Research
a dietetic practice group of the
Academy of Nutrition and Dietetics

TheDigest

Volume 47, Number 5 – Winter 2013

Dietary Nitrates and Nitrites Feed the Endogenous Nitrate/Nitrite/Nitric Oxide Pathway: Balancing Health Concerns with Potential Health Benefits

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ABSTRACT

Dietary nitrate (NO_3), nitrite (NO_2), and arginine can serve as sources for production of the nitrogen oxides or NO_x (a diverse group of metabolites including nitric oxide, nitrosothiols, and nitroalkenes) via ultraviolet light exposure to skin, mammalian nitrate/nitrite reductases in tissues, and nitric oxide synthase enzymes, respectively. NO_x are responsible for the hypotensive, antiplatelet, and cytoprotective effects of dietary nitrates and nitrites. Current regulatory limits on nitrate intakes, based on concerns regarding potential risk of carcinogenicity and methemoglobinemia, are exceeded by normal daily intakes of single foods, such as soy milk and spinach, as well as by some recommended dietary patterns such as the Dietary Approaches to Stop Hypertension diet. This review includes a call for regulatory bodies to consider all available data on the beneficial physiologic roles of nitrate and nitrite in order to derive rational bases for dietary recommendations.

> *Everything should be made as simple as possible, but not simpler.*
> ~Albert Einstein's comment on the philosophy of parsimony of William of Occam, English philosopher and Franciscan friar (c. 1285–1349)

INTRODUCTION

We have proposed that dietary nitrates and nitrites be considered nutrients.[1] This proposal contradicts regulatory exposure limits for nitrate and nitrite in ground water and processed meats due to hypothetical risk of methemoglobinemia in infants and gastrointestinal cancer, respectively.[2] This proposal is based on two lines of evidence: 1) the content of inorganic nitrate (NO_2^-) in vegetables and fruits provides a physiologic substrate for reduction to nitrite (NO_3^-), nitric oxide (NO) and other metabolic products (NO_x) that produce vasodilation, decrease blood pressure and support cardiovascular function in humans,[3-6] and 2) nitrates and nitrites are normal constituents of foods and diets rich in vegetables and fruits, including the Dietary Approaches to Stop Hypertension (DASH) or similar dietary patterns such as the Mediterranean diet, are protective against coronary heart disease and ischemic stroke risk.[7-10] We have also recently "shown" v. demonstrated that human milk contains nitrate and nitrite and, therefore, demonstrate that humans are adapted to receive these dietary components from birth.[11] The goals of this review are to 1) provide a physiological context for the potential cardiovascular benefits of dietary nitrite and nitrate from plant foods, and 2) support a growing consensus for a comprehensive reevaluation of the health benefits

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and risks associated with dietary sources of nitrates and nitrates. These goals require a brief primer on the dietary sources of nitrate and nitrite as well as the enzymatic and non-enzymatic mechanisms by which nitrate and nitrite-derived metabolites, termed NO_x, are produced in the vasculature and in tissues.

Dietary sources of NO_x

Nitrate enters the food chain through plant foods via the action of lightning and soil bacteria. In addition to being a required nutrient for plants, it is an approved food additive.[12] In humans, nitrate intake is primarily from vegetable sources while surface and ground water is a minor contributor. Nitrate intakes from vegetables are determined by the type of vegetable consumed, the levels of nitrate in the vegetables (including the nitrate content of fertilizer), the amount of vegetables consumed, and the level of nitrate in the water supply.[13] Approximately 80% of dietary nitrates are derived from vegetable consumption; sources of nitrites include vegetables, fruit, and processed meats.

The excessive concentration of nitrate in drinking water, typically a minor contributor to dietary nitrate and nitrite concentrations, must be considered a serious health concern, particularly for infants.[14] Even so, the Society of Agricultural and Biological Engineers hascalled for a more rational approach to setting exposure limits on nitrogen-containing effluents in wastewater treatment.[15] To wit,

> *"Considering that definitive evidence of nitrate health risks is conspicuously lacking, a more rational approach to setting effluent limits or waste treatment systems is needed, one that considers costs/ benefits and recognizes factors that act to limit nitrogen buildup in groundwater.*
>
> *Such factors include nitrogen removal by soil microorganisms, and aquifer hydrogeology."*

While ground water nitrate concentrations are regulated due, in part, to potential risk of infant methemoglobinemia, the practice of causal inference concerning this association has been questioned. Experts have questioned the veracity of the evidence supporting the hypothesis that nitrates and nitrites are toxic for healthy adolescent and adult populations.[3, 4, 16, 17] Many scientists now interpret the available data as evidence that the methemoglobinemia observed in infants was likely caused by bacterial infection-induced enteritis- which produces a high concentration of NO that is successively oxidized to nitrite and nitrate- rather than high nitrate concentrations in drinking water.[4, 17] Thus, it appears that the biologically plausible hypothesis of nitrite toxicity with regard to methemoglobinemia has transformed a plausible hypothesis into legal dogma[3], despite the lack of proof.[16, 17]

Physiologic resilience: redundant systems of NOx production in vasculature and tissues

Normal functioning of human vasculature requires both the presence of nitrite and nitric oxide along with the necessity to respond to these important signaling molecules.[18] Indeed, oxidative stress and reduced NO bioavailability are critically linked to endothelial dysfunction, hypertension and other forms of cardiovascular diseases. Mechanistically, the physiologic target for the metabolites of dietary nitrate is mitochondria and the regulation of oxidative stress.[19] The generation of up to ~70% of systemic nitric oxide is accomplished by endothelial nitric oxide synthase (eNOS), one of 3 members of the NOS family of enzymes, in the vascular endothelium.[20] These enzymes synthesize nitric oxide from the amino acid L-arginine and molecular oxygen to accomplish vasodilation, blood pressure regulation, inhibition of endothelial inflammatory cell recruitment, and platelet aggregation.[21] As a result, the normal production of nitric oxide and nitrite and the ability of the endothelium to respond to these species may prevent various types of cardiovascular disease, including hypertension, atherosclerosis, and stroke.[22]

The biological effects of nitric oxide are caused by the initiation of cyclic GMP (cGMP)–mediated intracellular signals in the vascular wall. In atherosclerosis, hypoxic conditions combined with an oxidative environment can limit eNOS-derived nitric oxide production; nitrite can directly induce vasodilation in hypoxic endothelium.[18] Remarkably, a low concentration of sodium nitrite in drinking water (50 mg/liter) can substitute for loss of eNOS-derived NO in eNOS deficient mice.[23]

Unlike the provision of eNOS-derived NO to the endothelium to maintain vasomotor tone, nitric oxide production from nitrite occurs primarily in tissues.[21] There are

two systems of reducing nitrate to nitrite in mammals. The first system identified to accomplish this was the action of commensal gram negative bacteria on the tongue to reduce salivary nitrate.[24] Concentrations of plasma nitrate in the saliva occur as part of enterosalivary circulation of dietary nitrate.[25] Approximately 25% of ingested nitrate is secreted in saliva, where some 20% (or ~5–8% of the nitrate intake) is converted to nitrite by commensal bacteria on the tongue. These anaerobic bacteria (e.g., sp. *Vionella*) on the dorsal surface of the tongue use nitrate as an alternative electron acceptor to produce energy. Indeed, use of an antibacterial mouthwash after consumption of dietary nitrate (10 mg/kg in water) attenuates the expected postprandial rise in plasma nitrite.[26] The nitrite supplied to the gastrointestinal tract serves to enhance gastric mucin production and can serve as a substrate for generation of nitrogen oxides for antimicrobial actions and support of gastric homeostasis.[27]

