Whole Food versus Supplement: Comparing the Clinical Evidence of Tomato Intake and Lycopene Supplementation on Cardiovascular Risk Factors

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ABSTRACT

Cardiovascular disease (CVD) is a major contributor to morbidity and mortality in the United States and worldwide. A link between diet and CVD is well established, with dietary modification a foundational component of CVD prevention and management. With the discovery of bioactive components beyond the essential nutrients of foods, a new era of nutritional, medical, botanical, physiologic, and analytical sciences has unfolded. The ability to identify, isolate, purify, and deliver single components that has expanded the dietary supplement business and health opportunity for consumers. Lycopene is an example of a food component that has attracted attention from scientists as well as food, agriculture, and dietary supplement industries. A major question, however, is whether delivering lycopene through a supplement source is as effective as or more effective than consuming lycopene through whole food sources, specifically the tomato, which is the richest source of lycopene in the Western diet. In this review, we examined clinical trials comparing the efficacy of lycopene supplements with tomato products on intermediate CVD risk factors including oxidative stress, inflammation, endothelial function, blood pressure, and lipid metabolism. Overall, the present review highlights the need for more targeted research; however, at present, the available clinical research supports consuming tomato-based foods as a first-line approach to cardiovascular health. With the exception of blood pressure management where lycopene supplementation was favored, tomato intake provided more favorable results on cardiovascular risk endpoints than did lycopene supplementation. Indeed, future research that is well designed, clinically focused, mechanistically revealing, and relevant to human intake will undoubtedly add to the growing body of knowledge unveiling the promise of tomatoes and/or lycopene supplementation as an integral component of a heart-healthy diet.

Introduction

The consumption of a diet rich in fruits and vegetables is associated with a reduced risk of cardiovascular disease (CVD) (1), in part through reductions in atherosclerosis, a main underlying mechanism leading to CVD. The established pathophysiologic (risk) factors for atherosclerosis impact regulatory functions of the endothelium, promoting an unstable, lipid accumulating, and inflexible vascular environment. Components of fruits and vegetables that influence these risk factors may beneficially impact atherosclerosis, endothelial function, and therefore CVD development.

Tomatoes are well recognized for their culinary versatility and multiethnic menu presence. Tomatoes also contribute significantly to the nutritional value of the diet, providing a number of essential nutrients and other bioactive components (2). However, tomatoes and tomato-based foods are best known as a rich source of dietary lycopene. Initially, tomato and lycopene intake was linked with reductions in prostate cancer (3–5). More recently, the association between tomato and/or lycopene consumption and CVD risk reduction has gained research interest. The discovery of lycopene as a potent antioxidant in vitro is largely

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3 Abbreviations used: AOX, antioxidant capacity; BP, blood pressure; CE, cholesterol ester; CRP, C-reactive protein; CVD, cardiovascular disease; FMD, flow-mediated dilation; MCP-1, monocyte chemotactic protein 1; ORAC, oxygen radical absorbance capacity; PON-1, paraoxonase 1; RNS, reactive nitrogen species; ROS, reactive oxygen species; SAA, serum amyloid A; sICAM, soluble intracellular adhesion molecule; SOD, superoxide dismutase; sVCAM, soluble vascular cellular adhesion molecule; TC, total cholesterol; TLR, Toll-like receptor; TRAP, total peroxyl radical-trapping antioxidant parameter.
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Polyphenols and the Human Brain: Plant “Secondary Metabolite” Ecologic Roles and Endogenous Signaling Functions Drive Benefits\textsuperscript{1,2}

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ABSTRACT

Flavonoids and other polyphenols are ubiquitous plant chemicals that fulfill a range of ecologic roles for their home plant, including protection from a range of biotic and abiotic stressors and a pivotal role in the management of pathogenic and symbiotic soil bacteria and fungi. They form a natural part of the human diet, and evidence suggests that their consumption is associated with the beneficial modulation of a number of health-related variables, including those related to cardiovascular and brain function. Over recent years, the consensus as to the mechanisms responsible for these effects in humans has shifted away from polyphenols having direct antioxidant effects and toward their modulation of cellular signal transduction pathways. To date, little consideration has been given to the question of why, rather than how, these plant-derived chemicals might exert these effects. Therefore, this review summarizes the evidence suggesting that polyphenols beneficially affect human brain function and describes the current mechanistic hypotheses explaining these effects. It then goes on to describe the ecologic roles and potential endogenous signaling functions that these ubiquitous phytochemicals play within their home plant and discusses whether these functions drive their beneficial effects in humans via a process of “cross-kingdom” signaling predicated on the many conserved similarities in plant, microbial, and human cellular signal transduction pathways. Adv. Nutr. 5: 515–533, 2014.

Introduction

It is generally accepted that the consumption of dietary polyphenols derived from fruit, vegetables, and other plant-derived foods may confer a number of health benefits, including to cardiovascular and brain function. An extensive and expanding literature describes how, in mechanistic terms, polyphenols may exert these effects, with the predominant current theory being that they do so via interactions with mammalian cellular signal transduction pathways. However, the question of why these phytochemicals have these effects has been primarily overlooked. Therefore, the current review summarizes both the evidence that polyphenols do indeed affect human brain function (with reference to cardiovascular function when relevant) and their suggested modes of action. It then goes on to consider why these compounds exert these effects, specifically whether their modulation of diverse physiologic variables in mammals is in fact related to the ecologic “secondary metabolite” roles and the endogenous signaling roles that the polyphenols are trying to play for, and within, their own home plant.

The “phenolics” represent a large group of ubiquitous phytochemicals that incorporate within their structure $\geq 1$ phenyl aromatic hydrocarbon ring with $\geq 1$ hydroxyl group attached. Within this broad chemical group, polyphenols represent a group of more complex phenolic structures that combine a shikimate pathway–derived cinnamic acid starter unit (cinnamoyl-CoA) with malonyl-CoA, which is derived via the acetate pathway. The majority of polyphenol groups incorporate 3 malonyl-CoA units and start with an identical polyketide before differentiating into stilbenes, which have a comparatively restricted distribution, and chalcones on the basis of their first enzymatic step (stilbene synthase vs. chalcone synthase). The chalcones go on to act as the precursors for the entire flavonoid group of structures, which incorporates $>8000$ secondary metabolite compounds that are found ubiquitously across plants and plant tissues. The flavonoids share a common underlying structure of 2 six-carbon rings, with a three-carbon bridge, which usually forms a third ring (1), and the wider group can be further subdivided into the groups represented in Figure 1.

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Beyond Blood Pressure: New Paradigms in Sodium Intake Reduction and Health Outcomes¹–⁴

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ABSTRACT

Since 1980, when inaugural national dietary guidance was to “avoid too much sodium,” recommendations have evolved to the 2010 Dietary Guidelines for Americans’ quantified guidance of 2300 and 1500 mg/d (USDA and U.S. Department of Health and Human Services. Dietary guidelines for Americans, 1st (http://www.cnpp.usda.gov/DGAs1980Guidelines.htm) and 7th (http://www.health.gov/dietaryguidelines/dga2010/dietaryguidelines2010.pdf) ed.). Too much sodium remains a valid concern, but are current targets too low for optimal health? New research moves beyond sodium’s effect on the surrogate marker of blood pressure to examine the relation between sodium intake and cardiovascular morbidity and mortality. Results show that sodium intakes both less than and greater than ~3000–5000 mg/d increase the risk of negative health outcomes. Additionally, newly compiled sodium intake data across populations show a uniformity that suggests that intake is physiologically set. Perhaps not coincidentally, the observed intakes fall within the range related to lowest risk. These findings are highly relevant to current efforts to achieve low sodium intakes across populations, because the data suggest that the efforts will be unsuccessful for healthy people and may cause harm to vulnerable populations. Remaining mindful of risks associated with both excessive and inadequate intakes is imperative with all nutrients, and sodium is no exception. Avoiding too much, and too little, sodium may be the best advice for Americans. Adv. Nutr. 5: 550–552, 2014.

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The organizer has indicated that related reviews of this symposium will be submitted for publication in an upcoming issue of Advances in Nutrition. One related review appears in this issue.

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Abbreviations used: CVD, cardiovascular disease; DGA, Dietary Guidelines for Americans; IOM, Institute of Medicine.

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Dr. King began the program with a general overview of sodium recommendations, highlighting the evolution from the 1980 DGA’s guidance to “avoid too much sodium” to the 2010 DGAs quantified guidance of 2300 and 1500 mg/d for those aged ≥51 y, and all people who are African American or have hypertension, diabetes, or chronic kidney disease (2). She compared the DGAs to the historical Institute of Medicine (IOM) recommendations. The first time sodium recommendations were quantified appeared in the Food and Nutrition Board’s 1989 publication *Diet and Health: Implications for Reducing Chronic Disease Risk* (3). The maximum intake goal was set at 2400 mg on the basis of observational data from the 1988 InterSalt study publication showing that blood pressure increased with age in individuals with intakes >2400 mg (4). The only groups who consumed less sodium were those living in primitive societies. In fact, when the primitive societies were omitted, there was no relation between sodium intake and increasing blood pressure with age. Nonetheless, the recommendation of 2400 mg as a maximum intake was adopted by authoritative bodies until 2005 when the DRI for sodium was set at 2300 mg as the upper level on the basis of 2 dose-response studies on blood pressure. The adequate intake was set at 1500 mg on the basis of modeling the minimum amount of sodium required to achieve a nutritionally adequate diet. (It should be noted that the modeled diet contained primarily reduced-sodium foods, many of which may not be readily available, such as reduced-sodium bread.) In 2005 and 2010 the DGA adopted these levels.

Perhaps due to the assumption that reducing sodium reduces blood pressure and therefore must reduce CVD, and assuredly because of the difficulty in conducting studies to examine sodium and health outcomes, the direct relation between sodium reduction and health outcomes had largely been overlooked in the literature until recently. Now, a critical mass of data relating both greater and lesser intakes of sodium to increased risk of outcomes such as death, CVD, and heart failure, has begun to emerge, and these data were reviewed in the 2013 IOM report “Sodium Intake in Populations: Evaluation of the Evidence” (5). Examination of the new evidence brought findings that were surprising, showing that current sodium intake recommendations may pose risk. But were they really surprising?

Dr. Heaney reminded the audience that these findings were exactly what could be expected based on the physiology of all nutrients. That is, the relation between a nutrient intake and health benefit is not a straight line that intersects with zero on the x and y axis, indicating that lower is better, but instead is a J-shaped curve that indicates risk at both ends of intake, with a rather wide range of “no harm” (or benefit) at intakes between these extremes. It is within this range, wherein the organism needs to exert minimal compensation, that nutrient requirements are typically set. Heaney outlined in his presentation the unexplained departure from the evidence-based approach for sodium. In fact, even with the use of blood pressure as a surrogate marker of benefit, the DASH (Dietary Approaches to Stop Hypertension) study shows that a focus on food and dietary patterns that provide adequate potassium, calcium, and magnesium create a more meaningful blood pressure effect and do not pose the potential harm of very low sodium intakes. Revisiting the sodium DRIs with consideration of the evidence on health outcomes and approaching the task adhering to the agreed-upon evidence-based process are critical to the integrity of nutrient recommendations, of which sodium should be no exception.

