567–1597.
10. Major GC, Alarie FP, Dore J, et al. Calcium plus vitamin D supplementation and fat mass loss in female very low-calcium consumers: potential link with a calcium-specific appetite control. Br J Nutr. 2009;101:659–663.
11. Melanson EL, Donahoo WT, Dong F, et al. Effect of low- and high-calcium dairy-based diets on macronutrient oxidation in humans. Obes Res. 2005;13: 2102–2112.
12. Melanson EL, Sharp TA, Schneider J, et al. Relation between calcium intake and fat oxidation in adult humans. Int J Obes Relat Metab Disord. 2003;27:196–203.
13. Christensen R, Lorenzen JK, Svith CR, et al. Effect of calcium from dairy and dietary supplements on faecal fat excretion: a meta-analysis of randomized controlled trials. Obes Rev. 2009;10:475–486.
14. Jacobsen R, Lorenzen JK, Toubro S, et al. Effect of short-term high dietary calcium intake on 24-h energy expenditure, fat oxidation, and fecal fat excretion. Int J Obes. 2005;29:292–301.
15. Lanou AJ, Barnard ND. Dairy and weight loss hypothesis: an evaluation of the clinical trials. Nutr Rev. 2008;66:272–279.
16. Major GC, Alarie F, Dore J, et al. Supplementation with calcium + vitamin D enhances the beneficial effect of weight loss on plasma lipid and lipoprotein concentrations. Am J Clin Nutr. 2007;85:54–59.
17. Soares MJ, Ping-Delfos WC, Sherriff JL, et al. Vitamin D and parathyroid hormone in insulin resistance of abdominal obesity: cause or effect? Eur J Clin Nutr. 2011; 65:1348–1352.
18. Boirie Y, Dangin M, Gachon P, et al. Slow and fast dietary proteins differently modulate postprandial protein accretion. Proc Natl Acad Sci U S A. 1997;94: 14930–14935.
19. Akhavan T, Luhovyy BL, Brown PH, et al. Effect of premeal consumption of whey protein and its hydrolysate on food intake and postmeal glycemia and insulin responses in young adults. Am J Clin Nutr. 2010;91:966–975.
20. Billeaud C, Guillet J, Sandler B. Gastric emptying in infants with or without gastro-oesophageal reflux according to the type of milk. Eur J Clin Nutr 1990;44: 577–583.
21. Miller MJ, Witherly SA, Clark DA. Casein: a milk protein with diverse biologic consequences. Proc Soc Exp Biol Med. 1990;195:143–159.
22. Douglas SM, Ortinau LC, Hoertel HA, et al. Low, moderate, or high protein yogurt snacks on appetite control and subsequent eating in healthy women. Appetite 2013;60:117–122.
23. Gilbert JA, Joanisse DR, Chaput JP, et al. Milk supplementation facilitates appetite control in obese women during weight loss: a randomised, single-blind, placebo-controlled trial. Br J Nutr. 2011;105:133–143.
24. Veldhorst M, Smeets A, Soenen S, et al. Protein-induced satiety: effects and mechanisms of different proteins. Physiol Behav. 2008;94:300–307.
25. Jones KW, Eller LK, Parnell JA, et al. Effect of a dairy- and calcium-rich diet on weight loss and appetite during energy restriction in overweight and obese adults: a randomized trial. Eur J Clin Nutr. 2013;67:371–376.
26. Lluch A, Hanet-Geisen N, Salah S, et al. Short-term appetite-reducing effects of a low-fat dairy product enriched with protein and fibre. Food Qual Pref. 2010;21: 402–409.
27. Doyon CY, Tremblay A, Rioux L-E, et al. Acute effects of protein composition and fibre enrichment of yogurt consumed as snacks on appetite sensations and subsequent ad libitum energy intake in healthy men. Appl Physiol Nutr Metab. In Press.
28. Tsuchiya A, Almiron-Roig E, Lluch A, et al. Higher satiety ratings following yogurt consumption relative to fruit drink or dairy fruit drink. J Am Diet Assoc. 2006;106: 550–557.
29. Ley RE, Turnbaugh PJ, Klein S, et al. Microbial ecology: human gut microbes associated with obesity. Nature. 2006;444:1022–1023.
30. Turnbaugh PJ, Ley RE, Mahowald MA, et al. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006;444:1027–1031.
31. Delzenne NM, Cani PD. Interaction between obesity and the gut microbiota: relevance in nutrition. Annu Rev Nutr. 2011;31:15–31.
32. Kadooka Y, Sato M, Imaizumi K, et al. Regulation of abdominal adiposity by probiotics (*Lactobacillus gasseri* SBT2055) in adults with obese tendencies in a randomized controlled trial. Eur J Clin Nutr. 2010;64:636–643.
33. Omar JM, Chan YM, Jones ML, et al. *Lactobacillus fermentum* and *Lactobacillus amylovorus* as probiotics alter body adiposity and gut microflora in healthy persons. J Func Foods 2013;5:116–123.
34. Ilmonen J, Isolauri E, Poussa T, et al. Impact of dietary counselling and probiotic intervention on maternal anthropometric measurements during and after pregnancy: a randomized placebo-controlled trial. Clin Nutr. 2011;30: 156–164.
35. Sanchez M, Darimont C, Drapeau V, et al. Effect of *Lactobacillus rhamnosus* CGMCC1.3724 supplementation on weight loss and maintenance in obese men and women. Br J Nutr. 2014;111:1507–1519.

Supplement Article

Microbiota and the gut–brain axis

John Bienenstock, Wolfgang Kunze, and Paul Forsythe

Changes in gut microbiota can modulate the peripheral and central nervous systems, resulting in altered brain functioning, and suggesting the existence of a microbiota gut–brain axis. Diet can also change the profile of gut microbiota and, thereby, behavior. Effects of bacteria on the nervous system cannot be disassociated from effects on the immune system since the two are in constant bidirectional communication. While the composition of the gut microbiota varies greatly among individuals, alterations to the balance and content of common gut microbes may affect the production of molecules such as neurotransmitters, e.g., gamma amino butyric acid, and the products of fermentation, e.g., the short chain fatty acids butyrate, propionate, and acetate. Short chain fatty acids, which are pleomorphic, especially butyrate, positively influence host metabolism by promoting glucose and energy homeostasis, regulating immune responses and epithelial cell growth, and promoting the functioning of the central and peripheral nervous systems. In the future, the composition, diversity, and function of specific probiotics, coupled with similar, more detailed knowledge about gut microbiota, will potentially help in developing more effective diet- and drug-based therapies.

INTRODUCTION

The gut microbiota is composed of trillions of microbes that influence normal physiology and alter the host's susceptibility to disease.[1] A growing body of evidence in animals supports the concept that the gut microbiota influences emotional behavior.[2–6] Changes in the gut microbiota or intestinal exposure to specific bacteria can modulate the peripheral and central nervous systems (CNS) in animals, resulting in altered brain functioning and suggesting the existence of a microbiota gut–brain axis.[7] There is good evidence from animal studies that gut bacteria influence brain chemistry and development and that the enteric nervous system, including the sensory vagus nerve, appears to be able to differentiate between nonpathogenic and potentially pathogenic bacteria[7] and may play a critical role in mediating the effects of gut microorganisms on behavior.[7,8] Because the nervous system has constant bidirectional communication with the immune system, the effects of bacteria on the nervous system cannot be disassociated from effects on the immune system. This type of crosstalk occurs regularly and can have profound neurological and immunological effects. However, the exact molecules responsible for host–microbe communication remain largely unknown.[9] Studies are currently addressing this neural circuitry and investigating the extent of the influence of gut microbiota on the CNS and on behavior,[7] as well as the therapeutic potential of probiotics for a range of immune disorders.[10] Microbiota products and metabolites also may promote metabolic benefits such as reduced body weight, reduced adiposity, improved glucose control, and improved insulin sensitivity via gut–brain neural circuits.[11]

Affiliation: *J. Bienenstock*, *W. Kunze*, and *P. Forsythe* are with the McMaster Brain-Body Institute at St Joseph's Healthcare Hamilton, Hamilton, Ontario, Canada. *J. Bienenstock* is with the Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada. *W. Kunze* is with the Department of Psychiatry and Behavioral Neurosciences, McMaster University, Hamilton, Ontario, Canada. *P. Forsythe* is with the Department of Medicine, McMaster University, Hamilton, Ontario, Canada.

Correspondence: *J. Bienenstock*, Professor of Medicine and Pathology, McMaster University, and Director, McMaster Brain-Body Institute, St Joseph's Healthcare Hamilton, 50 Charlton Ave. E, Hamilton, Ontario, Canada L8N4A6. E-mail: bienens@mcmaster.ca

Key words: behavior, brain, dysbiosis, gut, microbiota, probiotics.

doi: 10.1093/nutrit/nuv019

PROBIOTICS AND HEALTH

While the composition of the gut microbiota varies greatly among individuals, alterations to the balance of common gut microbes may affect production of the short-chain fatty acids (SCFAs), butyrate, propionate, and acetate, which are products of intestinal bacterial fermentation that regulate intestinal adaptive immune responses[12] and play key roles in CNS function.[11,13,14] Butyrate has direct effects on the growth, maturation, and functioning of gut epithelial cells,[13] on Treg cells of the immune system (regulatory T cells that play a key role in preventing autoimmunity), and on the nervous system, inhibiting IKca (an intermediate conductance Ca activated K+ channel).[15]

Germ-free mice have reduced concentrations of SCFAs compared with normal animals.[12] Smith et al.[12] fed germ-free mice SCFAs for 3 weeks and found that individual SCFAs or a combination of SCFAs increased immunoregulatory Treg cells. Ochoa-Reparaz et al.[16,17] found that *Bacteroides fragilis* or its exopolysaccharide, polysaccharide A (PSA), given to mice in experimental autoimmune encephalomyelitis models protected against CNS demyelinating disease both prophylactically and therapeutically.

A recent study also found that SCFAs, especially butyrate, positively influenced host metabolism by activating intestinal gluconeogenesis, both in insulin-sensitive and insulin-insensitive states, promoting glucose and energy homeostasis.[11] Diets high in nondigestible carbohydrates lower the pH in the proximal colon, which may be an important factor in butyrate production.[13] Propionate and acetate also have been found to promote satiety.[18] The metabolic benefits on body weight and glucose control induced by SCFAs or dietary fiber in normal mice are absent in mice deficient for intestinal gluconeogenesis, despite similar modifications in gut microbiota composition.[11] This finding suggests that while diet is a major factor in determining the composition of the gut microbiota and the production of SCFAs in mice, there is a strong interaction with genotype that influences outcomes.[11]

Altered gut microbiota can also be responsible for pathophysiology in the colon.[19,20] Gut microbiota–host misadaptation (dysbiosis) has been implicated in the rising incidence of inflammatory diseases such as inflammatory bowel disease.[1] Patients with inflammatory bowel disease or irritable bowel syndrome experience reduced levels of *Lactobacillus* and *Bifidobacterium* species in the gut.[19,20] Animal models indicate a role for bacteria in the adequacy of immune regulation and the development of intestinal inflammation. It is likely that reduced numbers and diversity of normal beneficial commensals such as lactobacilli and bifidobacteria play an important role in allowing detrimental microbes such as *Citrobacter rodentium* and *Escherichia coli* access to the epithelial surface.[21] Disease improvement occurs following manipulation of the gut microbiota with *Lactobacillus rhamnosus* and *Lactobacillus helveticus* probiotics.[21] Animals administered *B. fragilis* or even PSA, which initiates beneficial immune responses, can be protected from experimental colitis.[9] Probiotics can also improve gut dysfunction induced by stress, in part, by normalization of hypothalamic–pituitary–adrenal axis (HPA) activity.[21]

PROBIOTICS AND BEHAVIOR

The HPA reaction to stress is programmed in early life (at least in rodents). A landmark study from Japan demonstrated that early exposure to gut microbiota reduces the exaggerated HPA responses of germ-free mice in adulthood but not if given to adult animals.[22] Plasma ACTH and corticosterone levels were greater in response to stress in germ-free mice compared with specific pathogen-free mice.[22] Studies have shown similar results in normal, healthy mice and rats, i.e., that feeding a probiotic can attenuate the HPA axis response to stress.[15,23,24] Finally, it is important to recognize that stress itself has a major consequence on the composition and function of the gut microbiota.[25]

Mice fed *L. rhamnosus* JB-1 for 28 days experienced changes in certain Gamma amino butyric acid (GABA) receptors in different regions of the brain, increased anxiolytic behavior, and inhibition of corticosterone response to acute stress.[23] These changes were compatible with benzodiazepine effects. The neurochemical and behavioral effects were not found, however, in vagotomized animals, indicating that the vagus nerve is a major communication pathway between such bacteria in the gut and the brain.[23] Screening for this type of enteric nervous system activity could possibly provide potential treatments for anxiety and stress.

Recent unpublished research shows that the amount of the neurotransmitters GABA and glutamate can be increased in the brain by feeding the same probiotic bacteria to animals. However, the effects are limited, are time dependent, and depend on the continued presence of the starting probiotic.

It also has been demonstrated that when animals are fed *L. rhamnosus*, effects can be seen not only on the local nervous system but systemically as well.[26] Indeed, the intestinal microbiota can clearly influence brain chemistry and behavior in mice independent of the autonomic nervous system, gastrointestinal-specific neurotransmitters, or inflammation.[27] Fecal transplants from specific pathogen-free NIH Swiss mice, which are relatively not anxious, to BALB/c mice, which are

relatively anxious, surprisingly showed that the behavior of the animals was dependent on the source of fecal/microbiota material. Colonization of germ-free BALB/c mice with microbiota from NIH Swiss mice increased exploratory behavior, suggesting reduced anxiety, and increased hippocampal levels of brain-derived neurotrophic factor, which is important for the growth, differentiation, and maturation of neurons. In turn, colonization of germ-free NIH Swiss mice with BALB/c microbiota reduced exploratory behavior, suggesting an increase in anxiety. These changes were unaffected by vagotomy.

Diet can also change the profile of gut microbiota and, thereby, host behavior. Li et al.[28] showed that changing the composition of the diet of rodents altered the spatial memory of the recipients, indicating that nutrition and diet must be taken into account in such studies.

Research with a mutant bacteria devoid of PSA suggests that this component is necessary and sufficient for acute activation of intestinal sensory neurons, i.e., PSA can mimic the effects of the parent organism on the nervous system, much as it can mimic its immunological effects.[9,29] Thus, components of bacteria may themselves have the capacity to affect the functions of the nervous system. These findings support the concept that the luminal content of the gut and the bacteria contained within are important factors in determining behavior and even cognition in animals.[27]

HUMAN RESEARCH

The strong evidence in animals of a direct link between the gut microbiota and the brain has led to the suggestion that the effect might be similar in humans. Bercik et al.[27] suggested that intestinal dysbiosis might contribute to psychiatric disorders in patients with bowel disorders. However, to date, there has been very little evidence in humans that probiotics will have the same neurochemical and behavioral effects observed in animals. In a double-blind, randomized, placebo-controlled study, Messaoudi et al.[30] administered a probiotic formula (*L. helveticus* and *Bifidobacerium longum*) to healthy women for 30 days and then assessed the recipients' level of anxiety and depression and 24-h urinary-free cortisol levels. In the female volunteers, daily administration of the probiotic formula alleviated psychological distress as indicated in 3 behavioral assessments, and 24-h urinary cortisol was reduced in the treated women.

In another clinical pilot study, 39 patients with a diagnosis of chronic fatigue syndrome were randomly assigned to receive *Lactobacillus casei* Shirota or a placebo daily for 2 months. There was a significant decrease in anxiety symptoms in the treated group.[31] A more recent clinical study was performed in 23 healthy women volunteers with no gastrointestinal or psychiatric symptoms. The women were randomly assigned to groups given either a fermented milk product (*Bifidobacterium animalis*, *Streptococcus thermophilus*, *L. bulgaricus*, and *Lactococcus lactis*) or a placebo, which consisted of a nonfermented milk product adjusted for taste and texture, twice daily for 4 weeks.[32] Consumption of the fermented milk product had a robust effect on activity of the brain regions that control central processing of emotion and sensation, as observed with functional magnetic resonance imaging before and after consumption of the fermented milk product.

Some individuals diagnosed with autism spectrum disorders also display a spectrum of gastrointestinal abnormalities.[33] A recent study examined an animal model for the neurodevelopment disorders of autism in which pregnant mice were injected with a viral mimic (POLY I:C). This produced typical stereotypical autistic behaviors in the offspring that lasted into adulthood. Oral administration of *B. fragilis* to pregnant mice before and immediately after birth resulted in the development of significantly diminished autistic behaviors.[33] In this model, the administration of PSA did not prevent all of the abnormalities. However, the findings suggest that the incidence of viral infection over the course of pregnancy may produce lasting effects, which are potentially reversible by the oral administration of particular bacteria.