Contribution of dietary constituents to nitric oxide production

Endogenous reduction of nitrate to nitrite is the source of NO and NO_x in tissues and, in hypoxia, the vasculature.[22, 28] As such, dietary nitrates and nitrites, via successive reduction by mammalian nitrate and nitrite reductases, serve as the primary source for >50% of NO produced in the human body, resulting in about 1 mmol NO per day in those consuming Western-type diets.[29] Dietary protein intakes of ~90 grams of protein would contribute about 14.5 grams nitrogen; the amino acid arginine in protein serves as an organic substrate for nitric oxide synthase enzymes which produce about 1 mmol NO per day.[1] It has been estimated that one serving of a high nitrate vegetable, like spinach, contains more nitrate than what is endogenously formed by the all three NOS isoforms combined during a day.[21] It is noteworthy that dietary compounds such as vitamin C and polyphenols can enhance the formation of NO from nitrite and prolong the half-life of NO in the stomach, respectively.[30]

Biological actions of NO_x

Metabolic disposition of plasma and tissue nitrates is dependent upon local metabolic conditions, including tissue oxygenation, inflammatory state and exposure to ultraviolet light.[31] It has been demonstrated that the content of inorganic nitrate (NO_3) in certain vegetables and fruit can provide a physiologic substrate for reduction to nitrite (NO_2), and subsequently to nitric oxide (NO), which can lead, in a tissue-specific fashion, to the post-translational modification of proteins by nitration and S-nitrosylation (e.g., nitrosothiols) and nitration of fatty acids.[21] This collection of metabolites derived from the metabolism of nitrate, nitrite and NO are termed NO_x. Metabolism and regulation of NO and nitrite are at the local cellular and tissue level dependent upon oxygen tension, cellular redox status, redox active metal and thiol availability.[32] The production of these NOx species is associated with vasodilation, decreased blood pressure, and enhanced cardiovascular function in humans.[5, 33]

Nitrate, nitrite and cardiovascular effects of NO_x

Several authors have recently reviewed the extensive literature on the cardiovascular benefits of dietary nitrate and nitrite in animal models and humans.[6, 34, 35] The demonstrated cardioprotective effects of dietary nitrate and nitrite in humans include reductions in blood pressure, reduced platelet aggregation, enhanced endurance and improved endothelial function.[36] Indeed, systemic plasma levels of nitrite and nitrate reflect brachial flow-mediated dilation responses in young men and women.[37] Most of these effects had first been observed in animal models including efficacy in pulmonary arterial hypertension and intimal hyperplasia in the atherosclerosis.[38, 39]

It is now clear that nitrates in foods/beverages and sodium nitrite in beverages or intravenous infusions can, in a dose-dependent fashion, predictably lower acute blood pressure in humans and animal models. The magnitude of this effect ranges from decreased diastolic blood pressure (DBP) an average of ~4.5 mm Hg in human subjects consuming a variety of traditional Japanese foods high in nitrate (18.8 mg nitrate per kg body weight/day).[40] In elegant human studies by Kapil et al., subjects consumed, in a series of randomized crossover studies, either beetroot juice (500 ml containing ~341 mg nitrate) or inorganic potassium nitrate capsules (4, 12 or 24 mmol containing 248, 744 or 1488 mg of nitrate from KNO_3).[41] As expected, subjects experienced dose-dependent increases in plasma nitrate and nitrite. KNO_3 (24 mmol) ingestion caused reductions in both

systolic blood pressure (SBP) and DBP over 24 hours compared with KCl control. Peak differences were 9.4±1.6 mm Hg (at 6 hours) and 6.0±1.1 mm Hg (at 2.75 hours) for SBP and DBP, respectively. Interestingly, post-hoc analyses have revealed sex differences in processing of dietary nitrate load through the enterosalivary circulation and its consequences on blood pressure.[41] In another study by the same group, females had significantly higher baseline plasma nitrite compared to males (0.43 ± 0.03 vs. 0.36 ± 0.01 μM, $p < 0.05$), salivary [nitrite] (0.39 ± 0.05 vs. 0.26 ± 0.03 μM, $p < 0.05$), lower clinic (SBP: 105.5 ± 1.1 vs. 113.9 ± 0.9 mmHg, $p < 0.01$), home (SBP: 109.7 ± 1.0 vs. 119.3 ± 1.4, $p < 0.01$) and ambulatory BP (SBP: 115.1 ± 0.7 vs. 122.0 ± 0.8, $p < 0.01$). Females exhibited higher oral nitrite production compared to males (~2-fold for capsules containing 800 μM and 8 mM KNO_3, $p < 0.01$ for both).[42, 43] These results suggest that females produce more nitrite derived from enterosalivary circulation that may contribute to the reduced BP, and perhaps therefore the reduced risk of cardiovascular disease in this sex.

Dietary nitrates enhance physical endurance through improved efficiency of mitochondrial respiration in humans

In a series of landmark papers, it has been demonstrated that dietary nitrate lowers the oxygen cost of exercise,[44, 45] and enhances endurance[46] ostensibly by increasing efficiency of mitochondrial efficiency in humans.[19] These findings show that dietary sources of nitrate and nitrite are not only are the most efficacious hypotensive components of the diet but also improve physical performance in humans.

Regulatory limits and intake estimates for dietary nitrate and nitrite

The US Environmental Protection Agency limits human exposure to inorganic nitrates to 0.10 mg/L (or 10 ppm nitrate nitrogen) and nitrites to 1 ppm nitrite nitrogen. The Joint Food and Agricultural Organization/ World Health Organization has set the Acceptable Daily Intake (ADI) for the nitrate ion at 3.7 mg/kg body wt and for the nitrite ion at 0.06 mg/kg body wt.[12] Likewise, the Environmental Protection Agency has set a Reference Dose for nitrate of 1.6 mg nitrate nitrogen per kg body weight per day (equivalent to 7.0 mg nitrate ion/kg body wt per day).