Dr. Alderman presented the historic path of health outcomes–related sodium intake research. Published research as well as plausible physiologic mechanisms such as the renin-angiotensin-aldosterone system have long existed that refute benefit of sodium reduction to low amounts, but these data have been overshadowed until recently. Alderman was among the first to report the inverse relation between renin and myocardial infarction. Reduced sodium intake leading to increased renin concentrations is an example of sodium restriction not exerting the singular physiologic effect of blood pressure reduction, but instead shows how it exerts multiple effects, including negative consequences such as increased plasma renin activity, increased insulin resistance and sympathetic nerve activity, and elevated aldosterone and TGs. The net health effect cannot be predicted by the consequence on blood pressure alone. Alderman was also the first to suggest the J-shaped risk curve for CVD and sodium intake, and this hypothesis was supported by the 2013 IOM report. Subsequent to the 2013 IOM report, several additional papers have supported the J-shaped risk curve, including the 2014 Graudal et al. meta-analysis summarizing findings from 274,683 individuals from 25 studies (6). The idea that the blood pressure effect of sodium restriction can be extrapolated to a health benefit no longer retains scientific credibility.

Dr. McCarron capped the session by presenting a body of data showing the narrow sodium intake ranges observed in 69,011 people from 45 countries around the world gathered over the past 50 y, which are remarkably constant and appear to be independent of the food supply. The mean intake is 3600–3700 mg/d, and the mean population minimum and maximum are 2622 and 4830 mg/d, respectively. Mean intakes of the Adequate Intake of 1500 mg or the Tolerable Upper Intake Level of 2300 mg are not observed in these free-living healthy populations. He pointed out that interpreting reductions in sodium intake caused by reducing sodium in commercially prepared foods, such as in the United Kingdom, are in fact small variations within 1 SD of the mean. The data support that intake of sodium is not mediated by the food supply but is physiologically controlled through sodium appetite. The risk of reducing sodium below this “set point” is consistent with the increased morbidity and mortality observed at the lowest sodium intakes (similar to current recommendations) that have been reported by several researchers and reiterated in the IOM report.

Common ground among all who study sodium intake and health outcomes is that excess sodium intake carries
increased risk of morbidity and mortality. The controversy focuses on the lower end of sodium intake. Although public health guidelines continue to promote intakes <2300 mg/d, data suggest that this amount may be too low for optimal health. The recommended intakes do not cause concern for free-living individuals who have access to salt, but they do have direct implications for hospitalized patients, nursing home residents, and school feeding programs and other government-funded feeding programs that must adhere to these guidelines. Additionally, if, in fact, sodium intake is set physiologically, current resources being poured into sodium reduction by public and commercial entities could be more effectively spent on important innovations related to public health, such as increasing demand for smaller portion sizes, improving availability of lower-energy-dense food, and replenishing food deserts.

This session helped bring awareness to the potential risk associated with intakes at currently recommended amounts, amounts of intake that are lower than any observed in modern free-living healthy populations regardless of food supply. New data support a J-shaped curve for risk, with the intakes related to least harm being those between ~3000 and 5000 mg, a range that includes the current usual intakes of the majority of healthy individuals in the world.

The convergence of new data from research focused on health outcomes and newly compiled sodium intake amounts suggests that enforcing very low sodium intakes will at best fail for most people and at worst cause harm for vulnerable or ill individuals subjected to the recommended levels. Perhaps the 1980 DGA statement of “avoid too much sodium” really had it right, with 1 revision: “avoid too much—and too little—sodium.”

Acknowledgments
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References
The Science of Cocoa Flavanols: Bioavailability, Emerging Evidence, and Proposed Mechanisms1, 2

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3Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, Boston, MA; 4Department of Nutrition, Harvard School of Public Health, Boston, MA; 5Department of Biological Sciences, University of North Texas, Denton, TX; 6Department of Neurology and Friedman Brain Institute; 7Icahn School of Medicine at Mount Sinai; 8James Peters Veterans Affairs Medical Center; New York, NY; and 9University of California–San Diego School of Medicine, San Diego, CA

ABSTRACT

Over the past 20 y, evidence derived from in vitro experiments, animal models, observational studies, and clinical interventions have suggested that cacao (cocoa) flavanoids act through a variety of mechanisms to modify a number of risk factors associated with chronic conditions, including cardiovascular and neurodegenerative diseases. Recent studies have elucidated the synthesis of flavonoids by plants, making available for research specific flavonoids and their metabolites. The body of evidence suggesting that cocoa flavanols may play a role in reducing the risk of cardiovascular disease has been sufficient to generate several systematic reviews and meta-analyses. Studies are now being directed to identify the molecular pathways underlying the effect of cocoa flavanols, and clinical trials are being planned to test their impact on disease endpoints. Adv. Nutr. 5: 547–549, 2014.

Introduction

Among the broad array of dietary polyphenols, research has stimulated particular interest in the flavonoids, which comprise approximately two-thirds of the total of this class of bioactive compounds. Flavonoids are largely present in fruits, chocolate, and beverages such as coffee and tea. Cocoa contains the highest flavan-3-ol (flavanol) content of all foods on a per-weight basis and can contribute substantially to the total intake of dietary flavanoids. A growing body of basic research, observational studies, and clinical interventions strongly suggests that flavonoids, particularly the flavanols, from cocoa may affect multiple risk factors for chronic diseases, including elevated blood pressure, dyslipidemia, inflammation, insulin resistance, and vascular reactivity. Our knowledge of the mechanisms underlying these actions is limited but is steadily emerging to reveal interactions with signaling proteins, cell membranes, microRNA expression, and other targets. The goal of this symposium was to provide a brief update of recent research, from the farm to pharmacokinetics to physiology, exploring the putative role of cocoa flavanols on cardiovascular and cognitive functions.

Biosynthesis of Flavanols within Plants

The session was opened by Dr. Dixon who described the biochemical pathways used by plants for the biosynthesis of the major flavanols, catechin and epicatechin. Two important model systems used in this research are Arabidopsis thaliana (thale cress, a common weed) and Medicago truncatula (barrel medic, a forage legume in the Southern hemisphere and a close relative of alfalfa). Both species accumulate proanthocyanidins derived from flavanols in their seed coats. The genomes of both plants have been sequenced, and extensive collections of mutants are now available. Loss of proanthocyanidins in the seed coat leads to a transparent testa phenotype (clear seed coat), and understanding the genes affected in transparent testa mutants has greatly aided in the elucidation of the flavanol pathway. Catechin and epicatechin differ at the stereochemistry around the 2 and 3 positions of the central heterocyclic ring: catechin is 2,3-trans whereas epicatechin is 2,3-cis. The flavonoid precursors of both catechin and epicatechin are of the 2,3-trans configuration, established earlier in the pathway by the enzyme chalcone isomerase. Catechin is formed by direct reduction...
of 2,3-trans-leucoanthocyanidin by leucoanthocyanidin reductase. Epicatechin is formed via the reduction of an achiral anthocyanidin by the enzyme anthocyanidin reductase, allowing for introduction of 2,3-cis stereochemistry. Leucoanthocyanidin is the immediate precursor of anthocyanidin, formed by the action of anthocyanidin synthase, highlighting the close relation between the flavanol and anthocyanin pathways in plants.

Recent studies of the regulatory genes that control the biosynthesis of flavanols and anthocyanins reveal pathways that are regulated by ternary complexes of transcription factors, each containing a myeloblastosis family protein, a basic helix-loop-helix protein, and a β-transducin repeat domain protein. So, it is now possible to engineer plant tissues to accumulate increased amounts of flavanols and proanthocyanidins through overexpression of ≥1 of these types of transcription factors (1). Interestingly, naturally occurring mutants of some plants exist in which the fruits lack astringency due to loss of function of such regulatory genes.

After their biosynthesis in the plant, flavanols can be stored in the central vacuole of the cell as glycosides (epicatechin as the 3′-O-glucoside, at least in Medicago and Arabidopsis) or further converted to proanthocyanidins by mechanisms yet unknown. It is important to contrast the plant profile of these phytochemicals with their bioavailability and metabolism in vivo. For example, when mice are fed fractionated polymeric, oligomeric, and monomeric flavanol preparations, only the monomers (catechin and epicatechin) appear to be bioavailable. These monomers are recovered in plasma and, at much lower concentrations, in brain tissue as glucuronides of the parent molecule or of 3′-O-methylated derivatives. Importantly, these flavanol metabolites can now be synthesized and used in studies of their mechanisms of action.

Cocoa Flavanols in Alzheimer Disease Pathology: Experimental Approaches and Clinical Applications

Dr. Pasinetti noted that interest in developing polyphenols for treatment of dementia is warranted due to findings from basic research studies as well as a clinical trial showing that dietary supplementation with cocoa polyphenol preparations improve cognitive function in the elderly. It is pertinent that a new trial is now testing the cognitive benefits of pomegranate polyphenols in the elderly. However, our understanding of the mechanisms by which polyphenols benefit cognition is limited. Recent and ongoing studies of the role of brain-penetrating polyphenols on synaptic plasticity can provide important information supporting their potential application for Alzheimer disease (AD) prevention as well as treatment.

The profiles of flavanols in cocoa and grapes are similar, with catechin, epicatechin, catechin gallate, and epicatechin gallate as the principal flavonoids. Pasinetti and colleagues characterized the pharmacokinetics of these flavanols in Sprague-Dawley rats and found that they accumulate at submicromolar concentrations in brain. By using electrophysiology, some of these metabolites, such as 3′-O-methyl-epicatechin-5-O-β-glucuronide, were found to prevent acute oligomeric amyloid-β-induced long-term potentiation impairments and improve basal synaptic transmission and long-term potentiation through activation of the calmodulin kinase II-cAMP response element-binding protein signaling pathway in hippocampal slices from a genetically modified AD mouse model (2). Investigations of molecular pathways with the use of Luminex xMAP® multiplexed immunoassays revealed that, by modulating neuroplasticity mechanisms, flavanols may be able to promote synapse growth and increase receptor density. A minimally processed cocoa extract (“Lavado”) was found to attenuate erythroid transcription factor or GATA-binding factor 1 (GATA1)—mediated repression of presynaptic genes in the AD mouse model. Because exogenous GATA1 expression increases amyloid-β content through mechanisms influencing β-secretase 1, GATA1 presents an additional target through which cocoa flavanols may beneficially influence AD neuropathology. Thus, cocoa flavanols may attenuate the onset and progression of AD neuropathology through molecular mechanisms influencing GATA1 expression in the brain, a molecular index of major depressive disorders.

Synaptic alterations and dysfunction are among the earliest events in the development of AD-type cognitive decline, preceding neuronal loss and likely contributing to the progressive failure of neuronal systems leading to clinical dementia. Targeting the molecular mechanisms that contribute to synaptic dysfunction in the brain with cocoa flavanols and other dietary polyphenols should provide useful insight into the development of novel primary and secondary prevention strategies to preserve cognitive function as well as the rational design of future clinical studies.

Evidence-Based Assessment of Cocoa Flavanol Effects on Cardiovascular and Metabolic Risk Factors

In addition to laboratory experiments and case series reports suggesting benefits of cocoa flavonoids, epidemiologic and clinical evidence have been mounting in support of their action to reduce the risk of cardiovascular disease (CVD). Dr. Ding characterized the body of evidence for the clinical effectiveness of cocoa flavanols from short-term randomized trials as strong for traditional risk factors for CVD, such as metabolic mediators like blood pressure, lipids, and insulin resistance. However, long-term randomized controlled trials (RCTs) with hard CVD endpoints are necessary before recommendations for cocoa flavonoid can be proffered.

Systematic reviews of short-term cocoa flavanol trials and meta-analyses of cohort studies are now available and are largely consistent in their conclusions regarding metabolic endpoints. For example, Shrime et al. (3) analyzed studies with an average cocoa flavonoid dose of 400–600 mg/d and with isocaloric comparison to controls and found supplementation or fortification to improve multiple CVD risk factors, including lowered blood pressure, lower LDL and higher HDL cholesterol, improved insulin resistance, and enhanced flow-mediated dilation; however, total cholesterol,
TGs, pulse rate, BMI, and C-reactive protein were unchanged by the intervention. Although limited by their short duration (2–18 wk), these trials testing intermediate endpoints do provide evidence for an impact of cocoa flavonoids on metabolic factors associated with primary and secondary prevention of CVD.