The findings of these clinical studies are consistent with the findings in rats and mice and suggest that the communication between gut microbiota and the brain is modifiable and may provide targets for the treatment of patients with heightened stress responses associated with gut dysbiosis.[32]

CONCLUSION

The changes that occur in the microbial content of the gut as a result of ingestion of probiotic bacteria or changing the balance of gut microbiota in other ways can trigger a variety of mechanisms. These include effects on the host immune, nervous, and endocrine systems, which in turn affect each other, demonstrating an important role of the crosstalk between the gut and the host. Behavior, mood, and the response to stress can all be affected by the ingestion of probiotic bacteria. These data are very exciting and have aroused great popular and scientific interest. There is currently a significant gap between the experimental and clinical data. The challenge now is to translate these animal findings into clinical application. In the future, the composition, diversity, and function of specific probiotics, coupled

with more detailed knowledge of the composition of gut microbiota, could potentially help in developing more effective diet and drug therapies.

Acknowledgments

The content of this article was presented as part of the Second Global Summit on the Health Benefits of Yogurt, held as a satellite to the Experimental Biology meeting in San Diego, California, on 30 April 2014. The conference was organized by the American Society for Nutrition and Danone Institute International. The supplement coordinators are Sharon M. Donovan, University of Illinois at Urbana-Champaign, USA, and Raanan Shamir, Schneider Children's Medical Center, Israel.

Funding. The authors gratefully acknowledge support and sponsorship provided by the Giovanni and Concetta Guglietti Family Foundation, National Science and Engineering Research Council (371955-2009 to W.K. and 371513-2009 to P.F.).

Writing and editorial assistance were provided by Densie Webb, PhD, RD, who was contracted and funded by Danone Institute International. J.B. received financial reimbursement for travel expenses and an honorarium from the Danone Institute International for participation in the conference.

Declaration of interest. The authors have no relevant interests to declare.

REFERENCES

1. Lozupone CA, Stombaugh JI, Gordon JI, et al. Diversity, stability and resilience of the human gut microbiota. Nature. 2012;489:220–230.
2. Forsythe P, Sudo N, Dinan T, et al. Mood and gut feelings. Brain Behav Immun. 2010;24:9–16.
3. Cryan JF, Dinan TG. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. Nat Rev Neurosci. 2012;13:701–712.
4. Collins SM, Surette M, Bercik P. The interplay between the intestinal microbiota and the brain. Nat Rev Microbiol. 2012;10:735–42.
5. Forsythe P, Kunze W. Voices from within: gut microbes and the CNS. Cell Mol Life Sci. 2013;70:55–69.
6. Dinan TG, Stanton C, Cryan JF. Psychobiotics: a novel class of psychotropic. Biol Psychiatry. 2013;74:720–726.
7. Forsythe P, Kunze WA, Bienenstock J. On communication between gut microbes and the brain. Curr Opin Gastroenterol. 2012;28:557–562.
8. Diaz Heijtz R, Wang S, Anuar F, et al. Normal gut microbiota modulates brain development and behavior. Proc Natl Acad Sci U S A. 2011;108:3047–3052.
9. Mazmanian SK, Round JL, Kasper DL. A microbial symbiosis factor prevents intestinal inflammatory disease. Nature. 2008;453:620–625.
10. Forsythe P, Bienenstock J. Immunomodulation by commensal and probiotic bacteria. Immunol Invest. 2010;39:429–448.
11. De Vadder F, Kovatcheva-Datchary P, Goncalves D, et al. Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits. Cell. 2014; 156:84–96.
12. Smith PM, Howitt MR, Panikov N, et al. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. Science. 2013;341: 569–573.
13. Louis P, Flint HJ. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. FEMS Microbiol Lett. 2009; 294:1–8.
14. Schroeder FA, Lin CL, Crusio WE, et al. Antidepressant-like effects of the histone deacetylase inhibitor, sodium butyrate, in the mouse. Biol Psychiatry. 2007; 62:55–64.
15. Kunze WA, Mao YK, Wang B, et al. *Lactobacillus reuteri* enhances excitability of colonic AH neurons by inhibiting calcium-dependent potassium channel opening. J Cell Mol Med. 2009;13:2261–2270.
16. Ochoa-Reparaz J, Mielcarz DW, Ditrio LE, et al. Central nervous system demyelinating disease protection by the human commensal *Bacteroides fragilis* depends on polysaccharide A expression. J Immunol. 2010;185:4101–4108.
17. Ochoa-Reparaz J, Mielcarz DW, Wang Y, et al. A polysaccharide from the human commensal *Bacteroides fragilis* protects against CNS demyelinating disease. Mucosal Immunol. 2010;3:487–495.
18. Arora T, Sharma R, Frost G. Propionate. Anti-obesity and satiety enhancing factor? Appetite. 2011;56:511–515.
19. Swidsinski A, Ladhoff A, Pernthaler A, et al. Mucosal flora in inflammatory bowel disease. Gastroenterology. 2002;122:44–54.
20. Madden JA, Hunter JO. A review of the role of the gut microflora in irritable bowel syndrome and the effects of probiotics. Br J Nutr. 2002;88 (Suppl 1): S67–S72.
21. Gareau MG, Jury J, MacQueen G, et al. Probiotic treatment of rat pups normalises corticosterone release and ameliorates colonic dysfunction induced by maternal separation. Gut. 2007;56:1522–1528.
22. Sudo N, Chida Y, Aiba Y, et al. Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. J Physiol. 2004; 558(Pt 1):263–275.
23. Bravo JA, Forsythe P, Chew MV, et al. Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proc Natl Acad Sci U S A. 2011;108:16050–16055.
24. Ait-Belgnaoui A, Durand H, Cartier C, et al. Prevention of gut leakiness by a probiotic treatment leads to attenuated HPA response to an acute psychological stress in rats. Psychoneuroendocrinology. 2012;37:1885–1895.
25. Lyte M. Microbial endocrinology and the microbiota-gut-brain axis. Adv Exp Med Biol. 2014;817:3–24.
26. Forsythe P, Wang B, Khambati I, et al. Systemic effects of ingested *Lactobacillus rhamnosus*: inhibition of mast cell membrane potassium (IKCa) current and degranulation. PLoS One. 2012;7:e41234.
27. Bercik P, Denou E, Collins J, et al. The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. Gastroenterology. 2011; 141:599–609, 609, e591–e593.
28. Li W, Dowd SE, Scurlock B, et al. Memory and learning behavior in mice is temporally associated with diet-induced alterations in gut bacteria. Physiol Behav. 2009; 96:557–567.
29. Mao YK, Kasper DL, Wang B, et al. *Bacteroides fragilis* polysaccharide A is necessary and sufficient for acute activation of intestinal sensory neurons. Nat Commun. 2013;4:1465–1475.
30. Messaoudi M, Lalonde R, Violle N, et al. Assessment of psychotropic-like properties of a probiotic formulation (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175) in rats and human subjects. Br J Nutr. 2011;105: 755–764.
31. Rao AV, Bested AC, Beaulne TM, et al. A randomized, double-blind, placebo-controlled pilot study of a probiotic in emotional symptoms of chronic fatigue syndrome. Gut Pathogens. 2009;1:6–11.
32. Tillisch K, Labus J, Kilpatrick L, et al. Consumption of fermented milk product with probiotic modulates brain activity. Gastroenterology. 2013;144:1394–1401, 1401, e1391–e1394.
33. Hsiao EY. Immune dysregulation in autism spectrum disorder. Int Rev Neurobiol. 2013;113:269–302.

Supplement Article

Potential role of the intestinal microbiota in programming health and disease

Olivier Goulet

The composition of the microbiota varies according to prenatal events, delivery methods, infant feeding, infant care environment, and antibiotic use. Postnatal gut function and immune development are largely influenced by the intestinal microbiota. Emerging evidence has shown that early microbiota colonization may influence the occurrence of later diseases (microbial programming). The vast majority of microbial species (commensals) give rise to symbiotic host–bacterial interactions that are fundamental for human health. However, changes in the composition of the gut microbiota (dysbiosis) may be associated with several clinical conditions, including obesity and metabolic diseases, autoimmune diseases and allergy, acute and chronic intestinal inflammation, irritable bowel syndrome (IBS), allergic gastroenteritis (e.g., eosinophilic gastroenteritis and allergic IBS), and necrotizing enterocolitis. Based on recent advances, modulation of gut microbiota with probiotics, prebiotics, or fermented dairy products has been suggested as a treatment of, or prevention for, different disorders such as IBS, infectious diarrhea, allergic disease, and necrotizing enterocolitis.

INTRODUCTION

The microbial communities hosted by the human gut comprise a new, fascinating, and promising area for understanding the development of gut functions and some health disorders and diseases, as well as their treatment and prevention. The microbial communities, previously called the "intestinal microflora," are composed of approximately 10^{14} bacteria, which represent approximately 10 times the number of cells in the human body.[1,2] These bacterial communities have been forged over millennia of co-evolution with humans to achieve a symbiotic relationship that leads to physiological homeostasis. Although the terms "microbiota" and "microbiome" are often used interchangeably, microbiota refers to the organisms that comprise the microbial community, whereas the microbiome refers to the collective genomes of the microbes, which are composed of bacteria, bacteriophages, fungi, protozoa, and viruses that live inside and on the human body. The microbiota is now considered a human organ, with its own functions, i.e., modulating expression of genes involved in mucosal barrier fortification, angiogenesis, and postnatal intestinal maturation.[3,4] The intestinal microbiota is involved in normal digestion and affects energy harvest from the diet and energy storage in the host, fermenting unavailable energy substrates such as fiber to short-chain fatty acids (SCFAs).[3,4]

The diversity of gut microbiota has been revealed by the application of high-throughput sequencing of the microbial ribosomal RNA or DNA (metagenome).[4] This has clearly shown that the microbiota is represented by more than 1500 microbial species. Metagenomic analyses and 16 S rRNA gene sequencing have shown that

Affiliation: *O. Goulet* is with the Department of Pediatric Gastroenterology-Hepatology-Nutrition, National Reference Center for Rare Digestive Disease, Hôpital Necker-EnfantsMalades, University of Paris Descartes, Paris, France.

Correspondence: *O. Goulet*, National Reference Center for Rare Digestive Disease, Reference Center for Home Parenteral Nutrition, Hôpital Necker EnfantsMalades-University Paris Descartes, 149 rue de Sèvres, 75015 Paris, France. E-mail: olivier.goulet@nck.aphp.fr. Phone: 00-33-1-44-49-25-60.

Key words: cesarean delivery, dysbiosis, innate immunity, inflammatory bowel disease, microbiota, obesity, postnatal development.

doi: 10.1093/nutrit/nuv039

Firmicutes and *Bacteroidetes* are the 2 dominant bacterial phyla in most individuals. Other phyla include *Proteobacteria*, *Actinobacteria*, *Fusobacteria*, and *Verrucomicrobia*.[4] More recently, groups of bacterial families have been classified into enterotypes on the basis of their functions. For example, classification may be based on metabolism of dietary components and ability to handle drugs. The classification should help to further understanding of the role of enteric microbiota in health and disease.[5] Aging is associated with changes in diversity of noncultured species, with a greater proportion of *Bacteroides* species, a distinct abundance of *Clostridium* clusters, an increased enterobacteria population, and a lower number of bifidobacteria.[6,7]

From birth, the normal gut microbiota contributes to the development of gut function, educates the immune system, contributes to the regulation and maintenance of intestinal barrier function, provides protection against infection, and promotes tolerance of foods. The vast majority of microbial species give rise to symbiotic host–bacteria interactions that are fundamental for human health. Disruption of the establishment of a stable normal gut microbiota may be associated with, or even contribute to, the pathogenesis of disease. Unfavorable changes in the composition of gut microbiota, referred to as dysbiosis, may be associated with several clinical conditions such as nosocomial infection, necrotizing enterocolitis (NEC) in premature infants, inflammatory bowel disease (IBD), obesity, autoimmune diseases, and allergies.

This review aims to highlight factors that influence the gut microbiota soon after birth and the potential harmful effects that occur later in life. Indeed, the intestinal microbiome may be influenced by the environment, resulting in modification of the risk profile for childhood and adult diseases. Due to the association between dysbiosis and disease, an emerging concept is so-called "microbial programming," which is analogous to, or even a component of, "metabolic programming."

FACTORS THAT INFLUENCE INTESTINAL MICROBIAL COLONIZATION

The important role of the resident microflora in human health has gained increased recognition over the past few decades. However, it is not possible to define a "normal microbiome," as healthy individuals can harbor different microbial consortia. It is important to consider the functional capability or the genetic potential of the microbiome (e.g., the bacterial metagenome).[4,5]

Originally, the intestine was thought to be sterile during fetal life. However, the finding of microbial DNA in meconium of preterm and term infants offers the opportunity to further explore the intra-amniotic microbial milieu of newly born infants.[8] Studies have contributed to the characterization of the uterine microbiome, specifically that present in amniotic fluid, fetal membranes, and placenta.[9,10] When present in the uterine compartment, some bacteria such as *Ureaplasma* spp. and *Fusobacterium* spp. appear to be the most significantly associated with negative pregnancy outcomes (e.g., prematurity).[9] Upon delivery, the neonate is exposed to microbes from a variety of sources, including maternal vaginal, fecal, and skin bacteria. Initial colonization of the infant gut is highly influenced by the mother's vaginal and fecal bacterial communities, which include facultative anaerobes such as streptococci and enterobacteriaceae. Indeed, the first and most important phase of normal colonization occurs when the newborn fetus passes through the birth canal and ingests maternal vaginal and colonic microorganisms. These bacteria further proliferate when oral feeding is initiated. After 48 h, the number of bacteria is already as high as approximately 10^4–10^6 colony-forming units per milliliter of intestinal contents. Many factors may influence this process, including gestational length (preterm or full-term), mode of delivery (vaginal or cesarean section), infant diet (breastfeeding or formula), birth environment of neonatal intensive care unit, and use of drugs such as antibiotics and proton pump inhibitors[11–13] (Figure 1).

Infants delivered by cesarean section have a reduced number of bacteria compared with vaginally delivered infants, and colonization by bifidobacteria can be delayed by up to 6 months.[12] The microbiota of vaginally delivered infants mirrors the mother's vaginal and intestinal microbiota. These infants exhibit bacterial communities composed of prominent genera such as *Lactobacillus*, *Prevotella*, *Escherichia*, *Bacteroides*, and *Bifidobacterium*. Biasucci et al.[12] reported that after delivery by cesarean section, the intestinal microbiota is characterized by an absence of bifidobacteria. Vaginally delivered neonates, even if they showed individual microbial profiles, were characterized by predominant groups such as *Bifidobacterium longum* and *Bifidobacterium catenulatum*.[12] By using multiplexed 16 S rRNA gene pyrosequencing to characterize bacterial communities from mothers and their newborns, Dominguez-Bello et al.[14] found that in direct contrast to the highly differentiated communities of their mothers, neonates harbored bacterial communities that were undifferentiated across multiple body habitats, regardless of delivery mode. The results show that vaginally delivered infants acquired bacterial communities resembling their own mother's vaginal microbiota, dominated by *Lactobacillus*, *Prevotella*, and *Sneathia* spp.; cesarean section-delivered infants harbored bacterial communities similar to those found on the skin surface, dominated by *Staphylococcus*, *Corynebacterium*, and *Propionibacterium* spp.