The mean intake estimates for nitrate and nitrite from food in the United States and Europe vary from ~40–100 mg/d and 31–185 mg/d, respectively (as reviewed in).[1] Nitrite intakes vary from 0 to 20 mg/d. Nitrate intakes from sources other than vegetables, including drinking water and cured meats, has been estimated to average 35–44 mg/ person per day for a 60-kg human.[12] On the basis of a conservative recommendation to consume 400 g of different fruits and vegetables per day at median nitrate concentrations, the dietary concentration of nitrate would be ~157 mg/d.[12]

The International Agency for Research on Cancer's (IARC) Monograph Working Group concluded that "Ingested nitrate or nitrite under conditions that result in endogenous nitrosation is probably carcinogenic to humans (Group 2A).[47 48, 49] The American Institute for Cancer Research (AICR) has published the only dietary recommendations based on an extensive systematic review that addresses these conditions. AICR's *Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective* contains the following recommendation "Limit consumption of red meats (such as beef, pork and lamb) and avoid processed meats".[50] Systematic review indicated that up to ~500 grams (~18 ounces) a week of red meat can be consumed without raising cancer risk. However, review panelists could not determine a safe consumption level for processed meat; cancer risk was shown to increase with any consumption of processed meats.[50] As such, only processed meats, and not plant sources of nitrate and nitrite, were proscribed. In agreement with this conclusion is a panel convened by the European Food Standards Agency that concluded:

> *"Overall, the estimated exposures to nitrate from vegetables are unlikely to result in appreciable health risks, therefore the recognised beneficial effects of consumption of vegetables prevail."*[51]

Indeed, the direct evidence for the participation of nitrate and nitrite in human carcinogenesis is lacking despite extensive epidemiologic and animal studies.[17] Rodent toxicological studies[52] and human epidemiological investigations have not shown an unequivocal relationship between nitrite exposure and the risk of cancer.[53] These data suggest that nitrite

formation resulting from nitrate exposure from dietary sources pose no risk with regard to cancer. The association between nitrite consumption and gastrointestinal cancers was bolstered by findings that ingested nitrites may react with secondary amines or N-alkylamides to generate carcinogenic N-nitroso compounds (NOCs).[53] While NOCs have been shown in animal models to be carcinogenic,[54] proof in humans has been scant. The N-nitrosamides and N-nitrosoureas have been shown to be direct mutagens, while N-nitrosoamines do not act as direct mutagens but generally require activation by microsomal enzymes within the body, perhaps by microsomal enzymes.[55] The use of nitrites in bacon must be accompanied by the use of either sodium erythorbate or sodium ascorbate (vitamin C), antioxidants that inhibit the nitrosation effect of nitrites on secondary amines.[56] The use of these antioxidants, along with lower nitrate/nitrite levels in processed meats, has lowered residual nitrite levels in cured meat products in the U.S. by ~80% since the mid-1970s.[57]

A recent study has yielded new insights into the ability of vitamin C to modulate the formation of carcinogenic NOCs under conditions simulating the proximal stomach during the digestion of foods like processed meats.[58] Nitrite in processed meats may be converted to nitrosating species and NOCs by acidification in the presence of thiocyanate at low gastric pH. The formation of NOCs was examined in these conditions in the presence and absence of vitamin C and lipid. In the absence of lipid, vitamin C prevented the formation of N-nitrosodiethylamine and N-nitrosopiperidine and decreased the formation of N-nitrosodimethylamine and N-nitrosomorpholine five-fold and one thousand-fold, respectively. In the presence of 10% lipid (a food matrix component for processed meats), the presence of vitamin C increased the formation of nitrosodimethylamine, nitrosodiethylamine, and N-nitrosopiperidine 8-, 60- and 140-fold, respectively. Thus, the presence of lipid converts vitamin C from inhibiting to promoting acid nitrosation. This effect is attributable to the ability of vitamin C to assist in the generation of NO in the aqueous phase which enables the regeneration of nitrosating species by reacting with oxygen in the lipid phase.[58] While these data require confirmation in animal models and humans, it provides a biologically plausible mechanism for the observed association between processed meat consumption and gastrointestinal cancer risk. Others have postulated that gastric formation of NOCs may be inhibited by nutrients and other components of vegetables and fruits.[59] Clearly, more research is needed to address the potential mechanisms by which certain NOCs are related to cancer risk.

Dietary intakes in the context of WHO ADI levels

The appreciation of five facts regarding human exposures to nitrate and nitrite casts concern over current regulatory limits on nitrate and nitrite consumption. First, it is possible to approach or exceed World Health Organization Acceptable Daily Intake (WHO ADI) limits with usual intake levels of single foods, such as colostrum (at 100 ml intake in an newborn infant delivering 42% of the WHO ADI intake limit), soya milk (750 ml intake for a hypothetical 6.8 kg infant yields 104% of the WHO ADI intake limit), spinach[5] or a dessicated vegetable supplement.[1, 60] Second, recommended dietary intakes of vegetables and fruits, such as a DASH pattern with high nitrate food choices, exceeds the World Health Organization's Acceptable Daily Intake for nitrate by 550% for a 60-kg adult.[1] Third, for adults consuming the recommended intakes of vegetables and fruits, the origin of up to 95% of dietary nitrate and nitrite, the concentration of nitrate in saliva, via enterosalivary circulation, can reach up to three times the concentration in most global regulatory limits for drinking water. Fourth, provision of dietary nitrate, such as beetroot juice,[34] dietary nitrate,[61] or in a traditional Japanese dietary pattern,[62] are effective in lowering blood pressure in humans. Fifth, human infants consuming breast milk are exposed to nitrate and nitrite in human milk from birth.[11] In the absence of data from large prospective epidemiologic studies, including the European Prospective Investigation in Cancer (EPIC) and others,[63, 64] of a positive association between vegetable and fruit consumption (the largest source of nitrate and nitrite) cancer risk, we must conclude that the association between nitrate and nitrite consumption and cancer risk is limited to specific foods such as processed meats.

These facts indicate that WHO intake limits may not reflect optimal nitrate and nitrite concentrations from foods that confer health benefits. If nitrates and nitrites act as nutrients, it is likely that they do so to bolster the reserve of nitrite-derived NO_x metabolites required for optimal functioning through periods of physiologic stress (e.g., hypoxia and acidosis) and diseases characterized by endothelial dysfunction.[22]

Potential negative health effects of dietary nitrate and nitrite exposures

Two types of exposure place susceptible individuals at high risk of adverse effects of excess nitrite exposure. First, infants less than six months of age may be exposed to excess nitrates in bacterially-contaminated well water that reduces nitrate to nitrite.[65] Infants consuming excess nitrite experience methemoglobinemia or "blue baby syndrome" due to nitrite-mediated oxidation of ferric (Fe^{2+}) iron in oxyhemoglobin that leads to hypoxia and cyanosis.[3, 66] As such, an American Academy of Pediatrics consensus panel concluded that all prenatal and well-infant visits should include questions about the home water supply; if the water source is a private well, the water should be tested for nitrate.[14] The panel concluded that infants fed commercially prepared infant foods are generally not at risk of nitrate poisoning but that home-prepared infant foods from vegetables (e.g., spinach, beets, green beans, squash, carrots) should be avoided until infants are 3 months or older. Breastfed infants are not at risk of excessive nitrate exposure from mothers who ingest water with high nitrate content (up to 100 ppm nitrate nitrogen) as nitrate concentration does not increase significantly in the breast milk.[14]

It is noteworthy that the few human nitrate and nitrite exposure studies, including children and adults, have not produced methemoglobinemia. Infants exposed to 175 to 700 mg nitrate per day did not experience methemoglobin levels above 7.5%, suggesting that nitrate alone is not causative for methemoglobinemia.[67] A more recent randomized 3-way crossover study exposed healthy volunteer adults to single doses of sodium nitrite that ranged from 150 to 190 mg per volunteer to 290 to 380 mg per volunteer.[68] Observed methemoglobin concentrations were 12.2% for volunteers receiving the higher dose of nitrite ion and 4.5% for those receiving the lower dose. Recent nitrite infusion studies of up to 110 µg/kg/minute for 5 minutes induced methemoglobin concentrations of only 3.2%.[69] These data have led scientists to propose alternative explanations for the observed methemoglobinemia in infants, including gastroenteritis and associated iNOS-mediated production of eNOS induced by bacteria-contaminated water.[16, 17] These studies call into question the mechanistic basis for exposure regulations for nitrate and nitrite. At best, these findings highlight serious, but context-specific, risks associated with nitrite overexposure in infants. Experts have questioned the veracity of the evidence supporting the hypothesis that nitrates and nitrites are toxic for healthy post-infant populations.[3, 4, 12] It appears that the biologically plausible hypothesis of nitrite toxicity (e.g., methemoglobinemia) has essentially transformed a plausible hypothesis into sacrosanct dogma[3] in spite of a lack of proof.[16, 17]