Clinical evidence of hard endpoints from prospective cohort studies also supports the putative CVD benefits of cocoa flavonoids. For example, the Zutphen Elderly Study, a prospective cohort of 470 men in The Netherlands followed for 15 y with highly detailed assessment of cocoa intake, found a significant inverse association between cocoa intake and reductions of 50% and 47% in CVD mortality and total mortality, respectively, in the highest versus the lowest tertile. Overall, a systematic review and meta-analysis of 7 cohort studies showed that higher versus lower amounts of calorie-adjusted chocolate consumption were associated with a 37% reduction in the relative risk of total CVD (4). Recently, Larsson et al. (5) reported a 19% reduction in the risk of stroke in their analysis of calorie-adjusted chocolate consumption.

To solidify the body of evidence for a role of cocoa flavonoids in CVD prevention, long-term RCTs with hard endpoints are needed to build on the results from cohort studies and short-term trials. The Cocoa Supplement and Multi vitamins Outcomes Study (COSMOS), an RCT to test cocoa flavonoid supplementation with such hard endpoints led by investigators from Brigham and Women’s Hospital and Harvard Medical School, will commence enrollment in 2015. COSMOS will enroll 12,000 women aged ≥65 y and 6000 men aged ≥60 y who are free of CVD to determine the effect of high-quality cocoa flavanol supplementation at 750 mg/d (containing 75 mg epicatechin). This RCT has a 2 × 2 factorial design with multivitamin and placebo controls and a planned mean treatment duration of 4 y. At completion, this study is expected to yield strong evidence for or against cocoa flavonoid supplementation for the reduction in risk of CVD.

Flavanol Enhancement of Mitochondria Structure-Function as a Cardioprotective Mechanism

Central to the control of cell and organ bioenergetics are the mitochondria, organelles mainly responsible for the provision of cellular energy as ATP. Proper mitochondrial function is also necessary to minimize cell damage associated with the production of reactive oxygen species during ATP generation. Oxidative stress produced during myocardial ischemia can induce calcium overload in cardiac muscle, leading to the swelling of the mitochondria. Mitochondrion swelling is an established trigger of cell death, releasing from the inner membrane factors that activate cell apoptosis. Thus, mitochondria play a central in maintaining optimal cellular bioenergetics, so that loss of organelle structure or function is considered a critical aspect of the pathophysiology of many chronic diseases, including type 2 diabetes, AD, Parkinson disease, fibromyalgia, and various cardiovascular pathologies. Myocardial infarction (MI) is a major cause of worldwide morbidity and mortality, and pharmacologic strategies designed to limit heart damage are an important target for therapy. Dr. Villarreal noted that evidence on the association between consumption of modest amounts of cocoa products high in flavonols (specifically, epicatechin) and reductions in cardiometabolic risk has stimulated new research in this area.

Yamazaki et al. (6) implemented preclinical studies in rodents with the use of (−)-epicatechin to ascertain its potential to prevent or treat MI. In their pretreatment studies, 2 modalities of MI were tested: an ischemia-reperfusion injury in which blood flow to myocardium was transiently interrupted and a permanent obstruction of flow to the heart. In both cases, daily pretreatment with 1 mg/kg (−)-epicatechin for 10 d was able to reduce MI size by ~50% at 48 h after transient or permanent coronary occlusion. A second study was conducted to evaluate the participation of mitochondria in (−)-epicatechin–induced protection via assessment of organelle volume (density) and cristae abundance. After an i.v. dose of (−)-epicatechin, rats were subjected to a transient occlusion of the coronary artery. A dose-response effect on infarct size reduction was observed, with a single dose providing ~35% reduction in MI size and doubling the dose yielding a reduction of ~80%. Similarly, an acute dose-dependent benefit of (−)-epicatechin was obtained in assays of calcium-induced mitochondrial swelling. These preclinical studies indicate that (−)-epicatechin provides a cardioprotective action by limiting mitochondrial swelling, reducing apoptosis, and preventing cell death. Future studies are now warranted to evaluate the cardioprotective potential of (−)-epicatechin in other animal models to move closer to the potential translation of dietary flavonols for disease prevention and treatment.

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References

Neurocognition: The Food–Brain Connection1–4

James O. Hill,5,6 Kent Berridge,6 Nicole M. Avena,7 Hisham Ziauddeen,8 Miguel Alonso-Alonso,9 David B. Allison,10 Naiman A. Khan,11 and Michael Kelley12

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ABSTRACT

This article summarizes presentations from "Neurocognition: The Food–Brain Connection" symposium held at the ASN Scientific Sessions and Annual Meeting at Experimental Biology 2014 in San Diego, CA on 28 April 2014. Presenters reviewed research from several disciplines, including neurobiology, neuropsychology, cognitive neuroscience, and nutrition, concerning the role of the brain in food-intake regulation, reward, and addiction. A transdisciplinary approach was taken to evaluate the state of the science regarding addiction models, as well as research gaps and future research necessary to understand neurocircuitry and pathways involved in food-intake control and behavior in humans. Adv. Nutr. 5: 544–546, 2014.

Obesity prevalence in the United States remains elevated in both pediatric and adult populations. Concern about obesity led to public policies designed to discourage excessive caloric intake, especially from foods high in calories, sugars, and fats. These palatable foods were speculated recently to have addictive properties. However, there are several new human studies that fueled debate regarding the role of the brain in eating behavior.

The objectives identified for the speakers were as follows: 1) to introduce topics on relations between the brain and food-related behaviors; 2) to discuss the quality of evidence; and 3) to identify gaps in the literature and directions for future research.

The first speaker, Dr. Berridge, gave a presentation entitled “Food Reward and the Brain: Current Perspectives, Controversies, and Applications.” He said that it has become clear that the brain does not differentiate between food homeostatic and reward circuitry. Indeed, limbic and hypothalamic systems interact intimately in many regulatory pathways.

The limbic system of the brain separates food reward into liking vs. wanting. Increases in “wanting” to eat can occur in the absence of increases in “liking” for the same food being eaten. Wanting without liking is similar to incentive sensitization in drug addiction. Wanting to eat more food (e.g., extremely focused pursuit and consumption of sugar pellets) without liking it more can be produced in animals in the laboratory by microinjections of dopamine into the nucleus accumbens and even by opioids in some accumbens subregions. Alternatively, opioid, endocannabinoid, or orexin stimulation of hedonic hotspots throughout the limbic system increase both liking and wanting. Both liking and wanting are also increased together by most natural hungers. Therefore, wanting and liking circuits work together to increase eating behavior.

Extremely obese humans have reduced amounts of dopamine receptors, especially D2 receptors. However, whether this is the cause or consequence of obesity and overeating is unknown. The cause of the overeating may be more likely due to hyper-reactivity in dopamine-related limbic circuitry. This would be similar to incentive sensitization in drug addiction. Nevertheless, whether food addiction exists remains a compelling question.
Dr. Avena’s presentation, “Empirical Evidence Supporting the Construct of Food Addiction: A Focus on Animal Models,” centered on what she termed hyperpalatable, ultra-processed foods that might overactivate brain circuits linked to the reinforcement of food intake and reward.

Although drugs of abuse act on brain systems that reinforce natural behaviors, overlapping brain regions are activated by palatable foods and drugs of abuse. Her research suggests that normal-weight rats consuming excess sugar and rats that become overweight on a cafeteria-style diet show alterations in the mesolimbic dopamine and other brain systems that are consistent with what would be seen in animals using drugs of abuse.

In humans, the Yale Food Addiction Scale was used ostensibly to measure addiction-like responses toward food. Several studies were conducted using this scale, and, depending on the specific population studied, different percentages of people meet the criteria of the scale for food addiction. For example, ~11% of a general population meets the criteria for food addiction, but 56% of those who are obese with comorbid binge-eating disorder (BED) meet the criteria of the scale for having food addiction. Subsequent studies identified neurocorrelates between food addiction scores and activation of brain reward areas in response to the receipt or a cue for food, which maps to rat study findings.

After presenting evidence for the Diagnostic and Statistical Manual of Mental Disorders criteria for substance dependence, when the substance of abuse is palatable food, Dr. Avena dispelled areas of controversy in the comparison of food and drug addiction and then summarized where the field currently stands: “This is really just the start of a better understanding of food reward and brain mechanisms associated with eating, and how they may or may not lead to addictive-like behaviors.”

Dr. Ziaudddeen’s presentation, “Obesity and the Brain: How Convincing is the Addiction Model?”, drew on research in humans on the role of the brain reward system and normal and abnormal eating behavior. He noted that the brain–environment interaction in obesity development is a complex picture and that food addiction may have a specific place in it, albeit a small one.

Drug addiction/dependence results from the combination of a drug and a susceptible individual over time. Indeed, 85% of people who use an addictive substance never develop dependence. In addition, food addiction may not be associated with obesity. In fact, if there is a food addiction, one might expect to see more obesity in individuals who have had the condition for longer.

Long-term prospective studies are needed to develop a comprehensive model of food addiction that includes an addictive agent and charts the interaction between that agent and a susceptible individual over time, leading to the development of the addiction. However, typical research is cross-sectional, building models based on circumstantial evidence.

Evidence for food addiction to date includes the following: 1) the clinical overlap between drug addiction and BED/obesity; 2) familial co-occurrence of obesity/BED and substance use disorders; and 3) neuroscientific evidence. Many issues surrounding the concept of human food addiction need to be addressed. The addictive agents are not always clearly identified. The clinical overlap is unconvincing, and evidence from animal models does not translate well beyond the laboratory. Receptor and neuroimaging studies in humans are inconsistent. Despite this, comparisons and superficial similarities are noted with drugs, and these are misleading. According to Dr. Ziaudddeen, “At present there is little evidence to support a human food addiction syndrome.”

Dr. Alonso-Alonso agreed, noting that, beyond extreme phenotypes related to the BED spectrum, there is limited evidence for food addiction in humans. In his presentation, “Beyond Food Reward: Broadening the Picture, Cognitive Influences,” he summarized key issues in the food addiction model and discussed the importance of cognition as a moderator of responses to food reward.

Human FMRI studies indicate that palatable foods activate reward-related brain regions similar to other sources of pleasure in healthy individuals and drugs of abuse in the case of addicts. The majority of research focused on the identification of similarities, but recent studies comparing brain changes associated with drug addiction vs. obesity and eating disorders suggest that these overlaps are incomplete.

Dr. Alonso-Alonso reviewed several shortcomings in the use of functional neuroimaging in this field, including the following: 1) the impossibility to diagnose addiction on the basis of these data because addiction relies on the subjective experience of an individual; and 2) the limited external validity of the method.

Unlike laboratory rodents, humans live in a complex food environment that calls for >200 food-related decisions per day. These decisions rely on cognitive resources and, typically, the ability to balance immediate gratification against delayed reward on health or body image. Cognition influences rewarding responses to food via top-down mechanisms that are particularly developed in humans. Dr. Alonso-Alonso mentioned potential strategies to promote healthy eating via enhancement of cognitive control with interventions based on physical activity, meditation, neuro-modulation, or drugs. He highlighted recent research suggesting that certain foods and nutrients potentially strengthen the status of brain regions underlying cognitive control, such as the lateral prefrontal cortex.