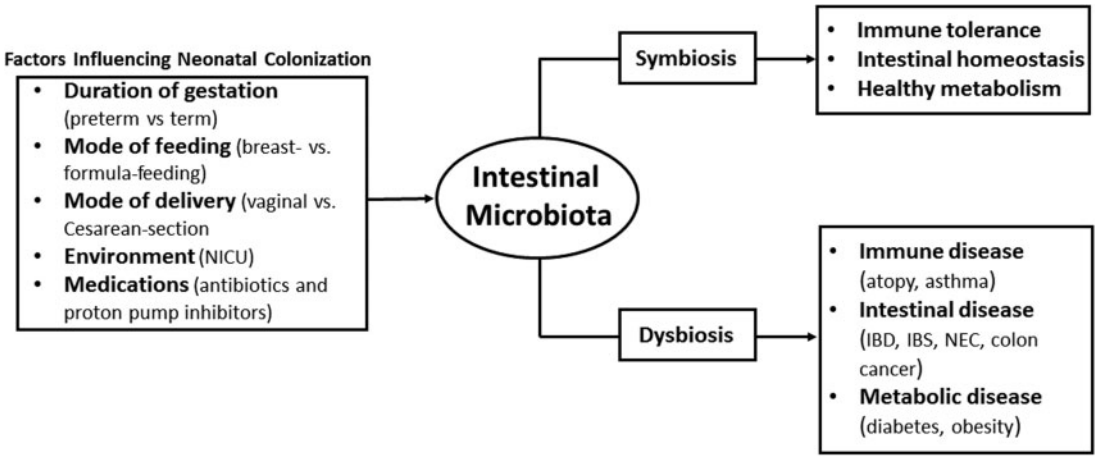

Figure 1 **Illustration of possible programming by the intestinal microbiota.** Abbreviations: IBD, irritable bowel disease; IBS, irritable bowel syndrome; NEC, necrotizing enterocolitis; NICU, neonatal intensive care unit

The pattern of bacterial colonization in the preterm infant differs from that in the healthy, full-term neonatal gut.[15] This "abnormal" colonization, mostly due to the routine use of sterile formula and antibiotics in neonatal intensive care units, could have a central role in feeding intolerance and in the development of NEC, a severe disease that primarily affects premature infants and often leads to death or extensive bowel resection (short bowel syndrome).[16]

The nature of oral feeding may influence the short-term composition of an infant's gut microbiota.[17] Human milk contains beneficial factors for the intestinal microbiota, such as human milk oligosaccharides (HMOs).[18] They function as prebiotics by stimulating the growth of *Bifidobacterium* and *Lactobacillus* spp., thereby selectively altering the microbial composition of the intestine.[18] It is likely that evolutionary selective pressure has equipped *Bifidobacterium infantis* with multiple enzymes for deconstructing human milk glycans. As a result, this subspecies is able to outcompete other bifidobacteria as well as other commensals and pathogens in the gut lumen of healthy, breastfed infants.[18] In formula-fed infants, enterococci, *Bacteroides* spp., and clostridia predominate.[19] In breastfed infants aged 1 month, there is a direct association between the levels of secretory immunoglobulin-A (IgA) in intestinal secretions and the number of bifidobacteria in the gut. Moreover, the level of the inflammatory cytokine interleukin-6 (IL-6) in intestinal secretions is inversely related to the number of *Bacteroides fragilis* organisms in the gut at 1 month of age.[20] Excessive inflammation in infancy may cause an increased risk of age-related gastroenteritis. It is suggested that HMOs not only stimulate *B. infantis* proliferation, they also activate important genes involved in the pro- and anti-inflammatory balance within the intestinal mucosa.[21,22] These observations provide additional evidence of the beneficial effects of breastfeeding for the newborn infant. In addition to HMOs, human milk contains other glycans with antimicrobial and prebiotic activity that are thought to have beneficial effects for the infant.[23] Moreover, there is accumulating evidence that human milk is not sterile but contains maternal-derived bacterial molecular motifs that are thought to influence the newborn's immune system development.[24] This procedure, referred to as "bacterial imprinting," requires further study.[24] However, comparative studies in infants fed infant formula have not carefully documented their effects on gut microbiota or health-promoting bacteria. Colonizing bacteria exist in a symbiotic relationship with the host, and immunologic homeostasis exists, protecting the infant from diseases. There is increasing evidence that the microbiome does not reach its adult composition until 2–3 years of age.[25] Finally, host defenses can be improved by feeding breast milk, which helps the immature intestinal mucosal immune system to develop and respond appropriately to highly variable bacterial colonization and food antigen loads. Later in life, the type of food consumed influences the intestinal microbiota profile.[26] In that regard, SCFAs, play a central role. SCFAs are organic fatty acids produced in the distal gut by bacterial fermentation of macrofibrous material that escapes digestion in the upper gastrointestinal (GI) tract and enters the colon. SCFAs are central to the physiology and metabolism of the colon. Resident bacteria can also metabolize dietary carcinogens, synthesize vitamins, and assist in the absorption of various molecules. Most of the SCFAs present in the colon (90%–95%) consist of acetate (60%), propionate (25%), and butyrate (15%). Butyrate is considered a major energy source for the colonic epithelium. SCFAs have been associated with improvement of metabolic functions in type 2 diabetes mellitus, including the control of blood glucose levels, insulin resistance, and Glucagon-like peptide

(GLP)-1 secretion.[27] These effects result from the different tissues that express SCFA receptors and, thus, become capable of responding to the beneficial effects induced by these molecules.[27]

Antibiotic usage changes gut microbiota. For example, administration of broad-spectrum antibiotics significantly reduced the relative abundance of *Bacteroidetes*, with a concurrent increase in *Firmicutes*.[28] Rapid reduction in microbial diversity is often observed after ingestion of antibiotics in infants aged <1 year, and complete recovery of the initial bacterial composition is not always achieved.[29] The understanding of the dynamics and mechanisms that underlie functional changes in the microbiome in response to antibiotic treatments remains limited. The response depends on the type of antibiotics, length of dosing, and baseline microbiome. A recent study provides an extensive description of gut microbiota responses to follow-up β-lactam therapy.[30] The results demonstrate that antibiotics that target specific pathogenic infections and diseases may alter gut microbial ecology and interactions with host metabolism to a much greater degree than previously assumed.[30]

Interestingly, it was found that in very low birth weight infants the meconium is not sterile and is less diverse from birth in infants who develop late-onset sepsis.[31] Prolonged use of antibiotics, which is common in preterm infants, profoundly decreased microbial diversity and promoted the growth of predominant pathogens such as *Clostridium*, *Klebsiella*, and *Veillonella* spp., which have been associated with neonatal sepsis. The authors suggested that there may be a "healthy microbiome" present in extremely premature neonates that may ameliorate risk of sepsis.[31] More research is needed to determine whether altered antibiotics, probiotics, or other novel therapies can reestablish a healthy microbiome in neonates. It was recently shown that disruption of the microbiota during maturation with low-dose antibiotic exposure can alter host metabolism and adiposity in mice.[32] By using low-dose penicillin delivered from birth in a mouse model, Cox et al.[32] demonstrated metabolic alterations and changes in ileal expression of genes involved in immunity. Administration of low-dose penicillin, even limited to early life, sufficiently perturbs the microbiota so as to modify body composition, indicating that microbiota interactions in infancy may be critical determinants of long-term host metabolic effects.

ROLES OF MICROBIOTA IN GUT FUNCTION DEVELOPMENT

Microbial colonization of the intestine is thought to play a particularly important role in postnatal development of the GI, metabolic, and immune systems. For example, Hooper et al.[33] reported that a single bacterial species, *Bacteroides thetaiotaomicron*, a prominent component of the normal mouse and human intestinal microbiome, modulates the expression of genes involved in several important intestinal functions, including nutrient absorption, mucosal barrier fortification, xenobiotic metabolism, angiogenesis, and postnatal intestinal maturation. Collectively, the gut microbiota also influences tissue regeneration, permeability of the epithelium, vascularization of the gut, and tissue homeostasis. More recently, Rakoff-Nahoum et al.[34] investigated changes in global intestinal gene expression through postnatal developmental transitions in wild-type mice. By using myeloid differentiation factor 88/TIR-domain-containing adapter-inducing interferon-β double-knockout mice, they reported profound alterations in small and large intestinal transcriptomes accompanying both weaning and puberty in wild-type mice. They defined the role of Toll-like receptors and IL-1 receptor family member signaling in postnatal gene expression programs and select ontogeny-specific phenotypes such as vascular and smooth muscle development and neonatal epithelial and mast cell homeostasis.[34]

The relationship between the gut microbiota and changes in GI motility has been investigated. For example, bacterial metabolites such as SCFAs and deconjugated bile salts have been shown to generate potent motor responses.[35] A study in mice showed that colonized mice had a faster intestinal transit time than germ-free mice.[36]

The gut microbiota protects against pathogens by competing for nutrients and receptors, by producing antimicrobial compounds, and by stimulating a multiple-cell signaling process that can limit the release of virulence factors.[37] Studies in germ-free mice have shown structural abnormalities such as reduced intestinal surface area and decreased epithelial cell turnover compared with colonized mice.[38] The gut microbiota also influences the development of the intestinal barrier and its functions.

The microbiota exerts many roles in the development of the gut immune system, especially by achieving appropriate programming of mucosal immunity. The roles of the gut microbiota include modulating development of the intestinal mucous layer and lymphoid structures, immune-cell differentiation, and production of immune mediators. Intestinal microbiota exert positive stimulatory effects on the intestinal innate and adaptive immune systems.[39] The intestine is an important immune organ, harboring approximately 60% of total immunoglobulins, $>10^6$ lymphocytes/g tissue, and the largest pool of immune-competent cells of the body within the intestinal mucosa. For instance, in response to

intestinal colonization, the number of T lymphocytes and plasmocytes within the intestinal lamina propria is clearly augmented. Whereas IgA-producing cells are virtually absent in germ-free mice, high IgA levels are detectable within the mucosa upon bacterial colonization.[40]

The innate immune system must discriminate between pathogens and harmless commensal bacteria of the intestinal microbiota. Pathogen recognition receptors such as Toll-like receptors and nucleotide-binding oligomerization domain receptors allow for recognition of a restricted number of bacterial motifs (either microbe-associated molecular patterns or, in the case of pathogens, pathogen-associated molecular patterns).[41] Both types of pathogen recognition receptors are naturally expressed by intestinal epithelial and antigen-presenting cells such as dendritic cells or macrophages, which enable them to easily sense any bacterial motifs. To avoid a permanent and unwanted stimulation of the innate immune system, the intestinal epithelial barrier is protected by a highly viscous microfilm, which prevents close contact between commensal bacteria and intestinal epithelial cells. However, upon contact, the enterocyte is able to send "alarm signals" in the form of chemokines or cytokines to the mucosal adaptive immune system and, at the same time, to secrete bactericidic peptides into the lumen.[42] This mechanism might be altered in some patients with IBD. Proinflammatory signals of enterocytes or antigen-presenting cells within the intestinal mucosa result in a rapid upregulation of homing the receptors on endothelial cells and the chemoattraction of inflammatory cells to the site of infection.

Intestinal mucosal barrier function can be defined as the capacity of the intestine to host the commensal bacteria and molecules, while preserving the ability to absorb nutrients and prevent the invasion of host tissues by resident bacteria. The dense communities of bacteria in the intestine are separated from body tissues by a monolayer of intestinal epithelial cells. The assembly of the multiple components of the intestinal barrier is initiated during fetal development and continues during early postnatal life. Thus, the intestinal barrier has not completely developed soon after birth, particularly in preterm infants. The central element is the epithelial layer, which physically separates the lumen and the internal milieu and is in charge of vectorial transport of ions, nutrients, and other substances. The secretion of mucus-forming mucins, sIgA, and antimicrobial peptides reinforces the mucosal barrier on the extra-epithelial side, while a variety of immune cells contributes to mucosal defense on the inner side. Thus, the mucosal barrier is physical, biochemical, and immune in nature. In addition, the microbiota may be viewed as part of this system because of the mutual influence that occurs between the host and the luminal microorganisms.

Alteration of the mucosal barrier function with accompanying increased permeability and/or bacterial translocation has been linked with a variety of conditions, including metabolic disorders (type 2 diabetes mellitus, insulin resistance, obesity) and IBD.[43] Genetic and environmental factors may converge to evoke a defective function of the barrier, which may, in turn, lead to overt inflammation of the intestine as a result of an exacerbated immune reaction toward the microbiota. IBD may be both precipitated and treated by either stimulation or downregulation of the different elements of the mucosal barrier, with the outcome depending on timing, the cell type affected, and other factors. Fermentation products of commensal bacteria have been shown to enhance the intestinal barrier function by facilitating the assembly of tight junctions through the activation of adenosine mono-phosphate (AMP)-activated protein kinase.[44] On the other hand, the deletion of all detectable commensal gut microbiota by a 4-week oral administration of 4 antibiotics (vancomycin, neomycin, metronidazole, and ampicillin) leads to more severe intestinal mucosal injury in a dextran–sulfate–sodium-induced mouse colitis model.[45] Early treatments with broad-spectrum antibiotics have been shown to alter the GI tract gene expression profile and intestinal barrier development.[46] This underlines the importance of normal bacterial colonization in the development and maintenance of the intestinal barrier. Antibiotic therapy between birth and age 5 years might increase the risk of Crohn's disease by disrupting the pattern of gut colonization.[47] A recent metaanalysis confirmed that antibiotic use is associated with increased risk of new-onset Crohn's disease, but not ulcerative colitis.[48]

EPIDEMIOLOGICAL EVIDENCE OF LINKS BETWEEN BACTERIAL COLONIZATION AND DISEASES

Epidemiological studies have suggested, or even established, an association between the mode of delivery or the use of antibiotics and the occurrence of health disorders or diseases. The use of cesarean section delivery has markedly increased in the past 2 decades in a large number of middle- and high-income countries in the world, reaching an unprecedented level of 50.1% in Brazil in 2009.[49,50] Although these operations can be lifesaving, both for mother and child, there is concern that increasing rates also may have short- and long-term deleterious effects. Studies suggested that children delivered by cesarean section could have increased risk later in life of atopy and allergies,[51] asthma,[52] and type 1 diabetes.[53] The main explanation for possible increased risk is that the lack of contact at birth with maternal vaginal and intestinal bacteria could make these

children more susceptible later in life to a number of diseases because of changes in the development of the immune system.[54]

Several authors have studied the risks of metabolic disorders and obesity linked to cesarean section. In a study by Huh et al.,[55] women were recruited during early pregnancy, and their children were followed after birth. Body mass index (BMI) z-score, obesity (BMI for age and sex ≥95th percentile), and sum of triceps + subscapular skinfold thicknesses were assessed at age 3 years in 1255 children. Among them, 284 children (22.6%) were delivered by cesarean section. At age 3 years, 15.7% of children delivered by cesarean section were obese compared with 7.5% of children born vaginally. In multivariable logistic and linear regression models adjusting for maternal prepregnancy BMI, birth weight, and other covariates, birth by cesarean section was associated with a higher odds of obesity at age 3 years (odds ratio [OR], 2.10; 95% confidence interval [CI], 1.36–3.23) and higher mean BMI z-score (0.20 units; 95% CI, 0.07–0.33). A study performed in Germany confirmed this trend of cesarean section to promote overweight and obesity,[56] as did Blustein et al. in the United Kingdom.[57] In 3 birth cohorts in Brazil, cesarean section did not lead to a significant increased risk of obesity during childhood, adolescence, or early adulthood.[49] Further studies are needed to confirm these findings and to explore mechanisms that underlie this association. Expectant mothers who choose cesarean delivery in the absence of an obstetrical or medical indication should be aware that their children might have a higher risk for obesity.[58]

The mode of delivery has been shown experimentally to shape gut colonization pattern and modulate regulatory immunity in mice.[59] Cesarean section has been considered a factor that contributes to IBD, especially Crohn's disease.[60,61] A metaanalysis of 9 studies evaluated the potential association between cesarean section and the development of IBD.[60] The pooled data from the 6 included studies indicated that cesarean section was a risk factor for Crohn's disease (95% CI, 1.12–1.70; $P = 0.003$). A positive association between cesarean section and pediatric Crohn's disease (95% CI, 1.06–1.35; $P = 0.005$) was observed. However, results from the 4 included studies for ulcerative colitis indicated the rate of cesarean section in ulcerative colitis patients was not higher than that in control patients (95% CI, 0.87–1.32; $P = 0.54$). Results of this metaanalysis support the hypothesis that cesarean section is associated with the risk of Crohn's disease, but not of ulcerative colitis. The overall rate of cesarean section in IBD patients was similar with that of controls. Another study aimed to investigate the relationship between mode of delivery and risk of IBD.[61] Seven eligible studies were included; 4 were of a retrospective cohort design and 3 were case-control studies. The total number of children born by cesarean section in the metaanalysis was 1354, and 11c355 were delivered vaginally. The proportion of IBD in the cesarean section group was 0.249% compared with 0.322% in the vaginal delivery group. The pooled OR for developing IBD when delivered by cesarean section was 1.00 (95% CI, 0.75–1.33). This analysis observed no significant difference in risk of IBD in offspring delivered by cesarean section compared with those born vaginally. The effect of cesarean section on IBD incidence in the age span 0–35 years was studied from a register-based national cohort study of 2.1 million individuals in Denmark born between 1973 and 2008. Cesarean section was associated with moderately increased risk of IBD at age 0–14 years (incidence rate ratio, 1.29; 95% CI, 1.11–1.49), regardless of parental disposition to IBD.[62] It is difficult to come to a conclusion regarding cesarean section as a risk factor for Crohn's disease. The possible impact of increasing cesarean section practices on the overall burden of IBD in childhood is likely to be small and probably associated with other factors yet to be identified.