Epidemiologic and clinical studies have weak associations between processed meat consumption (sources of nitrate and nitrite) and increased risk of gastrointestinal cancers, thyroid dysfunction and thyroid cancer,[70] and chronic obstructive pulmonary disease in women[71] as well as other conditions.[72] Nitrate and nitrite exposures have been associated with gastrointestinal cancer risk through the consumption of cured and processed meat.[73] When weak associations (relative risks < 2) exist and inconsistent outcomes are observed, as in the case of nitrate-cancer associations, epidemiologic evidence is insufficient to support an unequivocal positive association and, hence, public health recommendations.[74-76]

Although modestly increased associations between consumption of foods containing nitrite and nitrate and certain cancers have been reported in some prospective epidemiologic studies,[77-79] overall, findings across studies have been largely inconsistent and equivocal.[80-83] Consequently, the overall burden of proof remains inconclusive.[84-91] A biologically plausible mechanism for the carcinogenicity of ingested nitrate and nitrite involves endogenous N-nitrosation reactions. The only systematic review of nitrate and nitrite-containing foods which makes evidence-based recommendations is the American Institute for Cancer

Research's report on diet, physical activity and cancer risk[50] which includes the dietary proscription, "Avoid processed meats". The contexts in which nitrates and nitrites are consumed, whether through processed meats or from vegetables and fruits, need to be separated in order to clarify the physiological basis for associated health benefits and risks. As such, plant sources of nitrate and nitrite, the primary source of nitrates in the human diet, can be recommended for their potential health benefits.

Potential health benefits of dietary nitrates and nitrites: call for a new regulatory paradigm

Since the carcinogenicity of nitrate and nitrite from plant sources has not been demonstrated in rodents or humans, it is time for a comprehensive review of the evidence by a multidisciplinary panel of academic, governmental and industry scientists. Reviews sponsored by the Institute of Medicine of the National Academy of Science serve as excellent examples of the type of review recommended.

Current regulations do not take into account the endogenous biology of ingested nitrates and nitrites and the human data on the cardiovascular benefits of these compounds. Current research suggests that a more sophisticated regulatory approach to dietary nitrate and nitrite exposures are needed. The nitrate-nitrite-NO pathway has been demonstrated to serve as a backup system to ensure NO supply in situations when the endogenous L-arginine/NO synthase pathway is dysfunctional.[34] This redundant system of NO production in tissues has important implications for cardiovascular, gastrointestinal and immune function related to the provision of dietary nitrate and nitrite. As nitrite-dependent NO generation has been shown to play critical physiological and pathological roles, and is controlled by oxygen tension, pH, reducing substrates and nitrite levels, it is necessary to balance these contexts in a modern regulatory framework that acknowledges a physiological requirement for nitrate and nitrite supplied by dietary means.

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Note from the editor:

This article describes a controversial issue. Vegetables and fruits are the source of at least 80% of nitrate/nitrite in typical human diets even in healthy diet patterns such as the DASH or Mediterranean-type diets. In the largest prospective studies ever done in diet and cancer (e.g., EPIC, etc.), vegetables and fruits are associated with slightly lower cancer risk. Only processed meats, a minor source of nitrates/nitrites, are identified as being associated with cancer risk. Some experts believe all sources of nitrates/nitrites should be banned or restricted to a minimum. This article's main objective is in agreement with the suggested conclusion of suggest findings of an expert panel convened by the European Food Standards Agency. This panel concluded the following: "Overall, the estimated exposures to nitrate from vegetables are unlikely to result in appreciable health risks, therefore the recognized beneficial effects of consumption of vegetables prevail." (European Food Safety Authority, 2008, http://www.efsa.europa.eu/en/scdocs/doc/689.pdf, accessed February 14th, 2013).

Member Spotlight

Norman G. Hord, Ph.D., M.P.H., R.D.

Erin Gaffney-Stomberg, PhD, RD

Norman G. Hord, Ph.D., M.P.H., R.D.

For this edition of The Digest, we are spotlighting RDPG member Norman G. Hord, PhD, MPH, RD. Dr. Hord is an Associate Professor at the School of Biological and Population Health Sciences at Oregon State University in Corvallis, OR. Dr. Hord has an exciting career in translational nutrition research in which he uses his broad training in dietetics, public health and molecular nutrition to study the effects of dietary nitrate and nitrite on atherosclerosis and carcinogenesis while mentoring undergraduate and graduate students. Read below to learn how a tennis injury spurred his interest in nutrition and passion for research.

Dr. Hord, how would you describe your current position as an Associate Professor?

I am a professional 'ladder'. As a faculty member, I provide knowledge and skill-building 'ladder' services as a teacher, researcher and administrator in undergraduate and graduate programs in nutrition and dietetics. Through course work, research training and professional mentoring, my colleagues and I can help students reach their goals if they are willing to exert effort and demonstrate passion for the fields of nutrition and dietetics.

Dr. Hord, please tell us about your background. How did you get to where you are now?

My interest in nutrition began with an adverse reaction to a non-steroidal anti-inflammatory drug prescribed for a wrist injury (i.e., carpel tunnel syndrome) caused by playing tennis. The investigation of non-pharmacological approaches to common conditions motivated me to study nutrition as an undergraduate. My first job was as a food product developer for a large restaurant chain. I then received a master's degree in nutrition from Clemson University where I completed my dietetics courses and supervised practice requirements. I received my PhD in nutrition from Purdue University and performed my dissertation research in the laboratory of Dr. Gary Perdew. We published several papers detailing the subcellular localization and function of the aryl hydrocarbon receptor and its partner in transcriptional regulation called Arnt. These proteins sense and transduce signals from many dietary and diet-derived metabolites to regulate cellular detoxification reactions. After my doctoral program, I went on to complete the National Cancer Institute's (NCI) Cancer Prevention Fellowship. As a part of this fellowship, I completed a Master's in Public Health degree at Johns Hopkins University's Bloomberg School of Public Health. Additionally, I completed postdoctoral research training in the Laboratory of Nutritional and Molecular Regulation (headed by James Phang, MD) at the NCI. We demonstrated that dietary factors have genotype-dependent effects on phenotypes associated with colon cancer risk. Since then, I have held positions at several universities and taught in both coordinated (CP) and didactic (DPD) programs. At Michigan State University, I led the development of and wrote the self-study part for a new dietetic internship program. I am thankful to have had the opportunities to train in these areas and serve the profession of dietetics. I bring these perspectives to students and trainees each day.

What is your current research interest?