According to Dr. Alonso-Alonso, there is a need for integrated research to understand the interplay and relative contribution of 3 key factors underlying response to food reward: 1) the food itself, including the impact of different food types, nutrients, doses, thresholds, and patterns of consumption; 2) individual characteristics, such as genetic influences and phenotypes that can predispose or protect from overeating; and 3) the context, which refers to situational factors, personal attitudes toward food, and social norms, cultural values, and other environmental contributors.
The presentation also covered implications for treatment associated with the food-addiction model. Dr. Alonso-Alonso provided examples from past research in obesity to illustrate that abstinence may not be an adequate solution to reduce overeating and obesity in the general population. Additionally, numerous studies in the treatment of eating disorders concluded that, rather than advocating rigid dietary restrictions, the focus should be on addressing underlying issues and managing relations with food. There is also the potential for adverse unintentional consequences of defining food as addictive, including increased food-related anxiety and risk of reinforcing the overeating cycle.

The final presentation, by Dr. Allison, was “Neurocognition: The Food–Brain Connection—Methodologic and Epistemologic Considerations.” He addressed questions pertaining to the following: 1) determining appropriate research designs and how to do them well; and 2) defining food addiction. He lauded previous speakers for not just showing data but for considering its relevance: “This is a fairly new level of sophistication in the conversation about food addiction, and the first step toward a scientifically meaningful definition of addiction.”

An issue in the neuroscientific study of obesity is being “blinded by the light” of new technology. He cited the value of good experimental design principles and advised researchers to take a deep breath and ask themselves some questions when they evaluate studies, such as “Would I be convinced of this conclusion if it only showed a bar graph instead of brain images, and, if not, why do the pictures somehow make it seem like there’s something more here?” “No matter what approach is used,” said Dr. Allison, “we still need to correct multiple comparisons and publish the corrected results. We still need to stay grounded in the principles of sound investigation.”

He noted the value in considering differences between experimental conditions and reality and encouraged that outcomes be accurately presented to the media. Topics such as obesity and addiction draw great media attention. “By putting accuracy-reducing spin in abstracts, press releases will almost always pick it up, as will the news,” he said, adding that scientists need to be aware of their own complicity in misleading the public.

Other issues covered included skepticism about tools, such as the Yale Food Addiction Scale. “These approaches have been shown to be reliable and therefore measure something,” he said, “The question is what.” He emphasized the need to ensure that questions are meaningful, sample sizes are adequate, designs are valid, analyses are done properly, and results are interpreted fairly. “New technologies like functional magnetic resonance imaging may seem magical,” he said, “but they’re not magic and do not change the fundamental logic and statistics of experimental design.” Importantly, only with an objective and logically coherent definition of what it means for a thing to be addictive can we ask whether food is addictive. He noted that Diagnostic and Statistical Manual of Mental Disorders definitions are intended to determine whether specific persons are addicted to specific things and not whether specific things can, in general, be judged to be addictive or not.

In summary, speakers evaluated the knowledge of the neurocircuitry and pathway physiology for food intake, addiction, and overlap with neurocognition. Whereas pathways of food addiction were well demonstrated in animal studies, the challenges that arise when translating animal addiction models to humans were acknowledged. The speakers evaluated research on brain reward systems and food intake and underscored the need to stay grounded in the basics of sound experimental design and data analyses. All presenters agreed that the study of the food–brain connection has some way to go before science develops a meaningful definition of food addiction.

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Caffeine-Containing Energy Drinks: Beginning to Address the Gaps in What We Know

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ABSTRACT

Energy drinks are relatively new to the United States but are the fastest growing segment of the beverage market. Humans have a long history of consuming caffeine in traditional beverages, such as cocoa, coffee, tea, and yerba maté, but 2 workshops held at the Institute of Medicine (http://www.iom.edu/Activities/Nutrition/PotentialHazardsCaffeineSupplements/2013-AUG-05.aspx) and the NIH (http://ods.od.nih.gov/News/EnergyDrinksWorkshop2013.aspx) in 2013 highlighted many critical gaps in understanding the biologic and behavioral effects of the mixtures of caffeine, vitamins, herbs, sugar or other sweeteners, and other ingredients that typify caffeine-containing energy drinks (CCEDs). For example, different surveys over the same 2010–2012 timeframe report discrepant prevalence of CCED use by teenagers, ranging from 10.3% in 13–17 y olds to >30% of those in grades 10 and 12. Understanding of functional interactions between CCED ingredients, drivers of use, and biologic and behavioral effects is limited. The 4 speakers in the Experimental Biology 2014 symposium titled “Energy Drinks: Current Knowledge and Critical Research Gaps” described recent progress by their groups in extending our understanding of prevalence of CCED use, sources of caffeine in the United States, drivers of CCED use, and behavioral correlations and effects of CCEDs, including effects on attractiveness of both alcoholic and non-alcoholic beverages. Adv. Nutr. 5: 541–543, 2014.

The rapid growth of caffeine-containing energy drink (CCED) sales (1) and the observed increase in energy drink–associated emergency department visits (2) have drawn the attention of the biomedical and nutrition research communities, as well as many others. The objective of the Experimental Biology 2014 Symposium was to provide research updates addressing some of the critical gaps in our understanding of these products that were highlighted during the more extensive workshops in 2013.

The first 2 speakers presented new data on the shifting sources of caffeine in the United States and on the complex picture of caffeine use by young people and minorities and its interaction with environment, sleep, and health.

The first presentation, “Caffeine Intake in US Children and Adolescents,” by Dr. Naman Ahluwalia summarized new analyses of recently released data on caffeine consumption in 2–19 y olds from the 2009–2010 NHANES and compared these with other recently published analyses. The latest data from the NHANES 24-h diet recalls show little association of sex or socioeconomic status with prevalence of caffeine consumption in this age range. However, amounts of caffeine intake (total or milligrams per kilogram) increased with age and were significantly lower among non-Hispanic blacks than other major race- or origin-related population subgroups. Dr. Ahluwalia reported that 10% of all 12–19 y olds, or 25% of 12- to 19-y-old caffeine consumers, consumed more than the recommended maximum amounts of caffeine for their age and weight. Looking at dietary sources of caffeine, her analyses find 1.7% of 12–19 y olds consuming CCEDs, whereas Mitchell et al. (3), based on 7-d diaries collected for the Kanter Worldpanel Beverage Consumption survey, report 10.3% of 13–17 y olds consuming CCEDs.

Looking at longitudinal trends in dietary sources of caffeine, Dr. Ahluwalia summarized an analysis of NHANES data for 1999–2010 by Branum et al. (4) that finds a significant decrease in the percentage of caffeine that 2–22 y olds are obtaining from sodas and a significant increase in the percentage coming from coffee and CCEDs. The trend toward an increased percentage of caffeine intake coming from CCEDs was significant only for those subgroups aged ≥11 y.
Dr. Ahluwalia’s analysis shows that 4.4% of the caffeine consumed by 12–19 y olds was from CCEDs in NHANES 2009–2010. This is slightly more than Branum et al. reported for 12–18 y olds in their analyses of the 1999–2010 NHANES data but less than they reported for 19–22 y olds (10.3% of caffeine from CCEDs). In closing, Dr. Ahluwalia pointed out that the comparison of different epidemiologic studies of CCED use or caffeine intake is challenging due to differences, for example, in the age groupings used and in whether data analyzed are from the whole population or caffeine consumers only.

Dr. Michael A. Grandner summarized the epidemiologic associations among race and ethnicity, socioeconomic status, prevalence of sleep insufficiency, and worse health outcomes, including increased risk of cardiovascular disease and mortality, in his presentation on “Disparities in Energy Product Use, Sleep, and Health Outcomes.” For example, he pointed out that short sleep duration is not only associated with hypertension in epidemiologic studies but is a marker for significantly increased risk of incident hypertension, with differences in mean sleep duration apparently mediating the difference between white and black Americans in longitudinal change in diastolic blood pressure (5). Dr. Grandner then asked how social, cultural, and environmental differences might interact with CCED consumption to influence sleep patterns and other health-relevant behaviors. He reported new findings that blacks and whites differ substantially in their attitudes toward sleep, with whites significantly more likely to endorse connections between insufficient sleep and adverse consequences. These data, as well as the association of an overall energy-dense dietary pattern with poverty (perhaps abetted by the applicability of Supplemental Nutrition Assistance Program benefits to CCEDs and other sugar-sweetened beverages), reports that minority communities are exposed to more CCED advertising than white communities, which might lead one to hypothesize higher rates of CCED consumption by blacks and Hispanics than by non-Hispanic whites. The NHANES data that Dr. Ahluwalia reported for 2–19 y olds did not appear to support this hypothesis but instead showed higher rates of caffeine consumption (from all sources) for non-Hispanic whites and Mexican-Americans than for blacks. However, the NHANES data do not include a large enough number of CCED consumers to support meaningful analyses of consumption by population subgroups. An analysis of data on sports and energy drink use from the 2007–2010 National Health Interview Survey reported that prevalence of use of beverages from this substantially larger category was higher among the Hispanic/Latino group than among non-Hispanic blacks, whose prevalence of use was in turn higher than that of non-Hispanic whites. Each of the foregoing analyses has different strengths and weaknesses in its ability to address racial or ethnic differences in CCED use, and each differs from the others in substantial methodologic details, such that it is not possible to directly compare them. Dr. Grandner concluded that, because CCEDs are an emerging phenomenon, there are currently few clear population patterns in their use beyond the consistently higher prevalence of use by adolescents and younger adults.

The next 2 presentations took the symposium into experimental data on the behavioral effects of CCEDs.

Dr. Jennifer L. Temple presented on the “Physiological, Psychological, and Behavioral Effects of Caffeine in Children and Adolescents.” She summarized data showing that caffeine increased liking for sugar-sweetened, novel-flavored beverages. The change in “liking” was dose dependent (observed at 2 mg/kg caffeine but not at 1 mg/kg), and aspects of responses to the beverages depended on both sex and developmental status (prepubertal vs. postpubertal). Dr. Temple reported that analysis of data from the national Youth Risk Behavior Surveillance System for grades 9–12 showed a strong association of a number of risk behaviors with daily soda consumption. These associations also exhibited sex differences, with several associations (including those for involvement in physical fights and reporting multiple sex partners) stronger for females than males. Given the relatively high prevalence of CCED use by adolescents and young adults, there appears to be a strong need for additional research aimed at better understanding the relations between CCED use, sex, and risk behaviors.

Dr. Temple reported on a pilot study exploring factors that might influence CCED-purchasing behavior of 15–25 y olds. In a laboratory-based, model convenience-store setting, increasing the price of CCEDs reduced the number of servings purchased. Consistent with the association of CCED consumption and risk-taking, adding a warning label to the CCEDs increased the number of servings purchased.

Dr. Cecile A. Marczinski addressed “Energy Drinks Mixed with Alcohol: What Are the Risks?” in the final talk of the session. As background, Dr. Marczinski summarized recent data on prevalence of use of CCEDs combined with alcoholic beverages [alcohol mixed with energy drinks (AmED)]. According to the 2012 Monitoring the Future study (1), 26% of those in grade 12, 34% of college students, and 37% of young adults reported having used AmED in the past year. This prevalence of use is a concern because of the increasing numbers of emergency department visits associated with consumption of CCEDs either alone or in combination with alcohol (2) and because AmED consumption (compared with consumption of alcohol alone) was also reported to be associated with drinking to higher blood alcohol concentrations, greater risk of intending to drive while intoxicated or riding with an intoxicated driver, and increases in other risk-taking behaviors (6). Dr. Marczinski went on to describe experiments her group performed to begin to understand why AmED consumers may consume more alcohol than those consuming alcohol alone. In these studies, the effect of the CCED on desire to consume more of the beverage appears more pronounced than in the studies of non-alcoholic beverages described by Dr. Temple; clearly bringing alcohol into the mix is likely to bring more, and likely different, signaling mechanisms into play. In randomized, double-blinded studies of college students of both sexes (equal numbers in each experiment),
Dr. Marczinski and her colleagues find that, although consuming an alcoholic beverage (without caffeine) increases the rating of desire for more alcohol over ~40 min, those consuming AmED indicate substantially greater desire for more alcohol than those consuming a control (similarly flavored beverage with the same alcohol content but without added CCEDs) and that the increased desire for alcohol lasts substantially longer in those consuming AmED (6). This effect was dose dependent for the CCED but not for the doses of alcohol tested (equivalent to 2 shots of vodka). Dr. Marczinski hypothesized that the pharmacologic basis for these observations may be modulation of the pharmacologic or other effects of alcohol by the well-known inhibitory effect of caffeine on adenosine signaling in the central nervous system.