MODULATION OF INTESTINAL MICROBIOTA, GUT IMMUNE SYSTEM, AND HUMAN DISEASE BY PROBIOTICS

The administration of live microorganisms via food has a long history of practice. Today, both food and medicinal products containing live bacteria aim to modulate the intestinal microbiota. The term "probiotic" has been defined as "living micro-organisms which, upon ingestion in sufficient numbers, exert health benefits beyond basic nutrition." Probiotics are live, viable bacteria or other microorganisms such as yeasts that have a clearly identifiable positive effect on health and disease.[63] Nonviable bacteria or bacterial substrates are not considered to be probiotics. The most commonly used and studied species of probiotics belong to the genera *Lactobacillus*, *Bifidobacterium*, and *Saccharomyces*.[64] A wide variety of probiotic products and strains exist, and it is important to consider the term "probiotics" as a generic term for a range of microorganisms endowed with different properties and effects. The term "probiotics" is comparable to the term "antibiotics," which covers many different classes of drugs endowed with differing antibiotic activities. Thus, different antibiotics have different indications. If the term "probiotics" is used in a manner analogous to "antibiotics," it may prevent confusion with respect to the specific properties of probiotics. Some probiotics are used to prevent or treat infections, while others are of value in the prophylaxis

or treatment of allergic and inflammatory disorders. No single probiotic may achieve all clinical benefits. Probiotics have beneficial effects in the prevention and treatment of human disorders, as evidenced by clinical trials. The use of probiotic approaches is particularly helpful in young pediatric patients, since infants are particularly vulnerable to diseases and infancy is characterized by the delicate process of intestinal mucosa maturation and interaction with gut microbiota.[65]

Clinical benefits of probiotics depend on strain selection, dose and duration of administration, preservation in the GI tract, and, perhaps, combinations of probiotics.[66] Depending on the clinical setting, probiotics can be administered as drugs or combined with food such as yogurt and dairy products. Clinical benefits have been achieved using yogurt and dairy products. Interestingly, over a century ago, Élie Metchnikoff theorized that health could be enhanced and that senility could be delayed by manipulating the intestinal microbiome with host-friendly bacteria found in yogurt.[67] His theory flourished for a time, then drifted to the fringe of medical practice, only to reemerge in the mid-1990s as a concept worthy of mainstream medical attention.[68]

Over the last decade, new areas have opened in the use of probiotics in infants and children for treating or preventing infectious and antibiotic-associated diarrhea.[64–66] For allergy, current results of clinical trials are controversial and dependent on the clinical status of children and the probiotic strains used.[69] The use of probiotics to prevent NEC in very low birth weight infants is providing important and promising results.[69] However, controversies remain for a variety of reasons, including the following: the methodologies of metaanalysis involving different probiotic mixtures yield results that are debatable; the mechanisms by which probiotics are active are poorly understood; and in spite of their beneficial effects, probiotics, as live bacteria, make neonatologists anxious, especially regarding the safety of their use in very premature infants.[70] Nevertheless, one should consider the current results as well as hypotheses that might explain nonstrain-specific probiotic effects, such as providing a microbiological barrier against environmental pathogens and improved intestinal permeability from probiotics themselves or from their secreted products, thus protecting against the translocation of harmful bacteria. Moreover, a recent longitudinal analysis of the premature infant intestinal microbiome prior to NEC underlines the importance of microbial diversity.[71] It also demonstrated the impact of intravenously administered antibiotics on the microbial diversity present in fecal material.[71] Thus, while the provision of live bacteria might increase microbial diversity, these hypotheses need to be explored more extensively.

CONCLUSION

It is now well established that the intestinal microbiota play a major role immediately after birth by promoting intestinal function and by developing the gut immune system.[72–74] Numerous factors may influence early intestinal colonization (prematurity, cesarean section, breastfeeding, antibiotics) and the so-called immune phenotype programming.[75] Epidemiological studies suggest relationships between early colonization and occurrence of later human diseases such as obesity, allergic diseases, IBD, and autoimmune diseases. Causal relationships for many of the associations between the microbiome and disease states have yet to be proven. Understanding the links between the microbiome and human disease may provide prophylactic or therapeutic tools to improve human health. Modulation of intestinal microbiota with probiotics, prebiotics, and fermentation products is promising but requires further study to optimize the ingredients used, as well as the dose and duration, and to identify when in the life cycle they should be introduced.

Acknowledgments

The content of this article was presented as part of the Second Global Summit on the Health Benefits of Yogurt, held as a satellite to the Experimental Biology meeting in San Diego, California, on 30 April 2014. The conference was organized by the American Society for Nutrition and Danone Institute International. The supplement coordinators are Sharon M. Donovan, University of Illinois at Urbana-Champaign, USA, and Raanan Shamir, Schneider Children's Medical Center, Israel.

Funding. Writing and editorial assistance were provided by Densie Webb, PhD, RD, who was contracted and funded by Danone Institute International. O.G. received financial reimbursement for travel expenses and an honorarium from the Danone Institute International for his participation in the conference.

Declaration of interest. The author has no relevant interests to declare.

REFERENCES

1. Arrieta MC, Stiemsma LT, Amenyogbe N, et al. The intestinal microbiome in early life: health and disease. Front Immunol. 2014;5:427.
2. Saavedra JM, Dattilo AM. Early development of intestinal microbiota: implications for future health. Gastroenterol Clin North Am. 2012;41:717–731.

3. Dave M, Higgins PD, Middha S, et al. The human gut microbiome: current knowledge, challenges, and future directions. Transl Res. 2012;160:246–257.
4. Human Microbiome Project C. Structure, function and diversity of the healthy human microbiome. Nature. 2012;486:207–214.
5. Arumugam M, Raes J, Pelletier E, et al. Enterotypes of the human gut microbiome. Nature. 2011;473:174–180.
6. Claesson MJ, Cusack S, O'Sullivan O, et al. Composition, variability, and temporal stability of the intestinal microbiota of the elderly. Proc Natl Acad Sci U S A. 2011; 108 (Suppl 1):4586–4591.
7. Likotrafiti E, Tuohy KM, Gibson GR, et al. An in vitro study of the effect of probiotics, prebiotics and synbiotics on the elderly faecal microbiota. Anaerobe. 2014; 27:50–55.
8. DiGiulio DB. Diversity of microbes in amniotic fluid. Semin Fetal Neonatal Med. 2012;17:2–11.
9. Payne MS, Bayatibojakhi S. Exploring preterm birth as a polymicrobial disease: an overview of the uterine microbiome. Front Immunol. 2014;5:595.
10. Solt I. The human microbiome and the great obstetrical syndromes: a new frontier in maternal-fetal medicine. Best Pract Res Clin Obstet Gynaecol. 2015;29: 165–175.
11. Guarino A, Wudy A, Basile F, et al. Composition and roles of intestinal microbiota in children. J Matern Fetal Neonatal Med. 2012;25 (Suppl 1):63–66.
12. Biasucci G, Benenati B, Morelli L, et al. Cesarean delivery may affect the early biodiversity of intestinal bacteria. J Nutr. 2008;138:1796S–1800S.
13. Johnson CL, Versalovic J. The human microbiome and its potential importance to pediatrics. Pediatrics. 2012;129:950–960.
14. Dominguez-Bello MG, Costello EK, Contreras M, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci U S A. 2010;107:11971–11975.
15. Penders J, Thijs C, Vink C, et al. Factors influencing the composition of the intestinal microbiota in early infancy. Pediatrics. 2006;118:511–521.
16. Neu J, Walker WA. Necrotizing enterocolitis. N Engl J Med. 2011;364:255–264.
17. Morelli L. Postnatal development of intestinal microflora as influenced by infant nutrition. J Nutr. 2008;138:1791S–1795S.
18. Underwood MA, German JB, Lebrilla CB, et al. *Bifidobacterium longum* subspecies *infantis*: champion colonizer of the infant gut. Pediatr Res. 2015;77: 229–235.
19. Voreades N, Kozil A, Weir TL. Diet and the development of the human intestinal microbiome. Front Microbiol. 2014;5:494.
20. Sjogren YM, Tomicic S, Lundberg A, et al. Influence of early gut microbiota on the maturation of childhood mucosal and systemic immune responses. Clin Exp Allergy. 2009;39:1842–1851.
21. Chichlowski M, De Lartigue G, German JB, et al. Bifidobacteria isolated from infants and cultured on human milk oligosaccharides affect intestinal epithelial function. J Pediatr Gastroenterol Nutr. 2012;55:321–327.
22. Garrido D, Kim JH, German JB, et al. Oligosaccharide binding proteins from *Bifidobacterium longum* subsp. infantis reveal a preference for host glycans. PLoS One. 2011;6:e17315.
23. Newburg DS. Neonatal protection by an innate immune system of human milk consisting of oligosaccharides and glycans. J Anim Sci. 2009;87(13 Suppl): 26–34.
24. Perez PF, Dore J, Leclerc M, et al. Bacterial imprinting of the neonatal immune system: lessons from maternal cells? *Pediatrics*. 2007;119:e724–e732.
25. Yatsunenko T, Rey FE, Manary MJ, et al. Human gut microbiome viewed across age and geography. Nature. 2012;486:222–227.
26. David LA, Maurice CF, Carmody RN, et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature. 2014;505:559–563.
27. Puddu A, Sanguineti R, Montecucco F, et al. Evidence for the gut microbiota short-chain fatty acids as key pathophysiological molecules improving diabetes. Mediators Inflamm. 2014;2014:162021.
28. Faa G, Gerosa C, Fanni D, et al. Factors influencing the development of a personal tailored microbiota in the neonate, with particular emphasis on antibiotic therapy. J Matern Fetal Neonatal Med. 2013;26 (Suppl 2):35–43.
29. Perez-Cobas AE, Gosalbes MJ, Friedrichs A, et al. Gut microbiota disturbance during antibiotic therapy: a multi-omic approach. Gut. 2013;62:1591–1601.
30. Wall R, Ross RP, Ryan CA, et al. Role of gut microbiota in early infant development. Clin Med Pediatr. 2009;3:45–54.
31. Madan JC, Salari RC, Saxena D, et al. Gut microbial colonisation in premature neonates predicts neonatal sepsis. Arch Dis Child Fetal Neonatal. 2012;97: F456–F462.
32. Cox LM, Yamanishi S, Sohn J, et al. Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. Cell. 2014;158: 705–721.
33. Hooper LV, Wong MH, Thelin A, et al. Molecular analysis of commensal host-microbial relationships in the intestine. Science. 2001;291:881–884.
34. Rakoff-Nahoum S, Kong Y, Kleinstein SH, et al. Analysis of gene-environment interactions in postnatal development of the mammalian intestine. Proc Natl Acad Sci U S A. 2015;112:1929–1936.
35. Quigley EM. Microflora modulation of motility. J Neurogastroenterol Motil. 2011; 17:140–147.
36. Kashyap PC, Marcobal A, Ursell LK, et al. Complex interactions among diet, gastrointestinal transit, and gut microbiota in humanized mice. Gastroenterology. 2013; 144:967–977.
37. Di Mauro A, Neu J, Riezzo G, et al. Gastrointestinal function development and microbiota. Ital J Pediatr. 2013;39:15.
38. Sommer F, Backhed F. The gut microbiota—masters of host development and physiology. Nat Rev Microbiol. 2013;11:227–238.
39. Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. Cell. 2006;124:783–801.
40. Chung H, Pamp SJ, Hill JA, et al. Gut immune maturation depends on colonization with a host-specific microbiota. Cell. 2012;149:1578–1593.
41. Rakoff-Nahoum S, Medzhitov R. Role of the innate immune system and host-commensal mutualism. Curr Top Microbiol Immunol. 2006;308:1–18.
42. Ruemmele FM, Bier D, Marteau P, et al. Clinical evidence for immunomodulatory effects of probiotic bacteria. J Pediatr Gastroenterol Nutr. 2009;48: 126–141.
43. Sanchez de Medina F, Romero-Calvo I, Mascaraque C, et al. Intestinal inflammation and mucosal barrier function. Inflamm Bowel Dis. 2014;20: 2394–2404.
44. Peng L, Li ZR, Green RS, et al. Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of AMP-activated protein kinase in Caco-2 cell monolayers. J Nutr. 2009;139:1619–1625.
45. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, et al. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. Cell. 2004; 118:229–241.
46. Schumann A, Nutten S, Donnicola D, et al. Neonatal antibiotic treatment alters gastrointestinal tract developmental gene expression and intestinal barrier transcriptome. Physiol Genomics. 2005;23:235–245.
47. Hildebrand H, Malmborg P, Askling J, et al. Early-life exposures associated with antibiotic use and risk of subsequent Crohn's disease. Scand J Gastroenterol. 2008; 43:961–966.
48. Ungaro R, Bernstein CN, Gearry R, et al. Antibiotics associated with increased risk of new-onset Crohn's disease but not ulcerative colitis: a meta-analysis. Am J Gastroenterol. 2014;109:1728–1738.
49. Barros FC, Matijasevich A, Hallal PC, et al. Cesarean section and risk of obesity in childhood, adolescence, and early adulthood: evidence from 3 Brazilian birth cohorts. Am J Clin Nutr. 2012;95:465–470.
50. Hamilton BE, Martin JA, Ventura SJ. Births: preliminary data for 2009. Natl Vital Stat Rep. 2010;59:1–19.
51. Bager P, Wohlfahrt J, Westergaard T. Caesarean delivery and risk of atopy and allergic disease: meta-analyses. Clin Exp Allergy. 2008;38:634–642.
52. Thavagnanam S, Fleming J, Bromley A, et al. A meta-analysis of the association between caesarean section and childhood asthma. Clin Exp Allergy. 2008;38: 629–633.
53. Cardwell CR, Stene LC, Joner G, et al. Caesarean section is associated with an increased risk of childhood-onset type 1 diabetes mellitus: a meta-analysis of observational studies. Diabetologia. 2008;51:726–735.
54. Neu J, Rushing J. Cesarean versus vaginal delivery: long-term infant outcomes and the hygiene hypothesis. Clin Perinatol. 2011;38:321–331.
55. Huh SY, Rifas-Shiman SL, Zera CA, et al. Delivery by caesarean section and risk of obesity in preschool age children: a prospective cohort study. Arch Dis Child. 2012;97:610–616.
56. Pei Z, Heinrich J, Fuertes E, et al. Cesarean delivery and risk of childhood obesity. J Pediatr. 2014;164:1068–1073.
57. Blustein J, Attina T, Liu M, et al. Association of caesarean delivery with child adiposity from age 6 weeks to 15 years. Int J Obes. 2013;37:900–906.
58. Mesquita DN, Barbieri MA, Goldani HA, et al. Cesarean section is associated with increased peripheral and central adiposity in young adulthood: Cohort Study. PLoS One. 2013;8:e66827.
59. Hansen CH, Andersen LS, Krych L, et al. Mode of delivery shapes gut colonization pattern and modulates regulatory immunity in mice. J Immunol. 2014;193: 1213–1222.
60. Li Y, Tian Y, Zhu W, et al. Cesarean delivery and risk of inflammatory bowel disease: a systematic review and meta-analysis. Scand J Gastroenterol. 2014;49: 834–844.
61. Bruce A, Black M, Bhattacharya S. Mode of delivery and risk of inflammatory bowel disease in the offspring: systematic review and meta-analysis of observational studies. Inflamm Bowel Dis. 2014;20:1217–1226.
62. Bager P, Simonsen J, Nielsen NM, Frisch M. Cesarean section and offspring's risk of inflammatory bowel disease: a national cohort study. Inflamm Bowel Dis. 2012;18: 857–862.
63. Hill C, Guarner F, Reid G, et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 2014;11:506–514.
64. Floch MH, Walker WA, Madsen K, et al. Recommendations for probiotic use—2011 update. J Clin Gastroenterol. 2011;45 (Suppl):S168–S171.
65. Ashraf R, Shah NP. Immune system stimulation by probiotic microorganisms. Crit Rev Food Sci Nutr. 2014;54:938–956.