My research program addresses the potential mechanisms by which dietary nitrates and nitrites from plant foods modulate phenotypes associated with atherosclerosis and carcinogenesis. In my lab, we investigate the anti-inflammatory effects of dietary nitrates and nitrites in model systems and humans. We believe that current dogma and regulations concerning nitrate and nitrite are simplistic and based on the practice of poor causal inference. We believe the evidence supports our assertion that nitrates and nitrites from plant sources are nutrients that support vascular health via vasodilatory, anti-inflammatory and anti-thrombotic activities.

How did you become involved/ interested in your current line of research?

I was hooked by Dr. Nathan Bryan's (University of Texas) research showing that a small concentration of nitrite in drinking water could totally reconstitute nitric oxide (NO) homeostasis in an animal model that lacked endothelial nitric oxide synthase (eNOS), the enzyme that was thought to be the major source of NO in the body. We now know that a regular sized salad made of spinach can provide the body with enough nitrate to make more NO than all forms of nitric oxide synthases combined. The most interesting part about endogenous NO synthesis from dietary nitrate and nitrite is that it works best under conditions in which eNOS cannot (i.e. low oxygen or hypoxic and acidic conditions in the body). As such, these compounds can provide an 'endocrine reserve'

for NO production under ischemic or other stressful conditions. There is much to be learned about the dietary needs for nitrate and nitrite and the specific contexts in which they may support physiological functions or produce health risks.

What advice would you give to young researchers for developing a successful line of research?

I have two pieces of advice. First, young investigators should study diet-related problems whose solutions could provide high impact in human populations. Secondly, since excellent research requires multidisciplinary collaboration, investigators should not only be experts in their specific area but be able to apply their expertise as a part of collaborative teams. I followed my own advice: my training spans from cell biology, toxicology and public health in addition to nutrition and dietetics. Diet acts through thousands of relevant metabolic processes to influence health; we must all keep this broad perspective in mind lest we lose our focus on the narrow area of our specific expertise.

What are your career goals?

It is an odd but true statement to say, "I have already achieved all of my career goals!" Of course, the process of developing and refining goals is part of a healthy professional development (and our credentialing process!). As such, I strive to continue to serve students and the profession by contributing to discoveries in nutrition that benefit public health. These difficult goals can only be met through keeping current with research findings, effective teaching, academic leadership and professional service.

How has your affiliation with the Academy impacted your career progression?

Academy members have provided mentoring for me since the beginning of my career as an RD. One example is Dr. Nancy Lewis, current Speaker-elect of the Academy House of Delegates. Since my first position as a dietetic educator, Nancy has graciously provided balanced advice and wisdom on any issue I've asked her about. I have also benefited from Academy member invitations to speak at or organize FNCE events, to edit manuscripts or participate in position paper development. This mentoring has motivated me to want to contribute back to the profession.

If someone were to ask you to explain why research is important to the field of dietetics, what would you say?

It is our only defense against our poorly prepared competitors. There are many who would sell their advice or products to an unknowing public based upon little or no research and poor, if any, training. The 'loose' labeling laws of federal legislation in the U.S. (in contrast to Europe) allow for untrained people to sell poorly designed products that have little, if any, scientific support. While it has always been this way in the U.S., I am proud that the Academy not only supports evidence-based practice but also funds research to determine what works best. This leadership makes me proud to support the Academy in these efforts.

Chair's Report

Dear Research DPG Members

Chris Taylor, PhD, RD, LD

As another year begins it offers an opportunity for introspection. Social media are ablaze with posts of surviving the holidays, exultations of things for which we are thankful and the pending societal, political and economic issues that we also face. Regardless of how you approach these, health and nutrition pervade all of these issues, some at the very core. Out of these many successes and challenges we face are evolving layers of opportunity.

At the risk of sounding like a broken record, we are a group in a unique position to impact the trajectory and scope of our field. Our research spans the layers that now comprise the Clinical and Translation Science Awards that guide some federal health research. The work of bench to bedside has slowly transitioned into bench to community, no matter how big or small you define community. In a time of pinching purse strings and limited funds for research and programs, we can demonstrate the cost-effective impact of dietetics on many of the issues facing our nation and our world today.

Recently, your Executive Committee met to discuss the opportunities and obligations we have to you as members to provide member benefit and become enablers of the novel ideas you possess to impact health and charge the profession forward. We are looking to invest the resources of the RDPG into recognizing the great work of our members through new awards, facilitating research through mentorship, collaboration and seed grant awards and sharing the evidence we generate with policy-makers in Washington through the Public Policy Workshop. These discussions will begin shortly, but it is never too late to share your ideas of how we can serve you better.

One final thought in closing. Thank you all for your on-going support and for voting in both the Academy and DPG elections.

Sincerely,
Chris Taylor, Research DPG Chair, 2012-13

A Basic Glossary of Research Terms from A-Z: d-f

Inés M. Anchondo Dr.P.H., R.D., L.D., C.S.P.

Degrees of freedom – is the number of statistically independent values of a sample that are free to vary. All values can vary except for one, represented by n-1.

Descriptive study – is a research study design in which a behavior or phenomenon is observed, without influencing the behavior or phenomenon in any way.

Dependent variable – the outcome variable of interest.

Dichotomous variable – a variable that is categorized (separated) into two groups or categories.

Effect size – measures, defines, or estimates the strength of a relationship between two variables in a population to determine the sample size needed to generalize the results to the whole population.

Experimental error – the difference between an observed and predicted value, which can occur randomly or due to problems with research design or process. An experimental error can be a Type 1 error (a false positive, rejecting the null hypothesis although it is in fact true) or a Type 2 error (not rejecting the null hypothesis although it is factually not true, a false negative).

Experimental group – a group of individuals, objects, concepts etc. exposed to the independent variable in an experiment or research study.

Experiment – an act performed under controlled conditions in order to test a hypothesis.

Face validity – a test performed to determine whether the tool (questionnaire or survey) measures what it is intended to measure.

Factor analysis – a statistical procedure conducted to identify 'clusters' or groups of related items (known as factors) on a test. For example, in a multiple choice exam, a factor analysis can be done to see what types of questions (factual vs. conceptual) were answered better than others.

Focus group – a group of people gathered to ask them about their perceptions, opinions, beliefs, and attitudes towards something (situation, test, issue, etc.). Focus groups are used in qualitative research.

Frequency distribution – arranging or classifying statistics data from lowest to highest and including number of times each value occurred.

Note: this glossary was inspired by Mary Easaw, a clinical dietitian in a cardiothoracic hospital in Malaysia

Treasurer 's Report

Winter Greetings, Research DPG Members!

Karin Pennington, M.S., R.D., L.D.
RDPG Treasurer

Our annual budget allows for $24,035 of income and $24,076 in expenses. The fiscal year is from June 2012 to May 2013. Our current reserves are at 53%. This appears slightly lower than expected. The Academy would like our reserve to be near 100%. We expected the reserves to decrease a bit after FNCE, as FNCE is the largest expense of the fiscal year. FNCE was a success this year, and the RDPG provided a stipend for Executive Committee and HOD members to attend. The Academy has processed all expenses related to the stipend, and this is noted below in the "Expenses" part of the budget table. We are continuing to work with the Academy to process some of the revenue from our sponsors, the California Walnut Commission, The Beef Checkoff, and the National Cattlemen's Beef Association. We therefore expect the reserve to increase soon. Thank you to our sponsors for their generous support!