Many critical gaps in our understanding of CCEDs remain to be addressed. Among these are the contribution of ingredients other than caffeine and sugar to the metabolic, physiologic, and behavioral effects of CCEDs, elucidation of the mechanisms underlying the modulation of alcohol response by CCEDs, and better data on the prevalence of use of CCEDs and on their acute and long-term effects on appetite, metabolism, BMI, alertness, sleep patterns, and cognition.

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**References**

Polyphenols are an unavoidable component in the human diet. We obtain them from fruit, vegetables, cereals, seeds and beans, spices and herbs, oils, and all of the food products made from these basic components. Typically, the greatest quantities are consumed in the form of alcoholic and nonalcoholic beverages, such as wine and tea, fruit and fruit juices, and vegetables (2). Food diary studies suggest a wide variability in flavonoid consumption. For instance, the populations of the United States, Spain, and Australia were estimated to consume ~190, 313, and 454 mg/d flavonoids, respectively, with the largest part being taken in the form of flavanols and their oligomers and polymers (2–4). Evidence also suggests that the consumption of dietary flavonoids is typically outweighed by simpler phenolics. For instance, a study conducted in Finland demonstrated a mean consumption of 222 mg/d flavonoids and 640 mg/d phenolic acids (mainly via chlorogenic acid from coffee) (5). Similarly, a French cohort consumed a mean of 1193 mg/d phenolics, of which phenolic acids contributed 639 mg (6).

**Current Status of Knowledge**

**Polyphenols and human brain function**

Epidemiologic evidence suggests that the consumption of polyphenols confer a wide range of health benefits.
The Future of Nutrition Research at the National Institutes of Health1–3

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ABSTRACT
Cuts to the NIH budget decreased funding for nutrition research. It is even more necessary now to understand and elevate the role of nutrition research at the NIH. This symposium shed light on where nutrition research stands today and what the future holds for nutrition research at the NIH. In his introduction, the ASN president shared an overview of nutrition research at the NIH and a description of what the ASN is doing to advance the future of nutrition research. Nutrition program directors from various NIH institutes and offices, including the National Heart, Lung, and Blood Institute, the National Institute of Diabetes and Digestive and Kidney Disease, the National Cancer Institute, and the Office of Dietary Supplements, discussed nutrition research advances supported by past and present federal funding and highlighted nutrition research opportunities through forthcoming funding opportunity announcements of interest to ASN members.

Substantial reductions to the budgets of federal agencies limited funding for nutrition research. The NIH budget remained stagnant over recent years and was subject to substantial budget cuts that were associated with sequestration. Although some of those sequestered funds have since been restored, there is reason to believe that this issue will arise again. The NIH currently supports 2000 fewer research project grants than it did 5 y ago. In fact, today only 1 in 6 grant applications are funded, the lowest rate in NIH history. It is even more necessary now to understand and elevate the role of nutrition research at the NIH. This session focused on where nutrition research stands today and what the future holds for nutrition research at the NIH. Nutrition program directors from various NIH institutes and offices, including the National Heart, Lung, and Blood Institute (NHLBI)6, the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), the National Cancer Institute (NCI), and the Office of Dietary Supplements (ODS), discussed nutrition research advances supported by past and present federal funding and highlighted nutrition research opportunities through forthcoming requests for applications or program announcements that would be of interest to ASN members. They also discussed nuances when applying for grants at their specific institute, such as the importance of multidisciplinary and interdepartmental proposals.

Dr. Gordon Jensen, ASN President, opened the symposium with an overview of nutrition research at the NIH and a description of what the ASN is doing to advance the future of nutrition research. He provided a number of statistics related to nutrition funding: >90% of nutrition-related research and training is funded by the NIH and the USDA, only 8% of the NIH budget is devoted to nutrition and obesity research, and the NIH invested $1.7 billion for 4600 nutrition research projects in fiscal year 2012. More than half of all NIH nutrition-related expenditures each year are provided by the NIDDK, the NHLBI, and the NCI; each of these institutes invests more than $200 million annually in support of nutrition research. Through congressional outreach and testimony, ASN representatives have been very active in advocating for increased nutrition research support. As highlighted by Dr. Jensen, the ASN also developed a list of nutrition research priorities. These include the following: 1) variability in response to diet and food; 2) healthy growth, development, and reproduction; 3) health maintenance; 4) medical management; 5) nutrition-related behavior; and 6) food supply and environment. Cross-cutting tools are

1 This article is a summary of the symposium “The Future of Nutrition Research at NIH” held 28 April 2014 at the ASN Scientific Sessions and Annual Meeting at Experimental Biology 2014 in San Diego, CA. The symposium was sponsored by the American Society for Nutrition (ASN), the ASN Public Policy Committee, and the ASN Nutritional Sciences Council.
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6 Abbreviations used: DASH, Dietary Approaches to Stop Hypertension; FOA, funding opportunity announcement; NCI, National Cancer Institute; NHLBI, National Heart, Lung, and Blood Institute; NIDDK, National Institute of Diabetes and Digestive and Kidney Diseases; ODS, Office of Dietary Supplements.
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essential to advance nutrition research. “Omics” technologies will facilitate research on nutrient interactions with genes, proteins, and metabolites. Bioinformatics will enable researchers to more efficiently manage, analyze, and understand nutrition data. Databases are needed to track and observe trends related to nutrition. Biomarkers are essential to be able to determine and monitor nutrition status. Finally, economic analysis is needed to calculate and compare costs of research. Dr. Jensen finished his presentation by recommending that investigators read the nutrition research priorities (1).

Kathryn McMurry, Nutrition Coordinator at the NHLBI, provided an overview of nutrition research at the NHLBI. The NHLBI supports a broad spectrum of nutrition research related to obesity, cardiovascular diseases, pulmonary diseases, blood diseases, and sleep disorders. The NHLBI funding amount for nutrition research averaged approximately $230 million over the past 6 y; most study applications are initiated by investigators. Research methodologies span from knowledge to use: from basic research and epidemiology, which increases knowledge about biologic and behavioral associations and mechanisms, to clinical trials and feeding studies, which determine the efficacy of interventions under tightly controlled circumstances, to intervention studies in clinical and community settings, which determine the effectiveness of intervention in “real-world” settings, to research that translates and disseminates intervention approaches to clinical and community settings. Moreover, this path is bidirectional.

Recently, at the NHLBI, there has been increased interest in low-cost, pragmatic trials. These trials would be expected to do the following: 1) determine the effectiveness of an intervention in a real-world setting; 2) answer questions that greatly affect patients or healthcare providers; 3) leverage existing clinical practice settings/electronic resources; 4) minimize the use of separate infrastructure; and 5) use low-cost methods to collect, store, and utilize biologic specimens.

The seminal NHLBI-funded study Dietary Approaches to Stop Hypertension (DASH) serves to highlight some of the methods to collect, store, and utilize biologic specimens. The DASH project provides a mechanism for funding shared resources to complement current efforts, encourage application of state-of-the-art nutrition research, and to encourage applications that use application of nutrigenomics approaches to basic, translational, and clinical nutrition research. Gut Microbiota-Derived Factors in the Integrated Physiology and Pathophysiology of Diseases within NIDDK’s Mission (research project grant PAR-13-293) also was emphasized (3). The objective of this initiative is to support investigator-initiated multidisciplinary R01 research projects to define interactions between the gut and the gut microbiota that regulate normal physiology and pathophysiology of diseases within the NIDDK mission. The goal of the research projects is to define specific human gut microbiota genes or gene regulatory networks, metabolites, secreted proteins, or other molecular factors that affect or are affected by host physiology (including diet/nutrition), homeostasis, and disease pathophysiology. In addition to discovery of specific microbiota-derived factors, research projects will need to define the specific interactions and pathways by which they affect host processes locally within the gut and/or at distant organ sites.

The NIDDK also has a number of resources related to nutrition research. The Nutrition Obesity Research Centers program supports research infrastructure, including research services, enrichment programs, and collaborative activities, at an academic/medical institution or consortium of institutions, to foster interdisciplinary basic, clinical, and public health research. The Digestive Diseases Research Core Centers program provides a mechanism for funding shared resources (i.e., core facilities). Through these centers, the NIDDK aims to integrate, coordinate, and foster interdisciplinary cooperation between groups of established investigators that conduct high-quality research on digestive diseases. The Mouse Metabolic Phenotyping Centers program provides experimental testing services to scientists studying diabetes, obesity, diabetic complications, and other metabolic diseases in mice.

In the future, the NIDDK will continue to promote state-of-the-art nutrition research, encourage the introduction of novel technologies and concepts in nutrition research to complement current efforts, encourage application of...
high-throughput “omics” technologies to address diet–host–microbiome interactions, support collaborative and multidisciplinary research, and leverage existing resources and infrastructure developed across federal agencies. The NIDDK will meet the challenge of deploying its precious budgetary resources in the most effective and efficient ways to sustain research momentum and fully capitalize on research achievements.

Dr. Sharon Ross, Program Director at the NCI, discussed nutrition research at the NCI. At the NCI, there are 4 different extramural divisions that support nutrition research: 1) the Division of Cancer Biology; 2) the Division of Cancer Control and Population Sciences; 3) the Division of Cancer Prevention; and 4) the Division of Cancer Treatment and Diagnosis. The Nutritional Sciences Research Group, located in the Division of Cancer Prevention, supported mechanistic preclinical and clinical studies concerning the influence of bioactive food components on cancer prevention with an emphasis on understanding variation in response and biologic processes of cancer development and prevention. Current supported studies continue to focus on these areas and strive to understand the relationship between the microbiome, diet, and cancer prevention and understand the link between diet, obesity, and cancer prevention. The NCI encourages applicants to consider the totality of the evidence rather than selective inclusion of evidence when testing a hypothesis and to be mindful of adequately addressing power, sample size, multiple comparisons, and study design issues in both preclinical and clinical studies. In fiscal year 2012, the NCI funded 585 nutrition-related projects at a cost of more than $286 million, which represented 5.7% of the NCI budget (4).

Beginning in 2011, the NCI adopted a new approach to the selection of grant applications for funding. Rather than establish an absolute pay line, individual consideration of a broad range of applications were the hallmark of the NCI selection process for all competing applications. In 2013, most applications with scores up to percentile 9 were funded. Funded applications with higher scores were subject to divisional and Scientific Program Leaders review. The NCI awarded 1095 competing research project grants, resulting in a final success rate of 14% (5).

A number of recent funding opportunities at the NCI also were highlighted (6,7). Research Answers to NCI’s Provocative Questions—Group A (Cancer Prevention and Risk) is a funding opportunity announcement (FOA) for R01 and R21 applications that challenges investigators to seek answers to specific unsolved problems generally related to the investigation of changes in behavior and various exposure risks, mechanistic links between cancer risk factors and biologic events associated with cancer development, and how we might identify and better understand prevention mechanisms. The NCI omnibus R21 FOA supports the development of new research activities in all areas of cancer research (8). The R21 mechanism is intended to encourage exploratory and developmental research projects by providing support for the early and conceptual stages of these projects. These studies may involve considerable risk but may lead to a breakthrough in a particular area or to the development of novel techniques, agents, methodologies, models, or applications that could have a major effect on a field of cancer research (biomedical, behavioral, or clinical). The NCI omnibus R03 FOA supports small research projects on cancer that can be performed in a short period of time with limited resources (9). The R03 grant mechanism supports different types of projects, including the following: 1) pilot and feasibility studies; 2) secondary analysis of existing data; 3) small, self-contained research projects; 4) development of research methodology; and 5) development of new research technology.