66. Hsieh MH. The microbiome and probiotics in childhood. Semin Reprod Med. 2014;32:23–27.
67. Mackowiak PA. Recycling metchnikoff: probiotics, the intestinal microbiome and the quest for long life. Front Public Health. 2013;1:52.
68. German JB. The future of yogurt: scientific and regulatory needs. Am J Clin Nutr. 2014;99(5 Suppl):1271S–1278S.
69. Ismail IH, Licciardi PV, Tang ML. Probiotic effects in allergic disease. J Paediatr Child Health. 2013;49:709–715.
70. Mihatsch WA, Braegger CP, Decsi T, et al. Critical systematic review of the level of evidence for routine use of probiotics for reduction of mortality and prevention of necrotizing enterocolitis and sepsis in preterm infants. Clin Nutr. 2012;31:6–15.
71. Zhou Y, Shan G, Sodergren E, et al. Longitudinal analysis of the premature infant intestinal microbiome prior to necrotizing enterocolitis: a case-control study. PLoS One. 2015;10:e0118632.
72. Wallace TC, Guarner F, Madsen K, et al. Human gut microbiota and its relationship to health and disease. Nutr Rev. 2011;69:392–403.
73. Mondot S, de Wouters T, Dore J, et al. The human gut microbiome and its dysfunctions. Dig Dis. 2013;31:278–285.
74. Cho I, Blaser MJ. The human microbiome: at the interface of health and disease. Nat Rev Genet. 2012;13:260–270.
75. Weng M, Walker WA. The role of gut microbiota in programming the immune phenotype. J Dev Orig Health Dis. 2013;4:203–214.

Supplement Article

Update on protein intake: importance of milk proteins for health status of the elderly

Robert R. Wolfe

Loss of lean body mass that occurs with aging is the primary endpoint with which sarcopenia is defined. Furthermore, loss of muscle mass is central to the development of many adverse health issues in the elderly. Consequently, the response of lean body mass to nutritional interventions, particularly to dietary protein, has been a commonly measured endpoint. However, increased protein intake has been associated with improved markers for cardiovascular health, improved bone health, management of weight and metabolic diseases, and reduced all-cause mortality. Strength, rather than lean body mass, may be a more accurate indicator of health, especially in the elderly. The recommended dietary allowance for protein has been set at 0.8 g/kg/day. Because the average protein intake in the United States is approximately 1.2 g/kg/day, it appears that the average protein intake is above the recommended dietary allowance but below the low end of the acceptable macronutrient distribution range recommended by expert committees of the National Academy of Sciences and below the dietary intake levels suggested by the US Department of Agriculture in the Dietary Guidelines.

INTRODUCTION

Loss of lean body mass (LBM) that occurs with aging is the primary endpoint with which sarcopenia is defined.[1] Furthermore, this loss of muscle mass is central to the development of many adverse health issues in the elderly.[2] Consequently, the response of LBM to nutritional interventions, particularly to increases or decreases in dietary protein, has been a commonly measured endpoint in research, particularly in elderly patients. The effect of protein intake on LBM in the elderly has been extensively considered, often to the exclusion of many of the other potential health benefits of increased dietary protein. However, prospective studies that demonstrate the effect of changes in LBM on health outcomes are limited.

Historically, recommendations for protein intake have been based exclusively on measurements of nitrogen balance.[3] Presumably, nitrogen balance is taken as a surrogate for changes in LBM. However, problems exist with the nitrogen-balance approach, from technical considerations to the fact that nitrogen balance is not a physiological function. More importantly, even if nitrogen balance translates directly to short-term alterations in LBM, such results are of minimal importance in evaluating the health benefits of protein intake. This is because changes in LBM as a result of increases or decreases in dietary protein intake plateau after a few weeks and are rarely of demonstrable physiological significance. On the other hand, increased levels of dietary protein intake can translate to improvements in muscle strength and physical function, cardiovascular health, bone health, and weight management, which can affect long-term health outcomes. Determination of an optimal level of protein intake for the elderly should, therefore, take into account all of the physiological responses to varying levels of intake. The beneficial effects of increased protein intake on overall physiological

Affiliation: *R.R. Wolfe* is with the Department of Geriatrics, Center for Translational Research in Aging and Longevity, Donald W. Reynolds Institute on Aging, University of Arkansas for Medical Sciences, Little Rock, Arkansas, USA.

Correspondence: *Robert R. Wolfe*, 4301 W Markham St, Slot 806, Little Rock, AR 72205, USA. E-mail: rwolf2@uams.edu.

Key words: aging, lean body mass, milk, protein, sarcopenia.

doi: 10.1093/nutrit/nuv021

function in the elderly and how those effects relate to health outcomes are the focus of this article.

LEAN BODY MASS AND HEALTH OUTCOMES IN THE ELDERLY

Intake of amino acids or protein increases muscle mass by shifting the balance between muscle protein synthesis and breakdown.[2] Whereas many studies have demonstrated an acute stimulatory effect of amino acids, particularly the essential amino acids (EAAs), on net muscle protein synthesis, more recent studies have confirmed that this effect may translate to increases in LBM over a period of months.[4] Results of the Health ABC study suggest that the beneficial effects on LBM observed over a few months in prospective studies is sustained over a longer period of time. For example, in that study, protein intakes for more than 2000 elderly participants were divided into 5 quintiles, and LBM among the quintiles was compared. Those in the highest quintile of protein intake lost significantly less LBM over 3 years than those in the lowest quintile.[5] However, the highest intakes were not unusually high, about 1.2 g/kg/day, but the study does provide supportive evidence that increased protein intake may spare the loss of LBM. While it is tempting to interpret numerous studies as showing a relationship between the loss of LBM with aging and outcomes from diseases such as chronic obstructive pulmonary disease[6] or cancer,[7] the currently available data do not exclude the possibility that those who are the most ill and who ultimately die are more cachectic and, therefore, lose more LBM. A more striking relationship can be demonstrated between mortality and strength than exists for mortality and LBM. All-cause mortality as well as mortality from cancer are directly impacted by the level of strength, irrespective of LBM. In a study by Ruiz et al.,[8] individuals in the lower third of strength measurements had the worst health outcomes. This led to the concept of muscle quality being the most important criterion of muscle health in the elderly.[9] Muscle strength and function are affected by the level of dietary protein as a consequence of increased turnover (i.e., synthesis and breakdown) of muscle protein. Whereas the response of net protein synthesis to increased levels of protein intake will likely plateau at some point in time, the plateau in net protein balance will occur at higher rates of both synthesis and breakdown. Presumably, this results in newly synthesized proteins replacing damaged fibers that contract less efficiently. Thus, higher dietary protein intake under completely controlled circumstances improves muscle strength and function as a result of improved single-fiber contractile properties.[10] This indicates a direct relationship exists between the turnover rate of muscle protein synthesis and strength in the elderly, even when differences in muscle mass are taken into account.[11] Studies in which protein intake[12] or amino acid intake[13] was increased over a period of months confirm that increasing dietary protein intake significantly improves muscle function in the elderly. Studies in free-living participants are supportive of the concept that stimulating protein turnover through increased protein intake improves muscle strength.[12] However, in these types of studies, it is often difficult to control dietary intakes, which may be problematic. In a study performed in elderly volunteers, participants were confined to voluntary bed rest for 10 days during which all variables, including activity, caloric intake, and protein intake, were completely controlled.[14] In the setting of controlled inactivity, increasing EAA intake above the recommended dietary allowance (RDA) significantly ameliorated the decline in a variety of functional performance measurements that would have occurred otherwise.[14] However, the more muscle loss that occurs in older individuals, the more difficult it is to accrue LBM through diet alone.[12]

PROTEIN INTAKE AND CARDIOVASCULAR HEALTH

The beneficial effects of increased protein intake on cardiovascular health have been recognized for a number of years, but these effects have not been taken into account in the formulation of dietary recommendations and guidelines for protein intake.[2] An epidemiological study documented that the relative risk of ischemic heart disease among more than 80c000 women was greatest in those with the lowest protein intake and lowest in those with the highest intake of dietary protein.[15] One explanation for this effect is the reduction in blood pressure, which has been demonstrated to respond to a single supplement of 20 g of whey protein.[16] Nitric oxide synthesis is decreased in the elderly, and the reduction in blood pressure may stem from the stimulation of nitric oxide synthesis by the arginine component of dietary protein.[17] Another aspect of dietary protein/amino acid intake relates to dietary lipids and cardiovascular disease. Supplementation of the diets of elderly individuals with EAA for 1 month significantly reduced blood and liver triglycerides.[18]

PROTEIN INTAKE AND BONE HEALTH

Dietary protein intake has been implicated in the loss of bone due to the acidification of blood. Although the major contributor to this response is thought to be the sulfur-rich proteins, even a formulation of EAAs containing a minimal amount of sulfur has been found to acidify the blood and lead to increased excretion of

calcium, *n*-teleopeptide, and deoxypyridinoline in the context of bed rest.[19] However, this study, as well as others that indicated increased bone resorption in response to high protein or amino acid intake, did not consider the rate of bone formation and, thus, the net formation of bone. When net bone formation has been determined, higher rates of protein intake have been shown to have beneficial effects on bone health. For example, when 219 healthy volunteers aged 70–80 years were given either placebo or 30 g of whey protein per day for 2 years, the protein-supplemented group avoided the loss in femoral neck bone mineral density that occurred in the placebo group.[20] There may also be an indirect effect of protein intake on bone health. Bone strength is directly affected by the torque placed on the bones as a result of muscular contraction.[21] Because higher levels of protein intake increase strength in the elderly (see above), increased protein intake may have an indirect effect on bone strength by enabling the generation of greater muscular force.

BENEFITS OF PROTEIN INTAKE IN WEIGHT MANAGEMENT AND METABOLIC DISEASE

In the context of hypocaloric nutrient intake for the purpose of weight loss, the benefits of a diet in which protein comprises a relatively high proportion has been well documented in terms of maintaining LBM while fat mass is being lost. This could be explained, at least in part, by the fact that total caloric intake is reduced significantly during hypocaloric feeding, so that an increased percentage of dietary protein may be necessary just to achieve the same absolute amount of protein intake normally eaten in a conventional American diet. However, a high level of protein intake also provides beneficial effects on weight management even when caloric demands are equal to or greater than energy expenditure. A thermogenic response to protein intake, which results from the stimulation of protein turnover,[2] is one mechanism by which protein intake can benefit maintenance of energy balance. There is a metabolic cost of both protein synthesis and breakdown.[2] The energy cost of protein turnover constitutes a significant proportion of resting energy expenditure, and stimulation of protein turnover by increased protein intake increases energy expenditure via thermogenesis. If caloric intake remains constant, increased thermogenesis favors maintenance of a lower body weight. Increased protein intake may also aid in the maintenance of energy balance and weight management by having a satiating effect.[22] Increased protein and/or amino acid intake may benefit the metabolic state by improving insulin sensitivity[23] and reducing circulating lipid levels.[18] Importantly, an increase in protein intake also means a reduction in carbohydrate and/or fat intake to maintain caloric balance. Excess fat and excess carbohydrate, in particular, are linked to a variety of adverse health consequences in the elderly.[24,25] In contrast, adverse effects of high levels of protein intake have not been encountered in healthy individuals.[3] Thus, it may be that benefits of increased protein intake on weight management and metabolic disease are direct results of the increased availability of amino acids, as well as indirectly as a result of decreased intakes of carbohydrate and/or fat.

OPTIMAL PROTEIN INTAKE

A variety of recommendations for protein intake are currently available. Arguably the most well recognized is the RDA. The RDA for dietary protein was most recently considered by the Food and Nutrition Board of the National Academy of Sciences and published in the dietary reference intakes for macronutrients.[3] The RDA for protein represents 2 standard deviations above the average minimal amount of protein intake needed to maintain zero nitrogen balance (i.e., no net gain or loss of N over time). Thus, it represents the minimal amount of protein intake required to maintain nitrogen balance in approximately 98% of the population. It is important to recognize that the definition is based entirely on nitrogen balance and does not take into account any of the factors discussed above, which are impacted by the amount of protein consumed, particularly in older individuals.

The current RDA for protein (0.8 g/kg/day)[3] is a value that was first derived in 1943 in order to define the minimal amount of protein that would enable troops in World War II to avoid protein malnutrition.[26] Despite the quantity of data that have been generated since then on the topic of optimal protein intake, the value has remained unchanged. The RDA can be viewed as a reasonable minimal level of protein intake, but it was not intended to represent the optimal amount of protein intake in a circumstance of an abundance of food choices, as presently exists in the United States and other first world countries.

The optimal level of protein intake in the elderly is almost certainly greater than the RDA. There have been numerous studies in which a variety of endpoints have been used to compare the effects of consuming the RDA of protein to consuming greater amounts of protein, particularly in the elderly.[27] Although the magnitude of benefit from a higher protein intake varies among studies, depending on the specific experimental design, participants, and endpoint(s) measured, among other factors, there has never been a study in which individuals who consumed the RDA for protein

experienced benefits similar to those of individuals who consumed protein in excess of the RDA.

One practical limitation in translating the RDA for protein to the formulation of a complete diet is that dietary protein is not eaten in isolation and the RDA for protein does not account for other components of the diet. The Food and Nutrition Board recognized this practical limitation of the RDA and published an additional set of recommendations termed the acceptable macronutrient distribution range (AMDR), which considers macronutrient intake in the context of a complete diet.[3] The AMDR recommends that protein intake constitute 10%–35% of total caloric intake.[3] The minimal nature of the RDA in the context of a complete diet is evident when considered in light of the AMDR. The dietary reference intakes recommend a total caloric intake of 35 kcal/kg/day.[3] Thus, the RDA for protein represents <10% of total recommended caloric intake [(0.8 g/kg/day × 4 kcal/kg/day)/35 kcal/kg/day = 9.1%). The mid-range of the AMDR recommendation for protein intake is about 2.0 g/kg/day.

The most practical expression of the recommended protein intake is found in the *Dietary Guidelines for Americans* (DGA), promulgated by the US Department of Agriculture.[28] These recommendations are intended to translate the most recent nutrition research into dietary guidelines expressed in the context of real foods and daily meal plans. When recommended food intakes from the DGA are broken down into their components, the recommended protein intake is approximately 1.5 g/kg/day.[29] Thus, recommendations for dietary protein intake in the context of a complete diet range from 1.5 to 2 g/kg/day. These values are consistent with those presented in other studies that assessed the optimal level of protein intake based on multiple endpoints.[27] Because the average protein intake in the United States is approximately 1.2 g/kg/day,[29] it appears that the average protein intake is below the amount recommended by expert committees of the National Academy of Sciences and the US Department of Agriculture.

QUANTIFYING PROTEIN QUALITY

Digestible indispensable amino acid score

The dietary recommendations referred to above specify that protein should be of "high quality" but they do not specify how protein quality should be assessed. The Food and Agriculture Organization of the United Nations developed an approach for quantifying protein quality called the protein digestibility corrected amino acid score (PDCAAS).[30] The score was derived as a means to quantify dietary protein quality based on the amino acid profile and relative amounts of dietary EAAs in the test protein, corrected for digestibility using a single value for true fecal crude protein digestibility and expressed relative to a profile of amino acid requirements. Thus, a PDCAAS of 1 means that all of the minimal requirements for EAA intake would be met if an amount of the test protein equivalent to the estimated average daily requirement for protein (0.66 g/kg/day for adult men and women) was eaten. Most high-quality proteins have a PDCAAS > 1.0. However, at the time the score was created, it was deemed that excess dietary amino acids would not be utilized and should, therefore, not be included in the PDCAAS; as a result, all scores were truncated at 1.0. The truncation of PDCAAS at 1.0 does not allow a comparison of the relative quality of high-quality dietary proteins. The Food and Agriculture Organization of the United Nations recently released a document in which the adoption of a new scoring system to quantify dietary protein quality is recommended; the system is called the digestible indispensable amino acid score (DIAAS).[31] The DIAAS is meant to supplant the use of the PDCAAS. The conceptual goal of the DIAAS is similar to that of the PDCAAS. However, with the DIAAS, the quality of a protein is based on the relative digestible content of the EAAs and the amino acid requirement pattern. In contrast to the PDCAAS, the DIAAS is not truncated, thereby theoretically enabling a ranking of all dietary proteins by quality. An accurate quantitative ranking of protein quality has great potential for clarifying many aspects of protein nutrition in a general sense and could be of value specifically in the context of dietary recommendations and the creation of meal plans.