Karin Pennington, M.S., R.D., L.D.
RDPG Treasurer

Research DPG 2012-13 Budget

		Annual Budget ($)	As of January ($)
Revenue	Membership	17,535	7,235
	Grants/Contracts	6,500	2,500
	Publication Sales	—	1,155
	Interest Income	—	1,249
		24,035	12,139
Expenses	Lodging/ Subsistence	993	2,548
	Transportation	5,700	855
	Professional/ Consulting	2,000	495
	Postage	450	24
	Teleconferences	230	7
	Member Dues/ Fees	1,203	261
	Outside Services	3,200	0
	Awards	4,600	4,000
	Audio Visual	0	2,916
	Food Service	5,000	11,776
	Printing/Copying	650	281
	Other	50	0
		24,076	23,160
	NET	**-41**	**-11,021**
Reserve	**November 2012 Reserve**	**21,403**	**11,453**
	Reserve Percentage	**—**	**53%**

Nutrition and Epigenetics: Maternal Choline Intake and Fetal Cortisol-Regulating Genes

Marie Caudill, PhD, RD

Years of research demonstrate the importance of choline in fetal and infant brain development, with a role in preventing neural tube defects[1,2,3] and affecting the areas of the brain responsible for memory and life-long learning ability.[4,5,6] My lab at Cornell University focuses on the level of choline intake required to optimize maternal and fetal health, and we are especially interested in exploring choline and related nutrients in the context of epigenetics. One of our most recent studies[7] examined the effects of maternal choline intake during human pregnancy on epigenetic modulation of cortisol-regulating genes in the hypothalamic-pituitary-adrenal (HPA) axis. The results suggest that choline intake during human pregnancy may have implications for reducing stress-related metabolic disease risk later in life.[7]

Background

Nutrients such as choline, betaine and folate serve as methyl donors in cellular methylation reactions. Maternal intake of such nutrients has been shown to modify fetal epigenetic markers (e.g., promoter region DNA and histone methylation) in animal models, leading to sustainable functional alterations throughout the life span,[8-13] but little research has been conducted in humans.

My research group conducted a 12-week dose-response choline feeding study in pregnant women (baseline age 26-29 weeks gestation.)[14] The women consumed either 480 mg/d of choline, which is representative of the current adequate intake (AI), or 930 mg/d of choline, which is well below the upper level of tolerance of 3500 mg choline/d, from both their diet and supplements throughout the duration of their third trimester. The higher intake level was found to increase the use of choline as a methyl donor, as indicated by elevated levels of the metabolite dimethylglycine in maternal and fetal plasma.[14] The current study was undertaken as an extension of our previous 12-week feeding study to investigate whether the increased use of choline as a methyl donor might alter the epigenetic state of genes regulated by methylation, as had been observed in animal studies.[9,10]

Of particular interest were the cortisol-regulating genes, corticotropin-releasing hormone *(CRH)* and nuclear receptor subfamily 3, group C, member 1 *(NR3C1)*, since previous research indicates they may be particularly vulnerable to perinatal methylation.[8,15-17] These genes encode parts of the hypothalamic-pituitary-adrenal (HPA) axis, which is involved in stress response, immunity and glucose metabolism. In response to stress, the hypothalamus produces *CRH* which stimulates secretion of adrenocorticotropic hormone from the anterior pituitary leading to the release of glucocorticoids, such as cortisol, from the adrenal gland. The *NR3C1* encodes the glucocorticoid receptor important for negative feedback inhibition of those hormones.[18] Thus, modification of these genes could impact susceptibility to stress-related metabolic diseases later in life.[19-22]

Study Design and Methods

The participants of the study were healthy third-trimester (26- 29 weeks gestation) singleton pregnant women at least 21 years of age. They were recruited from Ithaca, NY and surrounding areas between January 2009 and October 2010. All participants completed a questionnaire regarding their age, education, work status, ethnicity and race, pre-pregnancy body mass index (BMI), parity, health history, medication and nutritional supplement use and physical activity. Participants also provided information regarding gestational weight gain, health insurance, mode of delivery, obstetrical complications and newborn sex and health characteristics after delivery (obtained through medical charts). Inclusion criteria included good health status (no chronic disease, normal kidney and liver function and no anemia), no tobacco or alcohol use, and a willingness to comply with study protocol. Twenty-six of the 29 pregnant women who began the study completed it. Of those 26 who completed the study, placental samples were taken from 24 (*n*=12 from each choline intake group) and cord blood samples from 23 participants. Written informed consent was acquired from all participants prior to study entry, and the study protocol was approved by the Institutional Review Board for Human Study Participant Use at Cornell University and Cayuga Medical Center (Ithaca, NY).

Intervention

The women were randomized to receive either 480 (approximately the choline AI; n=12) or 930 (*n*=12) mg choline/d. Of the choline provided, 380 mg was derived from the diet and supplemented with either 100

or 550 mg choline/d for the 480 and 930 mg/d intake groups, respectively. During the last 6 weeks of the study, about 20% of the choline provided was deuterium-labeled trimethyl d9-choline. Throughout the study, investigators provided all food and beverages to be consumed, and study participants consumed at least one meal/day on site as detailed in Yan *et al*.[14] Additionally, a prenatal vitamin and a supplement containing magnesium and potassium was provided three times per week. After the study, participants continued the same level of choline supplementation until delivery of their babies.

Fasting venous blood samples were obtained at the beginning and end of the study. Maternal EDTA-blood was obtained within 24 hours of delivery and cord venous EDTA-blood was collected immediately after delivery. Placenta samples were processed within 10-30 min of delivery, except for 3 cases: n=2 in the 930 and n=1 in the 480 mg/d groups, which were processed within 60-90 min. Since placenta is a heterogeneous tissue, biopsies from each quadrant of the placenta were used to obtain a representative subset of cells.

Cortisol concentration in maternal and infant cord blood was measured using liquid chromatography (LC)-mass spectrometry (MS) as part of a metabolite panel and then quantified using an ELISA kit. Site-specific methylation was analyzed using base-specific cleavage and MS of DNA samples from the placental tissue. RNA was extracted from placental tissues using a commercially available kit and analyzed using quantitative real-time PCR. Global DNA methylation was measured in placental tissues, cord blood leukocytes and maternal leukocytes using LC-MS, and global histone methylation was measured using Western blot analysis. S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) were measured using LC-MS.

Results

All babies were delivered without major complications and were apparently healthy. The mode of delivery, gestational age at birth, infant birth weight, and Apgar score did not differ (P=0.14-0.79) between the choline intake groups.

Cord plasma cortisol concentrations were approximately 33% lower in babies born to mothers consuming 930 versus 480 mg choline/d (P=0.07); however, maternal plasma cortisol did not differ between the choline intake groups nor did it correlate with cord plasma cortisol.

In placental tissue, higher maternal intake of choline (930 vs. 480 mg/d) yielded higher average cytosine-phosphate-guanine (CpG) methylation of the *CRH* promoter region (P=0.05). Although statistical significance was not achieved, methylation of each individual CpG unit was consistently higher in the 930 mg/d group. Higher average placental *NR3C1* promoter methylation was also observed (P=0.02).