The final speaker was Dr. Cindy Davis, Director of Grants and Extramural Activities at the ODS, who discussed the role of the ODS in nutrition funding. The mission of the ODS is to strengthen knowledge and understanding of dietary supplements by evaluating scientific information, stimulating and supporting research, disseminating research results, and educating the public to foster an enhanced quality of life and health for the U.S. population. Unlike the institutes at the NIH, the ODS is not able to directly fund or take primary responsibility for grants. Instead, the ODS co-funds grants that have already been through NIH peer review and subsequently funded through an NIH institute. ODS accepts applications from the program directors of the NIH institutes and centers 3 times a year and evaluates them on their relevance to the ODS mission and strategic plan, whether they fill gaps in the ODS research portfolio, and quality of the science. The ODS spends ~60% of its budget co-funding extramural grants. At its peak in 2007, the ODS co-funded 103 awards for $17.4 million compared with 96 awards in 2013 for $13.7 million with 14 different NIH institutes.

Research interests of the ODS are not limited to specific health conditions, organ systems, or population groups. The ODS supports all types of research, including preclinical, clinical, behavioral, and epidemiologic, in which the emphasis is on dietary supplements or their ingredients. However, the ODS will not entertain grants that have disease treatment as a focus. Primary consideration for support is given to proposals that stimulate dietary supplement research when it is lacking, clarify gaps, and focus on the use of supplements in improving or maintaining health and reducing the risk of chronic disease.

Dr. Davis also discussed a new FOA for fiscal year 2015 (10). The ODS will fund up to 30 administrative supplements that are designed to provide supplemental funds to relevant, active, NIH-supported research projects to incorporate dietary supplement research that is within the scope of the parent project.

The symposium ended with a panel discussion that also incorporated the scientific review aspects of nutrition research. Attendees now have a better understanding of how the different NIH institutes, offices, and centers fund nutrition-related research and future funding opportunities within the NIH.
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History and Impact of Nutritional Epidemiology

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ABSTRACT

The real and important role of epidemiology was discussed, noting heretofore unknown associations that led to improved understanding of the cause and prevention of individual nutritional deficiencies. However, epidemiology has been less successful in linking individual nutrients to the cause of chronic diseases, such as cancer and cardiovascular disease. Dietary changes, such as decreasing caloric intake to prevent cancer and the Mediterranean diet to prevent diabetes, were confirmed as successful approaches to modifying the incidence of chronic diseases.

As large databases from epidemiology studies become more available and papers using their large amount of information proliferate, there is a tendency for conclusions of those papers to achieve the status of answers rather than as the lead point for prospective studies to confirm or refute the associations suggested by epidemiologic methods. In this setting, it seemed appropriate to review the contributions that epidemiology made to the knowledge base in nutritional sciences to better understand the role of the plethora of papers in nutritional epidemiology that now fill our journals.

Dr. Alpers introduced the session by explaining the above rationale for the session. He pointed out that randomized controlled trials (RCTs) are the gold standard in clinical research and that these would be preferred for nutritional studies. However, there are difficulties in using foods or nutrients as interventions in RCTs. These difficulties include having the wrong proportion of food intake assigned to the diet, testing the wrong dose of nutrient, getting the duration of intervention wrong, intervening too late to alter the natural history of the disease under study, and not being able to correct for confounding factors, among which are lifestyle biases, genes, environmental effects on genes, or non-nutrient constituents of foods. For these and other reasons, data on the effect of diets or nutrients in chronic disease are dependent on observational studies to produce associations and derive hypotheses for additional testing. Such studies can be very useful, but when individual nutrient intervention were used to confirm hypotheses, the results did not in general confirm the implications from the associations identified in observational studies. Confirmation was found using whole diets or dietary patterns (e.g., Mediterranean) or whole food classes (e.g., whole grains), but the individual nutrients responsible for the confirmed observations found with food are not known. This symposium includes talks that use examples from the history of the field of nutritional epidemiology to demonstrate when epidemiology led to considerable advances but also to note the areas in which this methodology was not so successful and to exercise caution in the interpretation of the resulting associations.

Dr. Carpenter was unable to attend, but his talk was interpreted by Dr. Bier, who spoke on the “Historical Role of Epidemiology in Identifying Essential Nutrients.” He expanded on Dr. Carpenter’s selected example of the successful use of epidemiology in the discovery of the cause and prevention of beriberi. In his historical exposition, Dr. Carpenter chose to highlight the less well known story of Hamilton Wright, who studied beriberi in Malaya, then a British colony. Wright recognized that Malaya was an optimal location to study beriberi because the country was...
inhabited by 3 Asian populations whose different environmental conditions and habits might provide clues to the origin of the disease. He noted that, although Chinese brought in to work in the tin mines commonly developed beriberi, native Malays and Tamils imported from Sri Lanka did not. However, when imprisoned in a multiracial prison, all were similarly susceptible to the disease. Among other differences identified in the free-living populations, he realized that the Chinese ate “Siam” (white) rice and the Tamils ate “Bengal” (parboiled) rice. However, based on his medical training, including the recently appreciated germ theory of disease, and the limits of nutritional knowledge at the time, Wright persisted in his belief that the disease was caused by an unknown organism that entered the body by mouth with subsequent gastrointestinal production of a toxin responsible for the signs and symptoms of beriberi. Shortly thereafter, Dr. W. L. Braddon realized that Wright’s interpretation was mistaken and that the disease was a dietary disease. He recognized the importance of the fact that the Chinese ate Siam rice and that parboiling (Bengal) rice afforded protection against beriberi. He further appreciated that, although Malays ate Siam rice, it was often consumed freshly after winnowing. Thus, although he realized that beriberi was dietary in origin, he interpreted his findings as an indication of a toxin present in the rice. Understanding how to prevent the disease required a prospective experiment, then performed by Walter Fletcher, the senior physician at the Insane Asylum in Kuala Lumpur, where a beriberi outbreak had just occurred. Dr. Fletcher did not believe Dr. Braddon’s hypothesis, so he decided to test it by feeding inmates in 1 building Siam-style rice and in another building parboiled rice, cooked in the Tamil way. He found that 18 of 120 individuals fed Siam-style rice died compared with 0 of 120 fed Tamil rice. Fletcher rightly attributed the advantage as showing that white rice was deficient in a “dietetic value.” The irony of these findings was that, because of the poor medical communications of the time, the British experiments took place after earlier studies nearby in Asia already demonstrated the essential role of nutrient-deficient rice in the pathogenesis of the disease. In Japan, Kenehiro Takaki appreciated that kakké (beriberi) was the consequence of a rice diet, although he attributed the problem to protein deficiency, and Christiaan Eijkman in Indonesia, after an exhaustive series of experiments to eliminate alternative explanations possible from the observational data, came to the realization that the rice pericarp “silver skin” contained something essential for health. Although he did not identify the factor as thiamine, he shared in the Nobel Prize for this work that progressed from the observations on ingested rice to an identification of the source of the material that treated the disease. Dr. Bier concluded by noting that epidemiologists, clinical scientists, and chemists in this discovery process acted as collaborators, not rivals, but that the unraveling of the dilemma took time and studies in the field had to be designed to answer the hypothesis first established by epidemiologists.

Dr. Donald McCormick followed by speaking on the role of epidemiology in decision making for food fortification, using examples of many micronutrients. He initiated the discussion by noting that food fortification has clear benefits for certain portions of the population and, as examples, used folate addition to foods to aid pregnant women in preventing deficiency and lowering the incidence of neural tube defects in the fetus and vitamin D added to milk to prevent rickets. However, he noted the increasing tendency for the false expectation that food fortification at amounts higher than needed to prevent deficiency might decrease nondeficiency diseases. These expectations are often initiated by epidemiologic studies. The data with folate supplements and their role in preventing colorectal cancer are mixed, showing an inhibitory effect in individuals who are folate deficient but a promoting effect on the progression of established neoplasms. Similarly, the benefit suggested for vitamin D by epidemiologic studies in conditions as diverse as cancer and heart disease has yet to be confirmed by prospective RCTs. When there is no evidence of deficiency, current RDA amounts of intake should suffice for most people. However, the difficulty in defining and agreeing on a biochemical definition of the deficiency state continues to plague the field of micronutrients (e.g., vitamins B-12 and D) and led to additional confusion about how to translate the findings from epidemiologic studies into prospective trials that will provide definitive answers.

Dr. Anthony Miller then addressed the role of epidemiology in identification of foods and nutrients that influence the risk of cancer. He first discussed study designs beginning with correlative/ecologic studies. They further include case-control studies in which biases need to be recognized and cohort studies in which recognition of misclassification is important. Finally, intervention studies designed to confirm observed associations from the first 2 study types often use surrogate endpoints to detect premalignant changes, but when cancer is the endpoint in a study of finite length, the length of follow-up and timing in regard to natural history becomes very important. A number of non-interventional studies were reviewed initially, demonstrating that increased total calories were associated with increased risk of cancer but showing rather little specificity for specific macronutrients or food components. Although some studies demonstrated a reduced risk with increased intake of fiber or vegetable and fruit, other studies did not confirm these associations. The best associations continue to reflect cancer risk that is increased by higher caloric intake or decreased risk when following a total diet, such as the Mediterranean diet. Interventional studies, exemplified by β-carotene and vitamin A supplementation, mostly failed to reduce risk. The current period of increased interest in genetics was discussed, noting that multiple single-nucleotide polymorphisms in genes were tested for their association with cancer risk and that small effects were seen that need replication. These may indicate individual susceptibility, but in addition, these studies tend to ignore the effects of dietary factors. Thus, the role of cancer prevention by dietary...
change may have been downgraded in the recent literature. Dr. Miller concluded that improved calibration of nutrient intake improved our recognition of associations but that misclassification of dietary intake (e.g., red meat, fiber) impaired our ability to detect causal associations, if they truly exist. He also concluded that the effects of dietary patterns need to be pursued and that we not be misled or sidetracked by genetic associations, each of which may account for only a small portion of the cancer risk in a population-based study. This is important, because cancer risk seems to be increasing as a function of increased weight/obesity, but it is not certain whether this is all due to increased caloric intake or whether individual dietary components play a role.

Dr. Paul Jacques in his discussion of “The Relevance of Nutritional Epidemiology in the 21st Century” provided additional historical examples of successful confirmation of observational hypotheses with a focus on foods and dietary patterns and noted 1 future direction for the discipline. He reviewed the data on an association between ingestion of whole wheat and favorable health outcomes in diabetes and cardiovascular disease and the resulting interventional studies that confirmed the benefit of ingesting whole-grain foods. He followed this with the data on the Mediterranean-style dietary pattern, again confirmed by interventions on the incidence of diabetes and cardiovascular disease. These examples demonstrated the consistency between the evidence provided by the observational studies and intervention trials. However, as reviewed in cancer outcomes by Dr. Miller, clear epidemiologic data on the role of individual nutrients is more difficult to obtain. Thus, Dr. Jacques noted that 1 direction for the future of nutritional epidemiology was to use metabolomics to identify metabolites (not nutrients) associated with the individual foods and dietary patterns and by quantifying their potential to uncover diet–disease relations in populations. He concluded that, although traditional approaches continue to provide valuable knowledge about the cause of chronic diseases, new technologies will be essential to maximize the impact of epidemiology in the future.