Several DIAASs are shown in Figure 1. In general, proteins can be classified as being of high quality or lower quality. High-quality proteins provide at least 100% of all EAA requirements if 0.66 g/kg/day of the protein is ingested. For the most part, animal proteins constitute high-quality proteins, with dairy proteins among those with the highest quality. There are currently limited data to confirm that the rankings shown in Figure 1 are indicative of differences in physiological function. However, the existing evidence supports the validity of the DIASS rankings. Consistent with the respective DIAASs of milk protein or whey protein, ingestion of each stimulates muscle protein synthesis in human volunteers more than ingestion of the same amount of soy protein. Moreover, the response of muscle protein synthesis in rats to the ingestion of wheat, soy, egg, and whey proteins was found to be proportionate to the respective DIAASs. The authors attributed the effectiveness of whey protein to the relatively

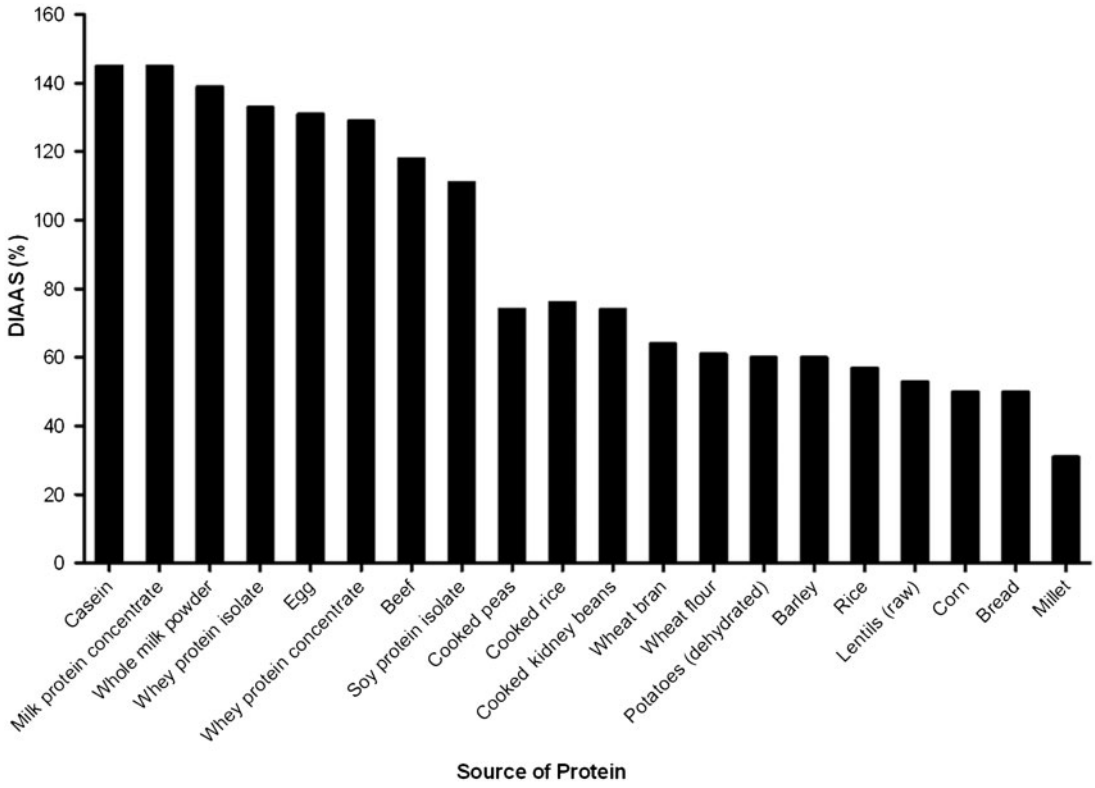

Figure 1 **Quality of common protein sources expressed as percent digestible indispensable amino acid score.** Values were calculated according to reference 31 and represent the percentage of the requirement for the most limiting essential amino acid in the test protein that will be met by ingestion of 0.66 g/kg/day of the test protein. *Abbreviation*: DIASS, digestible indispensable amino acid score.

high proportion of leucine and its affect on the increase in phosphorylation of p70 S6 kinase (p70S6K) and 4E binding protein 1 (4EBP1).[32] More measurements of protein synthetic rates following ingestion of specific proteins are important to fully understand the significance of the corresponding DIAAS.

DIAASs and milk proteins

Figure 1 shows that dairy proteins rank among the highest of the DIAASs. In addition to delivering a large amount of EAAs per gram of protein in a favorable profile relative to EAA requirements, milk proteins are absorbed at different rates, which likely further amplifies their effectiveness. Casein and whey are the principal proteins in milk. Whey protein is readily absorbed, resulting in a relatively rapid peak in plasma amino acid concentrations. In contrast, absorption of casein is prolonged due to coagulation in the stomach, resulting in a sustained but moderate increase in plasma amino acid concentrations. The rapid peak in amino acid concentrations, particularly of leucine, in response to the ingestion of milk proteins activates the process of synthesis, and the prolonged increased availability of amino acids enables sustained stimulation of protein synthesis. These aspects of milk proteins are not factored into the calculation of the DIAAS. Further, the DIAAS reflects only the ability of a test protein to meet EAA requirements, but that may not be the optimal profile of EAAs to maximally stimulate synthesis. Thus, whey protein stimulates muscle protein synthesis to a greater extent than casein, despite casein's higher DIAAS.[33] This could be due either to the more rapid absorption of whey or the fact that whey protein contains more leucine than casein. Both of these factors could have an impact on the ability of leucine to act as a "trigger" to initiate protein synthesis. According to the "leucine trigger" theory, it is necessary that intracellular initiation factors, including p70S6K and 4EBP1, be activated for protein intake to fully stimulate protein synthesis.[32] Thus, the amount of leucine in an ingested protein as well as how rapidly the amino acids in the protein are absorbed (and thus the peak leucine concentration achieved) are crucial determinants of the stimulatory effect on protein synthesis. These factors are not reflected by the DIAAS because the score is based on the most limiting amino acid in a dietary protein, and leucine is generally abundant in dietary proteins as compared to the minimal requirement for leucine. Thus, even the high DIAAS for milk proteins may underestimate their total anabolic value because of advantages that stem from the different rates of absorption of whey and casein as well as the

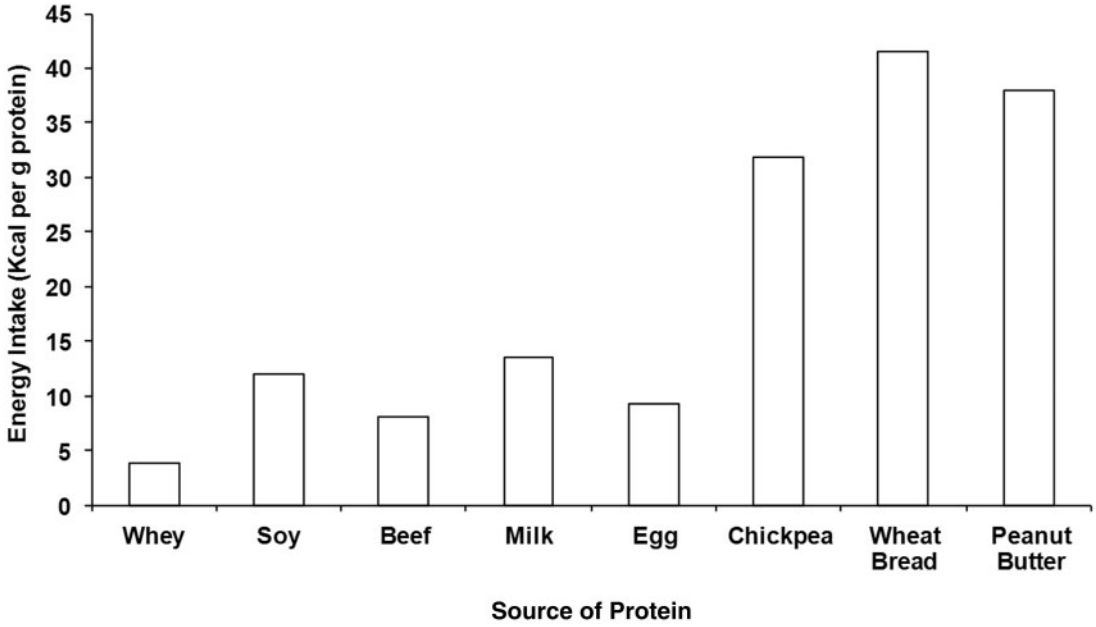

Figure 2 **Energy intake of different protein sources required to meet minimal requirements of adults for all essential amino acids.** Calculated from the US Department of Agriculture database for grams of protein per gram food source and the amount of each found source required to meet essential amino acid requirements, as derived from the digestible indispensable amino acid score.

high leucine content in whey that is not reflected by the DIAAS.

Relation of DIAAS to energy intake

Assuming that the DIAAS is substantiated by more studies that confirm the physiological relevance of its ranking of proteins, the ability to quantify protein quality has the potential for great practical significance. For example, the energy intake of particular foods required to supply enough protein to meet minimal EAA requirements could be expressed (Figure 2). The examples in Figure 2 show the wide discrepancies in the relative calorie content among various foods. Whole milk provides all of the EAA requirements, with fewer than 15 kcal of energy intake; skim milk would require significantly fewer calories. On the other hand, lower-quality proteins such as chickpea and wheat as well as foods that have a high caloric density, such as peanut butter, require that amounts in excess of the total daily caloric intake (35 kcal/kg/day) be ingested to provide the minimal requirement of EAAs.

CONCLUSION

In older individuals, protein intakes greater than the RDA promote better health outcomes by positively affecting a wide range of body systems. Rather than relying entirely on the results of nitrogen-balance studies, recommendations for protein should, therefore, take into account the impact protein has on a variety of endpoints related to health outcomes. The EAA-to-calorie ratio for high-protein foods must also be considered when comparing protein-rich foods. High-quality proteins, such as milk proteins, enable EAA requirements to be met with less caloric intake compared with lower-quality proteins. This is reflected by the scoring of their quality by the DIAAS.

Acknowledgments

The content of this article was presented as part of the Second Global Summit on the Health Benefits of Yogurt, held as a satellite to the Experimental Biology meeting in San Diego, California, on 30 April 2014. The conference was organized by the American Society for Nutrition and Danone Institute International. The supplement coordinators are Sharon M. Donovan, University of Illinois at Urbana-Champaign, USA, and Raanan Shamir, Schneider Children's Medical Center, Israel.

Funding. This work was supported in part by a National Institutes of Health grant (P30AG028718).
Writing and editorial assistance were provided by Densie Webb, PhD, RD, who was contracted and funded by Danone Institute International. R.R.W. received financial reimbursement for travel expenses and an honorarium from Danone Institute International for his participation in the conference.

Declaration of interest. R.R.W. has previously received grants from the National Cattleman's Beef Association and has received travel expenses and honoraria from

the National Cattleman's Beef Association and Dannon Yogurt.

REFERENCES

1. Paddon-Jones D, Short KR, Campbell WW, et al. Role of dietary protein in the sarcopenia of aging. Am J Clin Nutr. 2008;87:1562S–1566S.
2. Wolfe RR. The underappreciated role of muscle in health and disease. Am J Clin Nutr. 2006;84:475–482.
3. Institute of Medicine NA. Dietary Reference Intakes for Energy, Carbohydates, Fiber, Fat, Protein and Amino Acids (macronutrients). Washington, DC: Institute of Medicine; 2002–2005.
4. Dillon EL, Sheffield-Moore M, Paddon-Jones D, et al. Amino acid supplementation increases lean body mass, basal muscle protein synthesis, and insulin-like growth factor-I expression in older women. J Clin Endocrinol Metab. 2009;94:1630–1637.
5. Houston DK, Nicklas BJ, Ding J, et al. Dietary protein intake is associated with lean mass change in older, community-dwelling adults: the Health, Aging, and Body Composition (Health ABC) Study. Am J Clin Nutr. 2008;87:150–155.
6. Marquis K, Debigare R, Lacasse Y, et al. Midthigh muscle cross-sectional area is a better predictor of mortality than body mass index in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2002;166:809–813.
7. Prado CM, Baracos VE, Xiao J, et al. The association between body composition and toxicities from the combination of Doxil and trabectedin in patients with advanced relapsed ovarian cancer. Appl Physiol Nutr Metab. 2014;39:693–698.
8. Ruiz JR, Sui X, Lobelo F, et al. Association between muscular strength and mortality in men: prospective cohort study. BMJ. 2008;337:a439.
9. Conroy MB, Kwoh CK, Krishnan E, et al. Muscle strength, mass, and quality in older men and women with knee osteoarthritis. Arthritis Care Res. 2012;64:15–21.
10. Fitts RH, Romatowski JG, Peters JR, et al. The deleterious effects of bed rest on human skeletal muscle fibers are exacerbated by hypercortisolemia and ameliorated by dietary supplementation. Am J Physiol Cell Physiol. 2007;293:C313–C320.
11. Balagopal P, Ljungqvist O, Nair KS. Skeletal muscle myosin heavy-chain synthesis rate in healthy humans. Am J Physiol. 1997;272(1 Pt 1):E45–E50.
12. Tieland M, van de Rest O, Dirks ML, et al. Protein supplementation improves physical performance in frail elderly people: a randomized, double-blind, placebo-controlled trial. J Am Med Dir Assoc. 2012;13:720–726.
13. Borsheim E, Bui QU, Tissier S, et al. Effect of amino acid supplementation on muscle mass, strength and physical function in elderly. Clin Nutr. 2008;27:189–195.
14. Ferrando AA, Paddon-Jones D, Hays NP, et al. EAA supplementation to increase nitrogen intake improves muscle function during bed rest in the elderly. Clin Nutr. 2010;29:18–23.
15. Hu FB, Stampfer MJ, Manson JE, et al. Dietary protein and risk of ischemic heart disease in women. Am J Clin Nutr. 1999;70:221–227.
16. Townsend RR, McFadden CB, Ford V, et al. A randomized, double-blind, placebo-controlled trial of casein protein hydrolysate (C12 peptide) in human essential hypertension. Am J Hypertens. 2004;17(11 Pt 1):1056–1058.
17. Luiking YC, Ten Have GA, Wolfe RR, et al. Arginine de novo and nitric oxide production in disease states. Am J Physiol Endocrinol Metab. 2012;303:E1177–E1189.
18. Borsheim E, Bui QU, Tissier S, et al. Amino acid supplementation decreases plasma and liver triacylglycerols in elderly. Nutrition. 2009;25:281–288.
19. Zwart SR, Davis-Street JE, Paddon-Jones D, et al. Amino acid supplementation alters bone metabolism during simulated weightlessness. J Appl Physiol (1985). 2005;99:134–140.
20. Zhu K, Meng X, Kerr DA, et al. The effects of a two-year randomized, controlled trial of whey protein supplementation on bone structure, IGF-1, and urinary calcium excretion in older postmenopausal women. J Bone Miner Res. 2011;26:2298–2306.
21. Pang MYC, Eng JJ. Muscle strength is a determinant of bone mineral content in the hemiparetic upper extremity: implications for stroke rehabilitation. Bone. 2005;37:103–111.
22. Paddon-Jones D, Westman E, Mattes RD, et al. Protein, weight management, and satiety. Am J Clin Nutr. 2008;87:1558S–1561S.
23. Gannon MC, Nuttall FQ, Saeed A, et al. An increase in dietary protein improves the blood glucose response in persons with type 2 diabetes. Am J Clin Nutr. 2003;78:734–741.
24. Swinburn BA, Boyce VL, Bergman RN, et al. Deterioration in carbohydrate metabolism and lipoprotein changes induced by modern, high fat diet in Pima Indians and Caucasians. J Clin Endocrinol Metab. 1991;73:156–165.
25. Wolever TM, Gibbs AL, Chiasson JL, et al. Altering source or amount of dietary carbohydrate has acute and chronic effects on postprandial glucose and triglycerides in type 2 diabetes: Canadian trial of Carbohydrates in Diabetes (CCD). Nutr Metab Cardiovasc Dis. 2013;23:227–234.
26. National Research Council FaNB. Recommended Dietary Allowances. Washington, DC: National Research Council; 1943.
27. Wolfe RR, Miller SL, Miller KB. Optimal protein intake in the elderly. Clin Nutr. 2008;27:675–684.
28. US Department of Agriculture and US Department of Health and Human Services. Dietary Guidelines for Americans 2010. Washington, DC: US Government Printing Office; 2010.
29. Fulgoni VL, 3rd. Current protein intake in America: analysis of the National Health and Nutrition Examination Survey, 2003–2004. Am J Clin Nutr. 2008;87:1554–1557.
30. Food and Agriculture Organization of the United Nations. Protein Quality Evaluation: Report of the Joint FAO/WHO Expert Consultation. Rome: Food and Agriculture Organization of the United Nations; 1991.
31. Food and Agriculture Organization of the United Nations. Dietary Protein Quality Evaluation in Human Nutrition: Report of an FAO Expert Consultation. Rome: Food and Agriculture Organization of the United Nations; 2013.
32. Norton LE, Wilson GJ, Layman DK, et al. Leucine content of dietary proteins is a determinant of postprandial skeletal muscle protein synthesis in adult rats. Nutr Metab. 2012;9:67.
33. Wilkinson SB, Tarnopolsky MA, Macdonald MJ, et al. Consumption of fluid skim milk promotes greater muscle protein accretion after resistance exercise than does consumption of an isonitrogenous and isoenergetic soy-protein beverage. Am J Clin Nutr. 2007;85:1031–1040.