The higher maternal choline intake resulted in lower placental *CRH* gene transcription abundance. In cord leukocytes, the higher maternal choline intake yielded lower average promoter methylation of *CRH* and lower methylation of an individual CpG unit. Maternal choline intake did not affect maternal blood leukocyte average CpG methylation of *CRH*.

Placental global DNA methylation was 22% higher in the 930 mg/d maternal choline intake group compared with the 480 mg/d group (P=0.02). Global histone methylation analysis found that placental H3K9me2, a transcription repression and heterochromatin marker, was 20% higher (P=0.02) among women consuming 930 versus 480 mg/d choline and was positively correlated with placental global DNA methylation (Pearson's correlation r=0.40; P=0.05). Despite changes in global DNA and histone methylation, placental concentrations of SAM and SAH and the SAM/SAH ratio did not differ (P=0.38-0.97).

Discussion

This study provides compelling evidence that maternal choline intake during the third trimester of pregnancy can modify global and site-specific epigenetic markers in fetal-derived tissues which may have long-lasting effects. This was demonstrated in cortisol-regulating genes, suggesting an impact of maternal choline intake on programming of the HPA axis.

The present study demonstrated increased promoter methylation and reduced expression of *CRH*, the primary stimulator of the HPA axis, in the placenta of women consuming higher levels of choline. The increased promoter methylation may have modified expression of *CRH* by reducing the binding of

transcription factors to the promoter region, thereby leading to decreases in placental *CRH* expression among those in the higher choline intake group. As placental *CRH* enters the fetal compartment and stimulates the HPA axis, lower levels of *CRH* in the placenta are also consistent with the 33% lower cord plasma cortisol concentrations found in babies of mothers from the higher choline intake group.

Increased methylation of placental *NR3C1*, which encodes glucocorticoid receptors that stimulate placental *CRH* expression, may have contributed to decreased expression of *CRH* as well. However, our lab was unable to detect a change in transcript abundance for placental *NR3C1*.

In contrast to the placenta, cord leukocyte methylation of *CRH* and *NR3C1* was lower in infants born to women in the higher maternal choline intake group. This may represent a secondary response to the epigenetic alterations of the placental HPA axis genes and subsequent lower circulating levels of cortisol in the fetal compartment. In contrast to the placental compartment, decreased promoter methylation of *NR3C1* in the central HPA axis is associated with increased sensitivity to cortisol and improved feedback inhibition.[16]

Collectively, these data suggest that higher maternal intake of choline (930 vs. 480 mg/d choline) may lower fetal/neonatal circulating cortisol by altering the methylation state of cortisol-regulating genes in both the placental and fetal compartments. Previous studies have demonstrated an association between increases in placental *CRH* and obstetric complications, such as preeclampsia and intrauterine growth restriction, as well as elevated cord blood cortisol, which can lead to increased risk of stress-induced illnesses and chronic conditions like hypertension and insulin resistance later in life.[19,21-24] In addition, elevations in HPA axis activity are linked to impaired learning and memory.

Notably, the epigenetic state of the placenta was highly sensitive to varied maternal choline intake during the third trimester of pregnancy, with nearly all examined placental epigenetic markers (global and site-specific DNA methylation and global histone methylation) showing some degree of alteration.

As a mechanism of action, we propose that higher maternal choline intake alters the epigenetic state of cortisol regulating genes by influencing the supply of methyl groups. Figure 1 shows this proposed mechanism.

Conclusions & Implications

The study findings suggest that consuming extra choline during pregnancy beneficially "programs" the baby's responsiveness to stress and would be expected to lower the

Figure 1

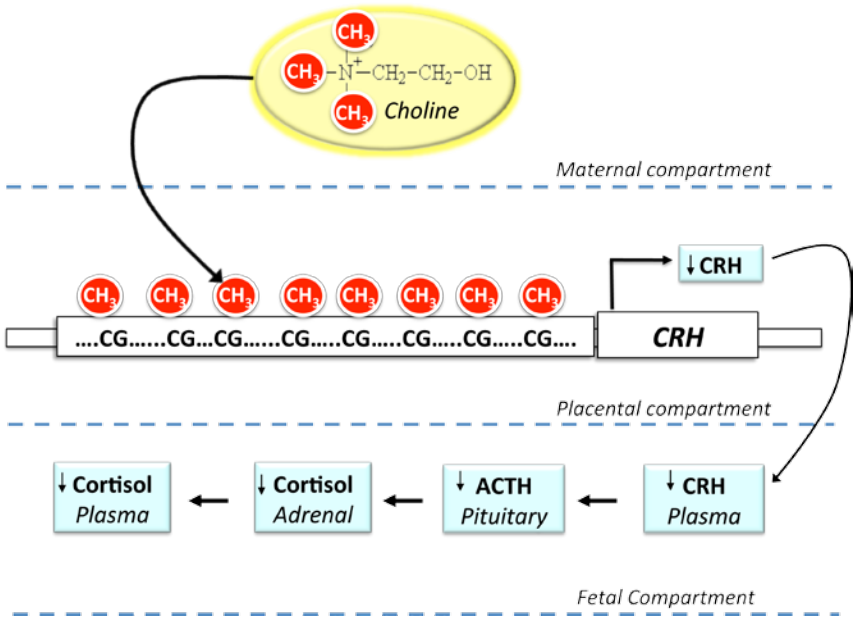

Figure 1. The proposed epigenetic mechanism by which extra maternal choline during the third trimester of pregnancy reduces fetal circulating cortisol concentrations. A higher consumption of choline, and its methyl groups (CH_3), by the mother (maternal compartment) enhances the methylation state of the corticotropin releasing hormone *(CRH)* gene in the placental compartment. This lowers the placental production of CRH and decreases the amount of CRH entering the fetal compartment. As a consequence, the production of adrenocorticotropic hormone (ACTH) by the fetal pituitary gland is reduced which leads to diminished production and secretion of cortisol by the fetal adrenal glands.

risk of stress-related diseases and possibly improve memory and learning.

The best way for pregnant women to achieve adequate choline intake levels is to include choline-rich foods, such as eggs, in the diet; however, higher levels of choline intake as investigated in this study may require supplementation. It is important for pregnant women to work with a Registered Dietitian, as most prenatal and regular multivitamins provide far less than the AI for choline. Before applying these findings to practice, additional studies are needed to replicate these findings in larger independent cohorts and to explore the long-term effects of these choline-induced epigenetic changes.

This work was funded by the Egg Checkoff, through the Egg Nutrition Center; the Beef Checkoff, through the National Cattlemen's Beef Association and the Nebraska Beef Council; the U.S. Department of Agriculture Cooperative State Research, Education and Extension Service (CSREES), special research grant 00444528; and the Affinito-Stewart Grants Program, through the President's Council of Cornell Women. The funding sources had no role in the study design, interpretation of the data, and/or publication of the results.