Acknowledgments
All authors read and approved the final manuscript.
example, the consumption of polyphenol-rich foods and drinks, such as chocolate (7) and tea (8), are inversely related to cardiovascular disease. Overall self-reported consumption of flavonoids has also been consistently shown to be associated with reduced mortality due to cardiovascular disease (9,10), with the inverse relation between flavonoid consumption and hypertension/blood pressure confirmed by urinary analysis (11). Intervention trials have also confirmed these cardiovascular benefits, although much of this research has focused on flavanols, most often derived from cocoa (12). Taken as a whole, these trials suggest a consistent beneficial effect of cocoa flavanols on cardiovascular variables, including inflammatory biomarkers related to atherosclerosis, insulin resistance, lipid profiles, blood pressure, and vasodilation/endothelial function (9,13,14). Meta-analyses suggest that these effects are achievable with an optimal dose of 500 mg/d flavanols (14) and within 2 h of consumption (15).

Naturally, cardiovascular variables are inextricably linked to cerebral blood flow and metabolism in the brain, and therefore, they covary with the incidence of age-related cognitive decline, dementia, and mood disorders (16–20). In line with this, the consumption of tea, polyphenol-rich foods, fruit and vegetables, and total amounts of flavonoids have been shown to be associated with protection against, or slowed progression of, cerebrovascular diseases, such as strokes, and neurologic disorders, including dementia (21–28), and cognitive impairment/decline in elderly populations (22,24,29–31).

Intervention studies

**Flavonoids.** A wealth of evidence in animals has shown that flavonoid-rich foods, extracts, and individual polyphenols can beneficially modulate cognitive function, typically in older and impaired rats that are suffering cognitive decrements as a consequence of age, brain insults, or induced pathologies. For instance, flavanol and anthocyanin-rich fruits and berries and single flavonoids consistently improve or prevent declines in all aspects of memory performance (32–35). These effects can also be accompanied by morphologic changes to structures relevant to memory, for example, by promoting or protecting neurogenesis and synaptic plasticity in the hippocampus (32,33,36,37).

There is less evidence pertaining to humans in this regard, and flavanols have attracted the most attention. Two studies, 1 using transcranial Doppler (38) and the other using fMRI (39), demonstrated that both high (900 mg) and low (150 mg) doses of flavanols administered for a short period (2 wk and 5 d, respectively) can increase cerebral blood flow in healthier older and younger adults. In terms of direct modulation of cognitive function, 2 placebo-controlled, crossover design studies demonstrated benefits in the cognitive function of healthy adults as measured by a “cognitive demand battery” (40) and by spatial memory and choice reaction time tasks (41) after single doses of flavanol-enriched drinks and chocolate, respectively. In the case of the latter study (41), 2 visual tests also demonstrated that the high-flavanol treatment increased the speed of detecting the direction of motion of stimuli and led to an improvement in visual contrast sensitivity. The evidence with regards longer-term administration is less persuasive. Although in 1 study cocoa flavanols (990/520 mg/d) reduced insulin resistance, blood pressure, and lipid peroxidation and improved performance on 2 of 3 cognitive tasks (42), several studies failed to elicit any improvements in cognitive function. For instance, there were no cognitive improvements reported after administration of chocolate products containing 750 mg/d flavanols for 6 wk in participants ≥60 y (43) and no improvement on a working memory task after 30-d administration of 250 or 500 mg cocoa flavanols to middle-aged volunteers, although steady-state visually evoked potentials assessed by electroencephalography (EEG) were modulated in a manner interpreted by the authors as showing increased neural efficiency (44). Finally, a recently reported study also assessed the effects of both single doses and 30-d administration of 250/500 mg/d cocoa flavanols in healthy middle-aged participants and failed to demonstrate any substantial cognitive effects, although the subjective psychological state of the participants was improved in terms of “calmness” and “contentedness” (45).

For other sources of flavanols, 2 studies reported modulation of brain function by single doses of the tea catechin epigallocatechin gallate (EGCG) in young participants, as assessed by EEG (40) and near-infrared spectroscopy (46), and 2 studies reported modulation of activity after green tea extracts (containing flavanols and other components) as assessed by fMRI after a single dose (47) and EEG after 16-wk administration (48) in the absence of cognitive/mood effects.

Two placebo-controlled trials also demonstrated improved subjective climacteric symptoms in menopausal females after 3-mo (60 mg/d) (49) and 6-mo (200 mg/d) (50) administration of Pycnogenol, a proanthocyanidin-rich pine bark extract. Memory function and oxidative stress markers were also improved after 3-mo administration of 150 mg of Pycnogenol to elderly participants (51). However, 2 studies assessing the effects of high doses (~1000 mg/d) of a similar extract, Enzogenol, for 5 or 6 wk did not show any improvements on a range of psychological or physiologic variables in healthy adults (52) and sufferers from mild traumatic brain injury (53).

Anthocyanins, the glycosides of anthocyanidins, are typically consumed by humans in brightly colored berries, grapes, fruits, and colored vegetables (54), and their consumption has been consistently shown to improve cognitive function in animal models of aging and neuropathology (55). However, human research has been methodologically inadequate, with the small sample sizes and control conditions in the 3 studies that involved the repeated administration

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3 Abbreviations used: COX, cyclooxegenase; EEG, electroencephalography; CREB, cAMP response element binding protein; EGCG, epigallocatechin gallate; eNOS, endothelial NO synthase; ER, estrogen receptor; ERK, extracellular signals-related kinase; iNOS, induced NO synthase; P38K, phosphatidylinositide 3-kinase; PKB, protein kinase B; SIRT or SRT, silent information regulator two protein; TOR, target of rapamycin.
of anthocyanin and phenolic acid–rich juice drinks rendering the modest findings essentially uninterpretable (56–58). Only 1 study assessed the effects of single doses with a comparatively healthy sample of 35 participants in a crossover design study, but this study found no substantial effects on a restricted range of cognitive, mood, and satiety measures (59).

**Phytoestrogens.** A range of flavonoids and other polyphenols have estrogenic properties as a consequence of their direct binding affinity at mammalian nuclear estrogen receptors (ER) α and β. These polyphenols include the isoflavones genistein and daidzein and the structurally related coumestans, such as coumestrol, all of which are particularly richly expressed in the legume (Fabaceae) family. Other phytoestrogens include lignans, which are converted by intestinal bacteria to estrogenic metabolites, such as enterodiol, enterolactone, and secoisolariciresinol (60), as well as a number of ubiquitous flavones, including, in order of descending potency, kaempferol, quercetin, apigenin, and luteolin, several flavanones, including naringenin, and several stilbenes, including resveratrol (61).

The phytoestrogens typically exhibit weak ER binding, with a preference for ERβ, but exist at comparatively high concentrations (62). They can also act as agonists or antagonists, the latter via blockade of the activity of endogenous ligands, can bind to and inhibit the activity of androgen or estrogen metabolizing enzymes, such as aromatase (60), and can exert non-receptor–binding properties, potentially via modulation of signaling cascades related to estrogenic functioning (63). This multiplicity of effects means that the activity of the phytoestrogen can be highly variable and can depend on a number of factors, including tissue type, receptor subtype, dose, and the amount of the endogenous ligand present (60).

Most animal and human research in this area has tended to use the “soy” isoflavones coumestrol, genistein, and daidzein, found most abundantly in the soya bean plant (Glycine max), although these compounds are actually expressed in substantial amounts by a wide range of plants, including the majority of the legume (Fabaceae) family, and other food crops, such as the *Coffea* (coffee) genus.

Daidzein itself can be metabolized by intestinal bacteria into the more potent estrogenic compound equol, although only 30–50% of individuals are equipped with the requisite intestinal flora to accomplish this, with the percentage rising in cultures that eat more daidzein-containing plant products (64).

Soy isoflavones have been shown to exert a number of effects relevant to general health, including upregulation of endogenous antioxidant activity, and modulation of cardiovascular and immune function variables, and the mechanisms underlying carcinogenesis, including the inhibition of aberrant mitogenic activity related to ERα activity (65). These factors may underlie the epidemiologic observations of a potential relation between soy-isoflavone consumption and breast cancer (66–69), in particular those cases that feature the overexpression of ER and progesterone receptors within tumor cells (67). Soy-isoflavone supplementation has also been associated with decreased bodyweight and improved glucoregulation in postmenopausal non-Asian women (70), decreased menopausal symptoms (71), and improved vascular variables, such as endothelial function (72) and blood pressure (73).

With regards effects on brain function, soy isoflavones have been shown to improve memory performance in intact and ovariectomized rodents (74–77). In humans, they have been shown to substantially improve the physical, but not psychological, symptoms of premenstrual syndrome (78), improve ratings of quality-of-life in postmenopausal women (79), decrease both follicle-stimulating hormone and luteinizing hormone, and increase circulating concentrations of 17β-estradiol (80). In terms of neurocognitive function and mood, a review by Lamport et al. (81) identified 13 methodologically adequate studies that assessed the effects of isoflavone treatments on cognitive function. Seven of these studies demonstrated modest beneficial effects of supplementation. The authors interpret the findings as being inconclusive, although it is interesting to note that most of the studies involved postmenopausal females (81). In addition, Greendale et al. (82) found that isoflavone consumption could be related to either cognitive benefits or decrements, depending on the stage of menopause. Taken with results showing that several years after ovariectomy primates lose the ability to respond to either 17β-estradiol or equol in terms of hippocampal receptor binding and activity (83), this would seem to confirm a theorized “window of opportunity” for hormone replacement therapy and isoflavone supplementation. This factor may also contribute to the somewhat equivocal nature of the human intervention literature. Interestingly, in the review by Lamport et al. (81), 3 of the 4 studies that included males in their samples demonstrated cognitive benefits, including a demonstration of selective isoflavone-related benefits on a spatial working memory task on which females usually outperform males (84). Evidence actually suggests both cognitive benefits and deficits in male rats after phytoestrogen consumption (74), so taken together, it would be interesting to see more data from male humans and, indeed, younger premenopausal females.

**Nonflavonoid polyphenols.** Consumption of the stilbene resveratrol (3,4′,5-trihydroxystilbene) is associated with numerous protective health benefits in animal models. These include increased longevity (85), anti-inflammatory (86) and antiviral (87) properties, and a protection against cancer and tumorogenesis (88), cardiovascular disease (89), and atherosclerosis (90). In terms of cognitive performance, a number of in vivo studies in rodents have demonstrated preserved behavior and cognitive performance in aged rats after laboratory induced brain insults (91–94).

Despite resveratrol being deemed safe for human consumption, the huge body of literature surrounding this compound mainly deals with in vitro and in vivo/ex vivo animal research. Human intervention trials include 2 studies that
demonstrated that both acute (30, 90, and 270 mg/d) (95) and chronic (75 mg/d) (96) administration of resveratrol improved peripheral vasodilation as measured by flow-mediated dilation in obese individuals. Similarly, 2 studies assessed the effects of resveratrol on cerebral blood flow and cognitive function. In the first, single doses of 250 and 500 mg of resveratrol increased cerebral blood flow in the frontal cortex in a dose-related manner during cognitive task performance, whereas both doses also increased deoxygenated hemoglobin concentrations, indicative of increased oxygen utilization, as measured by near-infrared spectroscopy (97). In the second study, 250 mg of resveratrol combined with 20 mg of the alkaloid piperine had exactly the same effect on cerebral blood flow variables (98). In neither study was cognitive function or mood modulated.

Interestingly, the less intensively researched stilbene pterostilbene, a dimethylether analogue of resveratrol, has greater oral bioavailability in plasma and brain tissue (99,100), has more potent cancer cell inhibitory effects (101) and exerts greater cognitive/neuroprotective and cellular signal transduction effects in rodent models than resveratrol (100). To date, its efficacy has not been tested in humans.