Supplement Article

Dairy in a sustainable diet: a question of balance

Toon van Hooijdonk and Kasper Hettinga

The demand for dairy products is growing rapidly, especially in emerging markets. Dairy products are nutrient rich and, therefore, an important food group for ensuring nutrient security in the future. In many countries, dairy contributes significantly to nutrient intake. Meta-analyses have shown that consumption of dairy may reduce the risk of chronic diseases and thereby lower healthcare costs. Milk production and processing contribute to greenhouse gas emissions, estimated at 2.7% (cradle-to-retail) of the world's total. Evaluating the position of dairy in the diet should take into account the impact of both nutritional and environmental factors. Local conditions are also important; in many parts of the world, the cow is an efficient converter of human-inedible resources into nutrient-dense food. Increased productivity of cows is a decisive factor in realizing sufficient milk production with optimal resource efficiency and minimal greenhouse gas emission. Models that optimize total diets, rather than individual food products, for their nutritional and environmental impact are the preferred approach for developing realistic alternative consumption strategies.

INTRODUCTION

Supplying a growing population with sufficient food to meet energy and micronutrient needs is one of the world's greatest challenges. It is estimated that world food demand will increase at an average rate of 1.1% per year between now and 2050.[1] Most demand exists in emerging markets where there is fast population growth and a continuous rise in income. The Food and Agriculture Organization (FAO) concluded in its updated analysis of 2012 that the challenge to realizing food security is determined more by socioeconomic and local-specific factors than by the capacity of the world to produce sufficient food.[1] However, many food-insecure countries, especially in sub-Saharan Africa, will still exist with an estimated undernourished population of more than 300 million people by the year 2050. At the same time, overconsumption of calories will dramatically increase the incidence of obesity (>50% of UK adults by 2050), with detrimental effects on health.[2] Food consumption projections indicate that in all parts of the world, the per capita consumption of commodities will increase, as shown in Figure 1.

Animal products play an important and growing role in diets worldwide, supplying, on average, 17% of the energy and 35% of the protein.[3] Animal products are nutrient rich and provide many highly bioavailable essential nutrients, which are especially important in the diets of children, pregnant and lactating women, and the elderly. Even small amounts of meat and dairy products can improve the nutritional status of those living in low-income households.[4] The estimated global demand for raw milk will increase from the current 704 million tons to 1077 million tons by 2050.[1,5] Because milk consumption in developed countries has stabilized, the 50% volume growth will mainly be due to increased consumption in developing countries.[5] This will require significant structural changes in the dairy supply chain, especially in countries where consumption will increase. The world dairy sector is characterized by a high degree

Affiliation: *T. van Hooijdonk* and *K. Hettinga* are with the Dairy Science and Technology Group, Wageningen University, Wageningen, The Netherlands.

Correspondence: *T. van Hooijdonk*, Dairy Science and Technology Group, Wageningen University, Postbox 17 / bode 30, 6700 AA Wageningen, The Netherlands. E-mail: toon.vanhooijdonk@wur.nl. Phone: +31653132828.

Key words: dairy, food security, greenhouse gas emissions, sustainable.

doi: 10.1093/nutrit/nuv040

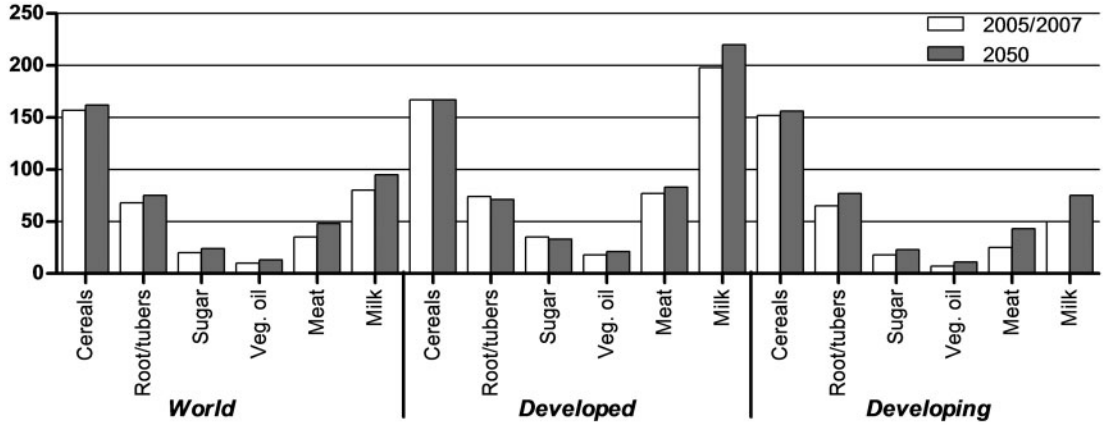

Figure 1 **Food consumption per capita, major commodities (kg/person/year).**[1]

of diversity in all parts of the chain. Farms range in size from 1 to 5 cows in many developing countries to more than 200 cows per farm in New Zealand.[6] According to the International Farm Comparison Network,[6] close to 1 billion people in the world live on dairy farms and the worldwide average is 3 or fewer cows per farm. Cows are efficient converters of human-inedible feed and by-products from feed and food chains into nutrient-rich milk, something that is especially relevant to small farms (see the Energy and Protein Efficiency section below). Also, the yearly milk production per cow shows a large variation—from 2539 kg/cow in China to 9682 kg/cow in the United States.[7] Since important environmental issues such as resource efficiency (feed, water) and greenhouse gas (GHG) emissions are strongly dependent on the milk yield per cow, there is significant potential to increase the volume of milk that will result in improved resource efficiency and reduced GHG emissions (see the Dairy and Sustainability section below). There is also large variation on the consumption side of the equation. The yearly average milk consumption per capita varies from 52 kg/person/year in developing countries to 202 kg/person/year in developed countries,[1] although that difference is expected to decrease.[5] While dairy contributes substantially to nutrient security,[4] the sector has been criticized for its environmental impact.[3] The future position of dairy in the diet should, therefore, be based on a balanced analysis of nutritional and environmental aspects. This article discusses these aspects of the dairy sector in more detail.

CONTRIBUTION OF DAIRY TO THE DIET

Intake of nutrients

Milk products are considered a basic food group in many diets. It is a nutrient-rich beverage, and consumption of dairy products is associated with better overall diet quality.[8] Therefore, dairy is a core part of dietary recommendations around the world, and many countries recommend 3 or more dairy servings per day in their dietary guidelines. The 2010 Dietary Guidelines Advisory Committee in the United States concluded that 3 servings of dairy per day would contribute proportionally more protein, calcium, magnesium, phosphorus, potassium, zinc, selenium, vitamin A, vitamin B_2, vitamin B_{12}, and choline than calories to the food pattern.[9] In developed countries, especially, dairy products contribute significantly to the intake of essential nutrients and protein.

Table 1 and Figure 2 provide an overview of the intake of selected nutrients in the Dutch and US populations relative to the European recommended daily allowances and US dietary reference intakes, respectively. Table 1 shows the contribution of dairy to the intake of these nutrients. It is clear that intakes of folic acid and vitamin D are below recommended levels. Although vitamin D can be synthesized in the skin when directly exposed to the sun, most people meet at least some of their vitamin D requirement through endogenous synthesis. However, numerous public health agencies recommend limiting exposure of skin to sunlight in order to lower the risk for skin cancer.[10] The Dutch population consumes more dairy than the US population; thus, dairy intake makes a larger contribution to nutrient intakes in the Netherlands. Two clear exceptions are vitamins A and D, which are supplemented in US dairy products, making dairy an important source of these nutrients in the United States. In many developing countries, the intake of milk is increasing but is unlikely to increase to the levels found in developed countries due to differences in dietary patterns. Governmental programs to increase consumption of dairy in developing countries typically target the most vulnerable groups, such as children and lactating women, for whom dairy products are an important

Table 1 **Contribution of dairy to the intake of nutrients (% of total) in the Netherlands and the United States**

Nutrient	Netherlands	United States
Vitamin A	8	28
Vitamin B_2 (riboflavin)	46	25
Vitamin B_6 (pyridoxine)	12	6
Vitamin B_{11} (folic acid)	19	10
Vitamin B_{12} (cobalamin)	35	26
Vitamin D	14	58
Calcium	67	51
Phosphorus	ND	28
Selenium	20	ND
Zinc	28	16

Data obtained from references[13–15]
Abbreviation: ND, not determined.

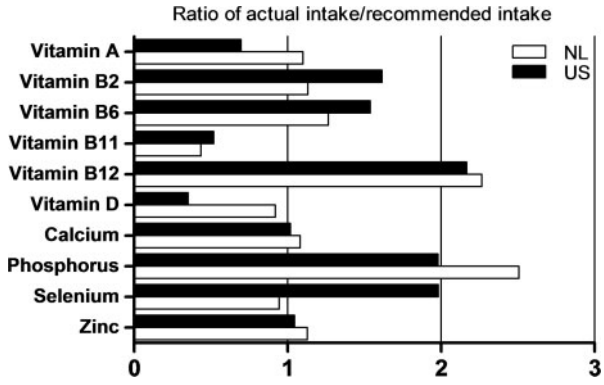

Figure 2 **Ratio of micronutrient intake vs the US recommended daily allowances.**[13,14]
Abbreviations: NL, the Netherlands; US, United States.

source of nutrients.[11] Although people with lactase nonpersistence can tolerate moderate amounts of milk (e.g., 250 mL), the existence of lactase nonpersistence together with the frequent occurrence of perceived lactose intolerance will always be factors that limit milk consumption, despite the fact that the number of lactose-reduced and lactose-free dairy products is increasing.[12]

Nutritional contribution and carbon dioxide footprint of milk compared with other beverages

The average person needs about 750 L of liquid per year. Milk has always been a significant contributor to the beverage category. However, over the years, milk has been replaced by soft drinks, especially in the Western diet (Figure 3), resulting in milk consumption that is below recommendations in almost all developed countries.[8] Compared with milk, soft drinks are often nutrient poor, contributing only sugar-based energy to the diet.

It is sometimes recommended[16] that plant-based beverages replace milk because of the lower carbon dioxide footprint. In absolute terms, this might be true;

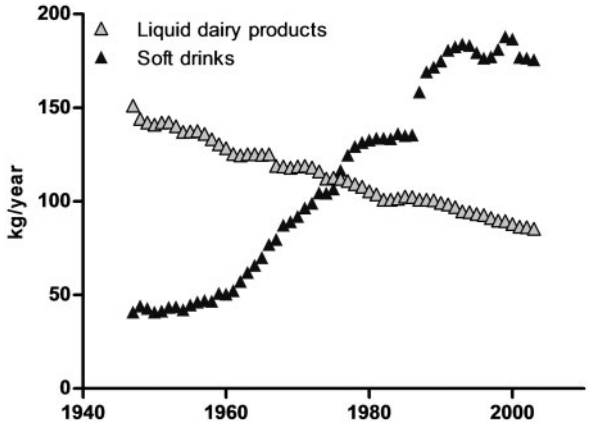

Figure 3 **Consumption of soft drinks vs dairy in the United States.**[15]

however, relative to the nutrient content, the picture is different, as Smedman et al.[16] showed. They assessed the contribution of several beverages to nutrient intake in relation to their carbon dioxide footprints. The conclusion was that the ratio of nutrient density to the carbon dioxide footprint was higher for milk than for beverages such as orange juice and unfortified soy beverages. It would be interesting to calculate this ratio for fortified plant-based protein beverages that have nutrient densities that are closer to that of milk.

Affordability of dairy nutrients

Dietary guidelines for nutrient intake can be effective only when consumer behavior can be changed. Important factors for consumer acceptance are that the foods that provide nutrients are appealing as well as affordable. To determine the most affordable way to consume required nutrients, the price per unit nutrient can be calculated for different food products. Figure 4 provides an overview of the price for consuming 10% of the recommended intake for several nutrients in the United States from vegetables, meat, or milk.[17] These data clearly show that milk is a relatively cheap source of several essential nutrients, which is especially important in developing countries where nutrient shortages still exist.

Contribution of dairy to the intake of essential amino acids

In addition to the contribution of dairy to micronutrient intake, dairy is also an important source of high-quality proteins. In the Dutch diet, dairy products contribute 25% of the total daily protein intake, while contributing only 15% of the total daily calorie intake.[18] Dairy protein is rich in essential amino acids and is highly digestible.[19] In the battle for food security,

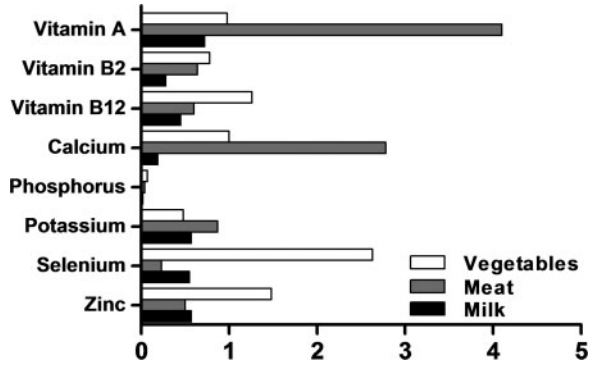

Figure 4 **Cost (in US$) of 10% daily value of nutrients.**[17]

dietary protein will be the decisive component.[4] It is, therefore, important to take protein quality into account when analyzing the nutrient adequacy of diets. The essential amino acid content, not simply the total amount of protein, should, therefore, be the basis when evaluating diets. Amino acid complementarity of proteins in a diet is important and is evident only by looking at the contribution of the essential amino acids provided by different proteins. High-quality proteins such as animal proteins can balance the amino acid pattern profiles of vegetable proteins in a mixed diet. An example is the combination of milk and wheat, in which the relatively high lysine concentration of milk proteins compensates for the low concentration of this essential amino acid in wheat.[19]

Dairy and diet-related noncommunicable diseases

Currently, 63% of deaths worldwide are attributable to chronic disease and the number is expected rise to 72% by 2020; 80% of such deaths presently occur in low- and middle-income countries.[20] Nutrition is seen as an important way to reduce diet-related noncommunicable diseases. Since full-fat dairy products contain saturated fat and saturated fat can lead to increased plasma cholesterol levels, which are associated with increased cardiovascular disease (CVD) risk,[21] most dietary guidelines recommend consumption of low-fat dairy products. However, evidence of a link between dairy consumption (including full-fat products) and CVD from observational studies shows a neutral or even modest beneficial effect.[22,23] Kratz et al.[24] concluded that the observational evidence does not support the hypothesis that dairy fat or high-fat dairy products contribute to obesity or cardiometabolic risk and suggest that high-fat dairy consumption, within typical dietary patterns, is inversely associated with obesity risk. The recent debates in the scientific literature on this controversy have not produced a conclusion on the relationship between full-fat dairy consumption and CVD. Prevention of chronic diseases with nutrition occurs via the intake of sufficient essential nutrients. However, nutrients do not work in isolation, and beneficial health effects will not result if the intake of 1 or more nutrients is suboptimal.[25] Bone health is a good example of disease reduction that depends on the adequate status of several essential nutrients including protein, calcium, phosphorus, and vitamin D,[26] all of which are found in dairy products. Overall, the results of meta-analyses provide evidence for a protective effect of dairy intake on chronic disease, which is also suggested in the overall survival advantage associated with dairy intake.[27]

DAIRY AND SUSTAINABILITY

For the global dairy sector, GHG emissions and resource efficiency are 2 major sustainability issues that play roles on a global scale.[28,29] Other factors such as water use and eutrophication are also important but are more local and will, therefore, not be discussed here.