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Hot Topics in Food Science and Health: Omega-3 Fatty Acids

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Studies showing a reduced risk of cardiovascular disease in Eskimos consuming a diet high in fish has focused attention on the health benefits of fish and their omega-3 polyunsaturated fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).[1] The 2010 Dietary Guidelines for Americans recommends consumption of 8 ounces of low-mercury seafood per week based on moderate evidence of decreased risk of death from cardiovascular disease. Many consumers have interpreted these recommendations to mean that dietary supplementation with fish oil is equal in benefit to eating fish. Advice for omega-3 fatty acid consumption seemed straightforward until a recent a meta-analysis[2] questioned those recommendations. This analysis included nearly 70,000 participants and reported that omega-3 fatty acid supplementation was not associated with reduced risk of heart attack, stroke, death from cardiovascular disease, or death from any cause.[2] Although the analysis is facing backlash from some scientists who say the analysis was flawed,[3] the study does bring omega-3 supplements into question.

Because humans are unable to synthesize omega-3 fatty acids, they are essential in the diet. In foods, omega-3 and other polyunsaturated fatty acids are usually esterified to a glycerol backbone, forming either a triglyceride or a phospholipid.[4] Triglycerides have three fatty acids attached to the glycerol, while phospholipids have two fatty acids plus a head group.[4] Because of the structural differences in these two types of molecules, triglycerides are hydrophobic, require bile salts and micelles to be absorbed, and are transported through the circulatory system inside chylomicrons. In contrast, phospholipids have both hydrophilic and hydrophobic ends, do not require bile salts or micelles for absorption, and form the outside of the chylomicron. As a result, omega-3 fatty acids esterified to phospholipids may have higher bioavailability[5,6] and greater distribution to tissues, compared to those esterified to triglycerides.[4,7]

There is also some evidence that omega-3's delivered through phospholipids may result in improved health outcomes. In a recent mouse study comparing outcomes in mice consuming chow with EPA/DHA-triglycerides versus EPA/DHA-phospholipids for nine weeks, the phospholipid group had reduced glucose intolerance, post-prandial lipemia, hepatosteatosis, and plasma insulin compared to the triglyceride group.[7] This is supported by a study in children with diagnosed ADHD who were given a supplement for three months that contained either EPA/DHA-triglycerides from fish oil or EPA/DHA-phospholipids.[8] While both groups had improved attentiveness compared to a placebo, the group receiving EPA/DHA-phospholipids had greater improvement than the triglyceride group.

The percentage of EPA/DHA esterified to phospholipids in fish depends on the species, but can be up to 30-40%.4 Phospholipid-EPA/DHA is approximately 40-75% in roe (fish eggs) and krill. However, rather than consuming fish, consumers are increasingly turning to supplements and fortified foods to obtain these essential fatty acids,[9] and many fish oil supplements contain EPA/DHA esterified to only triglycerides, although krill oil supplements do contain a high percentage from phospholipids. As more foods are fortified with omega-3 fatty acids, such as margarine spreads, orange juice, breakfast cereals, infant formulas, and possibly milk, the findings about phospholipids versus triglycerides should be considered when formulating foods and supplements with omega-3 fatty acids. Perhaps this is one explanation for the lack of benefit found in the meta-analysis of omega-3 supplementation, which did not distinguish between EPA/DHA from triglycerides or phospholipids. Or, perhaps we should just eat more fish instead?

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Upcoming Conferences

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(Florence, Italy)
Call for abstracts: Closed
Website: *www2.kenes.com*

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Society of Adolescent Medicine and Health Annual Meeting
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March 22-24, 2013
Women's Health 2013: The 21st Annual Congress
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April 20-24, 2013
Experimental Biology
(Boston, MA, U.S.A.)
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May 2-4, 2013
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Student Research

Overview of R

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Introduction

R is a relatively new software environment and programming language that has become popular for statistical analysis and graphic production.[1] It has the ability to compute both basic and advanced statistics,[2] ranging from simple arithmetic to multivariate analyses.[3] The name "R" was derived both from the developers' names and from its influencer, the programming language S.[2] R's increasing popularity is not a surprise, for it generally surpasses the capabilities of many commercial statistical programs.[4] The current niches for R include medical and public health applications, bioinformatics, environmental statistics, and econometrics,[2] all of which include nutrition research.

Challenges and Benefits

For researchers without a background in programming languages, R may have a steep learning curve. There are some underlying concepts, including operators, scripts, and dataframes, to learn when getting started with R that may sound daunting. However, with some effort and good introductory material, R can be very intuitive and user-friendly.[3] As such, there are increasing numbers of scientists, statisticians, and engineers without experience in other programming languages using R.[4] There are several benefits of R. First and foremost, R is available to download and use for free on several platforms including Windows, Mac, and Linux.[5] Since R commands are written using a true programming language, R easily handles large data sets[3] and is very flexible in reading, manipulating, and analyzing data.[2] Additionally, R is well-known for its high quality graphing capabilities. Line and bar graphs are easy to create and edit with options to modify axes, lines, points, color, background, title, legends, and other text. These graphics are publication ready and easily exported as a PDF, PNG, or PostScript file.[3]

Nutrition Research

Currently, nutrition researchers tend to use SAS, SPSS, and other common statistical applications to conduct statistical analyses.[6] R is an attractive alternative and has the ability to perform the statistical analyses often used in nutrition research, including but not limited to, power and sample size calculations, descriptive statistics (such as mean and standard deviation), t tests, analysis of variance,[6] analysis of covariance, post hoc testing, correlations,[7] regressions, survival analysis,[8] and non-parametric methods (such as Kruskal-Wallis test and Spearman's correlation).[9] R also performs multivariate statistics needed for bioinformatics. For example, a nutritional metabolomics profiling study could analyze principal components analysis as well as partial least squares discriminant analysis, all within R.[2]

Getting Started

R is easy to download and install from www.r-project.org.[5] The "base" R installation includes many tools for data analysis and graphics, but for specialized analyses, there are more than 4,000 "packages" (collections of functions and example data) available for download on the Comprehensive R Archive Network at cran.r-project.org/.[3]

Additionally, add-on applications may enhance R. For example, RExcel integrates R into an Excel spreadsheet, so that data and statistical analyses are all contained within the spreadsheet. RExcel is available for download at www.statconn.com.[10] A similar add-on application is imDev, but it focuses on multivariate analyses and data visualization specifically for –omics data. imDev is available for download at www.sourceforge.net/projects/imdev/.[11]

There are many resources to assist with learning R.[2] On the R project website, there are hyperlinks to manuals and mailing list archives from a very active online community.[3] Many books have been published on R, too.[2] Springer has published a *UseR!* series that includes 39 books, including the highly-rated, *Introductory Statistics with R*,[1] which may be purchased at a reduced cost with a subscription to SpringerLink.[3]

Personal Experience

Recently, I attended a three-day workshop on R.[3] I had a steep learning curve to overcome since my programming skills were limited to an advanced statistics course on SAS. After the first day of the workshop, I began to understand the advantages of using R. Installing it was quick and data was easy to import. The greatest benefits were that R ran natively on my Mac, and I did not have to run a Virtual Machine and boot Windows, as needed when using SAS.

Conclusion

For many researchers, the benefits of R outweigh the challenges and, as such, R has been increasing in popularity.[4] R is especially well-known for performing multivariate statistics and creating graphics. Ultimately, R is a statistical programming language that nutrition researchers should consider.

Acknowledgements

Thank you to Sasha Hafner, PhD, for his review of this article.

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Access to the RDPG listserv is an optional member benefit. If you are an RDPG member, and would like to join our RDPG listserv, please email me at rdpggroup@gmail.com, with the following information:

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