Greenhouse gas emissions

In 2006, the FAO published the report *Livestock's Long Shadow*, with the conclusion that 18% of the GHG emissions in the world are caused by livestock.[28] This report not only brought a high level of awareness to the subject but also generated serious pressure on the livestock sector from politicians and nongovernmental organizations to develop new knowledge, support the public debate on global warming, and guide mitigation strategies. Because the private dairy sector, represented by the International Dairy Federation, needed more objective information to identify effective mitigation strategies, it supported the FAO in a systematic investigation of worldwide GHG emissions from the dairy sector. In 2010, the FAO finalized this study and concluded that the total contribution to global warming from milk production was 2.7% (cradle to retail), which amounts to an average of 2.4 kg carbon dioxide-eq/kg liquid milk.[29] There is, however, considerable regional variation due to differences in farming systems, with GHG emissions ranging from 1 to 7.5 kg carbon dioxide-eq/kg milk. In the Netherlands, which has an intensive mixed farming system, i.e., the practice of combining agriculture and raising livestock, the contribution is 1.4 kg carbon dioxide-eq/kg milk. If dairy cattle–related meat production (slaughtered dairy cows and surplus fattened calves) is included, the total sector contribution increases to 4.0%. Methane has the largest global warming effect, responsible for 52% of GHG emissions, followed by nitrous oxide (35%) and carbon dioxide (13%).[29] The cradle-to-farm-gate contribution is about 80% of the total emissions from the dairy chain,

suggesting that mitigation initiatives are likely to be most effective on the farm. The post-farm GHG contribution is related to fossil energy use and waste.

Energy and protein efficiency

Land use and methane emissions are highly dependent on the productivity of individual cows. For highly productive cows, more ingested energy is used for milk production relative to the maintenance requirements. The same is true for ingested protein. For example, increasing yield from 6000 to 10 000 kg/cow/year reduces the energy input per kilogram of milk by almost 20%.[30] Because methane production is directly related to feed intake, the total GHG emissions per kilogram of milk will also decrease. Models predict that if the yearly yield of a cow increases from 2000 to 9000 kg, the GHG emissions decrease from 2.4 to 1.4 kg carbon dioxide-eq/kg milk.[30] Another aspect of the discussion regarding the contribution of dairy to nutrient security is the degree to which cow feed directly competes with human edible food crops. Figure 5 shows the conversion efficiency of an average Dutch cow fed a mixture of roughage (72%), concentrate (25%), and wet byproducts (3%).

Dairy cows' rations consist, for the most part, of resources that humans cannot or do not consume.[31] These resources include not only grass and other cell wall–rich crops but also byproducts from feed and food chains. Because only a small fraction of the feed is edible by humans, ruminants only marginally compete with the human food resources. In fact, they convert human-inedible resources into high-quality human food. Although the efficiency of total input may not be greater than 22% and 27% for energy and protein (nitrogen), respectively, in the Netherlands, the return as edible food for humans is very efficient—357% for energy and 438% for protein. In countries with less intensive farming systems, the return on human-edible energy and protein is even greater due to the low input of concentrate in the feed.[3] In addition, the cow converts lower-quality proteins such as grain and soy protein into proteins of the highest quality, based on the protein-digestibility–corrected amino acid score.[32] The question of which food production system offers the most efficient and effective use of land strongly depends on local conditions and available infrastructure, but such an analysis is outside the scope of this article.

Mitigation strategies

An agenda for action is urgently needed to fulfill dairy's role in establishing nutrient security while maintaining a responsible socioeconomic and environmental position. For this reason, the dairy sector has launched the worldwide initiative, the Global Dairy Agenda for Action. The purpose is to create a high level of awareness among all stakeholders and to initiate a series of actions devoted to mitigating dairy's environmental impact.[33] Given the expected growth in the milk supply, promising effective mitigation strategies include the following. 1) Increase the milk supply by increasing the productivity of cows. As discussed previously, an increase from 704 to 1077 million tons of milk by 2050 could theoretically be obtained by fewer cows with greater productivity, which could potentially reduce the average GHG emissions per kilogram of milk by more than 40%. 2) Reduce the number of cows by extending the number of lactations per cow. In practice, this means a reduced culling rate through improved animal health (less mastitis and hoof problems and higher fertility). 3) Use the potential energy from manure (Figure 4). In theory, the amount of biomass energy present in manure can replace a significant amount of the fossil energy consumed in the dairy chain, although this can only be done in confined farming systems. In the Dutch case, for instance, it would be possible to replace one-third (18 PJ) of the fossil energy used, partly by capturing the energy from the manure of the country's 1.4 million dairy cows.[34] Improved technologies are needed to capture the potential energy of manure in an economically feasible way. Furthermore, when it becomes economically feasible, the on-farm production of solar energy will be a huge potential energy source. 5) Improve manure and fertilizer management in order to reduce the soil nitrous oxide. Although the emission of nitrous oxide is not as unique to dairy farming as methane is, it still contributes a substantial amount to the dairy's total GHG emissions. Effective reductions can be achieved by nitrification inhibitors.[5] 6) Reduce losses and waste. The FAO concluded that roughly 20% of milk for human consumption is lost or wasted globally, suggesting that considerable resources are used in vain and that GHG emissions are emitted without any yield.[36] Although part of these losses and wastes are recycled through animal feed, there is a sufficient potential to reduce the environmental impact of the dairy sector by taking preventive measures. In developed countries, most of the wastes are at the consumer level, whereas in developing countries, it is mainly at the production level.[36]

Multidimensional optimization of total diets

Food security means first ensuring adequate diets for all people, now and in the future. "Adequate" in this sense means that the diet fulfills all the nutrient and energy requirements for healthy growth and aging. Basic foods

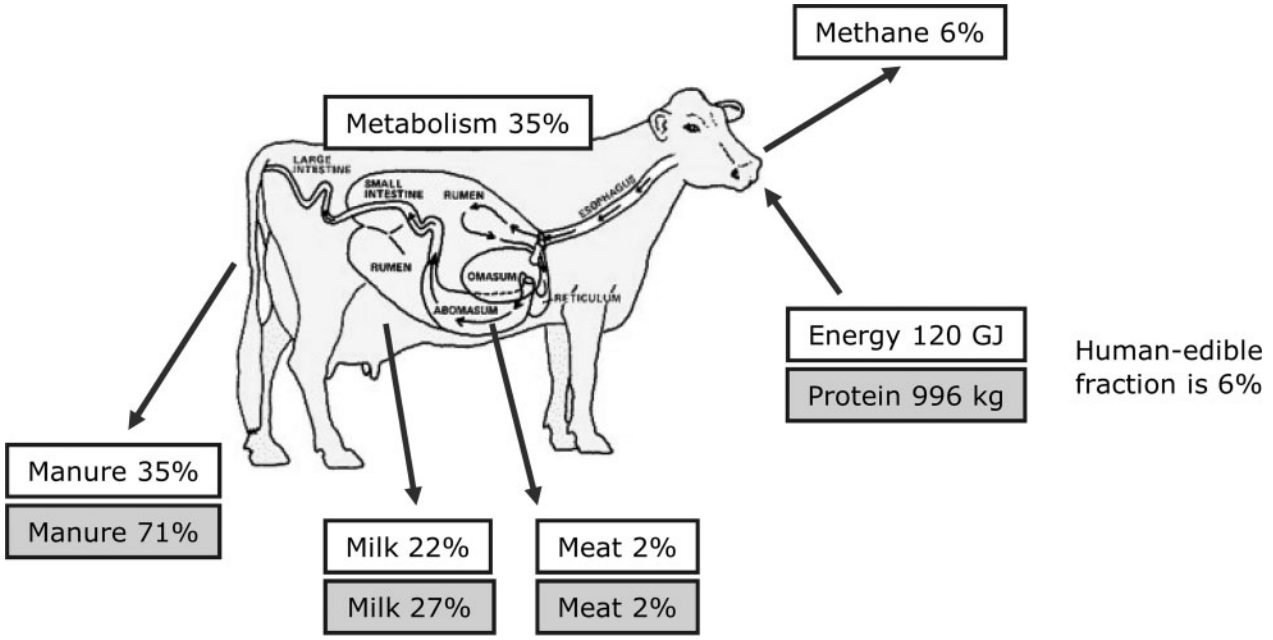

Figure 5 **The energy and protein (nitrogen) conversion of an average Dutch dairy cow (input per year).**[30]

such as animal products (dairy, meat, fish), grain products (bread, rice, pasta), and vegetables and fruits are the most important contributors to nutrients and energy intake. On average, all people have roughly the same requirements for nutrients and energy, but the means by which they are delivered through the diet may depend on the geographic region of residence, socioeconomic status, age, activity profile, and individual preferences. Moreover, such diets should be based on production systems that minimize the environmental impact (including low emissions, no pollution, and minimal impact on biodiversity). Finally, such diets should be acceptable, affordable, and safe. The science of evaluating diets based on all these dimensions is still in its infancy, but some first attempts have been made. Macdiarmid et al.[37] recently published a good example of this. The authors mathematically modeled a diet with respect to nutrient adequacy and minimal GHG emissions through a linear programming technique. They found that a reduction of 36% in GHG emissions could be achieved by optimizing existing food groups in a typical UK diet, while still maintaining the recommended nutrient intake. To guarantee a realistic outcome, acceptability and affordability constraints were added to the model. Due to the favorable nutrient profile of dairy, only a very small reduction in dairy consumption resulted from these model calculations.

CONCLUSION

The increased demand for dairy products needs to be realized, with the highest contribution to nutrient security and with improved resource efficiency and reduced GHG emissions. Infrastructural changes in milk production will be required to increase the volume of milk by as much as the predicted 50%. An increase in the productivity of dairy cows is crucial to obtain the required milk supply with minimal impact on scarce resources and global warming. When evaluating the position of dairy in a given diet, local aspects with respect to resource efficiency, as well as other environmental parameters, should be taken into account. Ruminants are excellent converters of human-inedible resources into high-quality foods that do not require sophisticated extraction technologies. This is especially true in developing countries. Infrastructural changes and new technologies are needed to mitigate the environmental impact of the dairy sector. Capturing the potential energy from manure together with the on-farm production of solar energy could significantly reduce fossil energy use by the dairy production chain. The modeling of diets with respect to nutrient adequacy and minimal environmental impact is a promising tool that refocuses the attention from comparing food products in isolation to evaluating complete diets. Modeling should also take into account affordability, food safety, and taste.

Acknowledgments

The authors thank Jan Dijkstra from the Department of Animal Nutrition of Wageningen University for his data input for this paper. The content of this article was presented as part of the Second Global Summit on the Health Benefits of Yogurt, held as a satellite to the

Experimental Biology meeting in San Diego, California, on 30 April 2014. The conference was organized by the American Society for Nutrition and Danone Institute International. The supplement coordinators are Sharon M. Donovan, University of Illinois at Urbana-Champaign, USA, and Raanan Shamir, Schneider Children's Medical Center, Israel.

Funding. Writing and editorial assistance were provided by Densie Webb, PhD, RD, who was contracted and funded by Danone Institute International. Dr van Hooijdonk received financial reimbursement for travel expenses and an honorarium from the Danone Institute International for his participation in the conference.

Declaration of interest. The authors have no relevant interests to declare.

REFERENCES

1. Alexandratos N, Bruinsma J. World agriculture towards 2030/2050: the 2012 revision. ESA Working paper No. 12-03. Rome, Italy. 2012.
2. UK Government Office for Science. Tackling Obesities: Future Choices—Project Report. 2nd edn. United Kingdom. 2007.
3. Council for Agriculture Science and Technology. Animal Agriculture and Global Food Supply, Task Force Report no. 135. Ames, Iowa, USA. 1999.
4. FAO. World Livestock 2011: Livestock in food security. Rome, Italy. 2011.
5. FAO. FAOSTAT. 2013. http://faostat3.fao.org/faostat-gateway/go/to/browse/Q/QL/E. Accessed September 12, 2014.
6. IFCN. Dairy Report 2011 For a Better Understanding of Milk Production Worldwide. Kiel, Germany. 2011.
7. IDF. The World Dairy Situation 2012. Bulletin of the IDF. 2012; No. 458/2012.
8. Heaney RP. Dairy intake, dietary adequacy, and lactose intolerance. Adv Nutr. 2013;4:151–156.
9. Dietary Guidelines Advisory Committee. Report of the Dietary Guidelines Advisory Committee on the Dietary Guidelines for Americans, 2010, to the Secretary of Agriculture and the Secretary of Health and Human Services. Washington, D.C.: U.S. Department of Agriculture, Agricultural Research Service; 2010.
10. American Academy of Pediatrics Committee on Environmental Health. Ultraviolet Light: A Hazard to Children. *Pediatrics*. 1999;104:328–333.
11. Dror DK, Allen LH. The importance of milk and other animal-source foods for children in low-income countries. Food Nutr Bull. 2011;32:227–243.
12. Brown-Esters O, Namara MP, Savaiano D. Dietary and biological factors influencing lactose intolerance. Int Dairy J. 2012;22:98–103.
13. Elmadfa I, ed. European Nutrition and Health Report. Karger, Basel. 2009.
14. Centers for Disease Control and Prevention, National Center for Health Statistics. National Health and Nutrition Examination Survey Data (2003–2006). http://www.cdc.gov/nchs/nhanes.htm. Accessed April 2013.
15. U.S. Department of Agriculture. Economic Research Service Food Availability. http://www.ers.usda.gov/data-products/food-availability-(per-capita)-data-system.aspx. Accessed April 2013.
16. Smedman A, Lindmark-Mansson H, Drewnowski A, et al. Nutrient density of beverages in relation to climate impact. Food Nutr Res. 2010;5170.
17. Drewnowski A. The contribution of milk and milk products to micronutrient density and affordability of the U.S. diet. J Am Coll Nutr. 2011;30(5 Suppl 1): 422S–428S.
18. National Institute for Public Health and the Environment (RIVM). VCP Voedselconsumptiepeiling 2003. The Netherlands; 2003.
19. Schaafsma G. The protein digestibility-corrected amino acid score. J Nutr. 2000; 130:1865S–1867S.
20. World Health Organization. Global Status Report on Noncommunicable Diseases. Geneva, Switzerland; 2010.
21. Pedersen JI, James PT, Brouwer IA, et al. The importance of reducing SFA to limit CHD. Br J Nutr. 2011;106:961–963.
22. Astrup A, Dyerberg J, Elwood P, et al. The role of reducing intakes of saturated fat in the prevention of cardiovascular disease: where does the evidence stand in 2010? Am J Clin Nutr. 2011;93:684–688.
23. Soedamah-Muthu SS, Ding EL, Al-Delaimy WK, et al. Milk and dairy consumption and incidence of cardiovascular diseases and all-cause mortality: dose-response meta-analysis of prospective cohort studies. Am J Clin Nutr. 2011;93:158–171.
24. Kratz M, Baars T, Guyenet S. The relationship between high-fat dairy consumption and obesity, cardiovascular, and metabolic disease. Eur J Nutr. 2013;52:1–24.
25. Heaney RP. Nutrients, endpoints, and the problem of proof. J Nutr. 2008;138: 1591–1595.
26. Bonjour JP. Calcium and phosphate: a duet of ions playing for bone health. J Am Coll Nutr. 2011;30(5 Suppl 1):438S–448S.
27. Elwood PC, Pickering JE, Givens DI, et al. The consumption of milk and dairy foods and the incidence of vascular disease and diabetes: an overview of the evidence. Lipids. 2010;45:925–939.
28. Food and Agriculture Organization of the United Nations. Livestock's Long Shadow: Environmental Issues and Options. Rome, Italy; 2006.
29. Food and Agriculture Organization of the United Nations, Animal Production and Health Division. Greenhouse Gas Emissions from the Dairy Sector, a Life Cycle Assessment. Rome, Italy. 2010.
30. Dijkstra J, France J, Ellis JL, et al. Production efficiency of ruminants: feed, nitrogen and methane. In: E Kebreab, ed. Sustainable Animal Agriculture. Wallingford, UK: CAB International; 2013.
31. Gerber P, Vellinga T, Opio C, et al. Productivity gains and emissions intensity reduction in dairy systems. Livestock Sci. 2011;139:100–108.
32. Food and Agriculture Organization of the United Nations. Protein Quality Evaluation: Report of the Joint FAO/WHO Expert Consultation. Rome, Italy; 1991.
33. Action TGDAf. http://www.dairy-sustainability-initiative.org. Accessed April 2013.
34. Krebbekx J, Lambregts E, de Wolf W, van Seventer M. Melk, de grene motor. Utrecht, The Netherlands: Berenschot; 2011.
35. Subbarao GV, Nakahara K, Hurtado MP, et al. Evidence for biological nitrification inhibition in Brachiaria pastures. Proc Natl Acad Sci U S A. 2009;106: 17302–17307.
36. Food and Agriculture Organization of the United Nations. Global Food Losses and Food Waste—Extent, Causes and Prevention. Rome, Italy; 2011.
37. Macdiarmid JI, Kyle J, Horgan GW, et al. Sustainable diets for the future: can we contribute to reducing greenhouse gas emissions by eating a healthy diet? Am J Clin Nutr. 2012;96:632–639.