The curcuminoid curcumin is formed from 1 cinnamic acid starter unit with 2 malonyl-CoA units (1). It is responsible for the bright yellow color of the Indian spice turmeric (Curcuma Longa) and has been used for centuries within the Ayurvedic system of medicine in the treatment for a host of ailments, including inflammation (102).

Despite a number of clinical trials that generated results suggesting potential utility in treating inflammation, cardiovascular disease, and diabetes (103), little research has focused on brain function. Curcumin is associated with an attenuation of cognitive deficits in rodent models of Alzheimer’s disease (104,105). Epidemiologic data also suggest a relation between better cognitive performance and curry consumption in humans (106). However, to date, the results of only 2 small studies in humans, both assessing the effects of curcumin in Alzheimer’s sufferers, have been reported, and neither demonstrated symptomatic or biochemical efficacy (107,108).

Although the evidence from intervention studies of direct modulation of human brain function by polyphenols, as reviewed above, is currently weak, it is notable that this may primarily reflect the limited research in this area and the methodologic limitations of the small number of studies. Certainly, the results of the many animal studies investigating brain function, the epidemiologic evidence, and the evidence of cardiovascular benefits after polyphenol interventions all point in the direction of a specific benefit to central nervous system function associated with polyphenol consumption. Naturally, this raises the question of how these benefits are achieved.

**Polyphenols: mechanisms of action relevant to human brain function**

The notion that polyphenols owe their beneficial effects on physiologic variables and disease states to direct antioxidant effects has been replaced by a growing consensus that their effects are more likely attributable to direct interactions with cellular signal transduction pathways (109). Comparatively, low bioavailability certainly precludes antioxidant properties as a key factor in their neuroprotective and neuroenhancing effects (110). Naturally, access to the brain is a necessary precondition for exerting direct effects on brain function, and it is notable that, after dietary supplementation, flavonoids and their metabolites were shown to be present in the brain at the low concentrations (10–300 nM) that would be sufficient for them to exert pharmacologic effects at receptors and within signaling pathways (33). This ability to cross the blood–brain barrier seems to be dictated by both the lipophilicity of the individual molecule and its interactions with transmembrane proteins, such as the multidrug resistance permeability glycoproteins, which transport molecules across cellular membranes and the blood–brain barrier (36,111). Interestingly, several classes of flavonoids become more concentrated and are retained in neural tissue longer than they persevere in plasma (32). In addition to direct interactions with neurotransmitter receptors, polyphenols can also interact directly within diverse downstream neuronal and glial protein kinase and lipid kinase signaling cascades, such as the ubiquitous MAPK and phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB) and target of rapamycin (TOR) signaling cascades (36,112,113). These ubiquitous signaling cascades transduce signals received either by receptors spanning the membrane of the cell or in its cytosol via a chain reaction in which a series of kinases activate each other in turn, by phosphorylation, ultimately leading to either the removal of proteins attached to transcription factors in the cytosol, allowing them to translocate to the nucleus, or direct interactions with transcription factors already in the nucleus. The summed and interacting activity in multiple signaling pathways dictates the response of the cell to environmental or stressor-related information, for instance, by modulating the activity of transcription factors, such as NF-κB or CREB (cAMP response element binding protein), in turn leading to a wide range of cellular responses, including cell proliferation, apoptosis, and the synthesis of growth factors, such as neurotrophins, and inflammatory molecules, such as induced NO synthase (iNOS), cytokines, and cyclooxygenase-2 (COX-2). Overactivity or dysregulation within these signaling pathways is implicated in the pathogenesis of cardiovascular and neurodegenerative diseases and cancers (36,114). Recent research demonstrates that a range of flavonoids bind directly to individual protein kinases within these cascades, modulating their phosphorylation state and thereby modifying the activity and outcome of the signaling pathway (115). The effects of polyphenols within the central nervous system can then be primarily attributed to interactions with signal transduction pathways that both have a direct effect on cognitive function and an indirect effect via the attenuation of inflammatory processes and the enhancement of cerebrovascular function.
**Cognitive function.** Flavonoids modulate cellular signaling pathways most directly by interacting with a range of receptors to which neurotransmitters and other signaling molecules bind. Dietary flavonoids have been shown to interact with estrogen (see above), GABA<sub>A</sub> (116), adenosine (117), opioid (118), nicotinic (119), and receptor tyrosine kinases. The latter include receptors such as tyrosine-related kinase B, which responds to key neurotrophins such as brain-derived neurotrophic factor (120). Evidence also suggests the existence of brain plasma membrane and nuclear binding sites that have yet to be fully characterized but for which flavonoids have a high affinity (121). In terms of downstream effects within signal transduction pathways, flavonoids may exert beneficial effects on cognitive function by activating components of the extracellular signal-regulated kinase (ERK) signaling cascade that leads to increased activity of transcription factors, such as cAMP response element binding protein, with a resultant increase in the expression of neurotrophins, such as brain-derived neurotrophic factor. This can ultimately lead to an increase in the synaptic plasticity and long-term potentiation that underlies long-term memory consolidation. Alternatively, upregulatory interactions within the PI3K/PKB pathways may lead to increased activity in the nutrient-sensing TOR signaling pathways or the increased expression of endothelial NO synthase (eNOS) and therefore NO synthesis (36,112).

**Cerebrovascular function.** The modulation of “good” NO synthase activity via eNOS leads to a brain-specific increase in local blood flow, angiogenesis, and neurogenesis, all of which may contribute to neuroprotection and neuronal repair in the face of aging and insults (36,112). Modulation of eNOS synthesis also underpins the ability of flavonoids to improve peripheral vascular variables, such as endothelial function, blood pressure, and platelet aggregation, and in turn beneficially modulate gross cerebral blood flow and hemodynamic responses to neural activity. Notably, polyphenol-related increases in vasodilation, cerebral blood flow, and NO synthesis are also implicated in hippocamal angiogenesis and neurogenesis, processes that are implicated in learning, memory, and neuroprotection (36,122).

**Neuroinflammation.** Flavonoids can also selectively inhibit deleterious overactivity in signaling pathways. The beneficial effects of flavonoids on cardiovascular health and cancer has been attributed to an attenuation of the inflammatory cascades implicated in the pathogenesis of cardiovascular disease and tumorogenesis (123). As with other tissues, short-term inflammation of brain tissue can be a beneficial, natural, defensive reaction to injury, infection, stroke, and toxins. However, sustained neuroinflammation as a consequence of continued activation of microglia, the primary immune cells of the nervous system, and their subsequent sustained release of damaging proinflammatory mediators may contribute to the neuronal damage associated with neurodegenerative diseases, such as multiple sclerosis, Parkinson’s disease, and various dementias, as well as the deterioration in cognitive function seen with aging (124).

Evidence suggests that disparate flavonoids, including a number that act as integral parts of the human diet, such as flavonols and isoflavones and their metabolites, can suppress the neuroinflammatory activity of microglia by inhibiting each stage of the inflammatory signaling process in activated glia: inhibiting the activity of proinflammatory transcription factors, the release of cytokines, the generation of reactive oxygen species, and the synthesis or activity of iNOS, COX-2, and lipoxygenase and the resultant overproduction of NO, PGs, and leukotrienes. These effects in turn may be related to the ability of polyphenols to modulate the activity of multiple components of a range of neuronal and glial signaling pathways, including the individual components of MAPK and other kinase pathways involved in the inflammatory and apoptotic responses described above (114,123). As an example, polyphenol-rich acai fruit pulp was shown to attenuate inflammatory responses in microglial cells and protect neurons from induced stress by inhibiting both MAPK and TOR pathways (125,126).

Evidence does suggest that interactions with the components of these signaling pathways is dependent on the exact structure of the polyphenol molecule in question, for instance, in terms of the number and location of hydroxyl groups or the nature of specific bonds. This means that different polyphenols will exert markedly different cellular effects (36). However, a recent meta-analysis of 25 human intervention studies that included an assessment of the effects of a wide range of flavonoids on inflammatory biomarkers noted a substantial reduction in TNF-α associated with consuming flavonoid-rich foods or supplements and several flavonoid subgroups (127). Similarly, oral administration of 40 mg of resveratrol to humans was shown to lead to reductions in oxidative stress variables, the expression of proinflammatory kinases in mononuclear cells, the activity of the proinflammatory transcription factor NF-κB, and concentrations of related downstream proinflammatory cytokines (128), and increase nuclear factor-E2-related factor-2 activity with a consequent upregulation of endogenous antioxidant variables (129). The attenuation of neuroinflammation could certainly underpin the epidemiologic evidence of protected brain function in elderly humans.

**Metabolites and the role of the gut microbiota.** Of course, there is a proviso to the above interpretation of the mechanisms of action of polyphenols. In reality, only a small percentage of polyphenols are absorbed intact in the upper gastrointestinal tract, with the vast majority hydrolyzed to aglycones and/or metabolized by gut microbiota before absorption and additional phase I/II metabolism (130,131). Emerging evidence suggests that the gut microbiota makes a substantial contribution to the eventual palette of circulating, potentially bioactive, compounds, including the generation of entirely new compounds, such as a range of conjugated lactones and simple phenolic acids (131–133).
To add more complications, the eventual palette of polyphenol derivatives will depend on the individual’s own gut microbiota profile, and the consumption of differing polyphenols will change the makeup of the microbiota itself (131,134). Given that the composition of the gut microbiota plays multifarious roles in organism development, digestion, the bioavailability of bioactive nutritional components, immune function (130), and even brain development and behavior (135,136), it has been suggested that many of the health benefits of polyphenols may be related to modulation of gut microbiota populations (130).

The extensive metabolism of polyphenols does bring into question the relevance of much of the in vitro research that has informed the above. This research has often used naturally unattainable concentrations of polyphenols and parent compounds rather than the many metabolites that would be present in vivo (114). Similarly, it is not possible to attribute the many in vivo biologic effects, including those seen in human trials, directly to the consumed parent molecules rather than their metabolites. However, it is notable that the conjugated forms of polyphenols and the phenolic metabolic derivatives created by the gut microbiota typically have similar or greater bioactivity than the parent compounds, including within signaling pathways (133,134,137).

It is also notable that the gut microbiota play an important evolved role in the tailoring of a wide range of exogenous dietary nutrients for increased bioactivity, including essential amino acids and several vitamins (138). Given that polyphenols formed part of the human diet throughout evolution, it would be surprising if the microbiota community did not fulfill similar roles for this group of compounds.

**Why Do Polyphenols Affect Human Brain Function?**

The ecologic roles of polyphenols and other phenolics

Although they are the dominant life form on earth, covering and harvesting the light from the vast majority of the terrestrial surface of the planet, plants have 1 major problem: they are autotrophs, rooted in place and feeding themselves by synthesizing the complex organic compounds that they require for life, from simple inorganic molecules that they are autotrophs, rooted in place and feeding themselves by synthesizing the complex organic compounds that they require for life, from simple inorganic molecules that they synthesize, using the energy of light. This process of photosynthesis enables plants to produce the carbohydrates, fats, and proteins that are the building blocks of all living tissues. In addition, plants also produce a wide range of secondary metabolites, which are compounds that are not essential for growth and development but serve a variety of roles in defense, communication, and reproduction.

These secondary metabolites, also known as phytoalexins, are produced in response to stress and injury, such as mechanical damage or infection by pathogens. They are often released into the rhizosphere, the area around the root system, where they can act as antibiotics against soil-borne microorganisms. In the case of herbivores, which consume plant tissues, polyphenols can act as antifeedants, disrupting the digestion of the plant tissue or attracting and deterring predators. In addition, polyphenols have antioxidant and anti-inflammatory properties, which can help to protect the plant from oxidative stress caused by environmental factors such as UV radiation or extreme temperatures.

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phenolic secondary metabolites (155,156), many of which are exuded into the soil in response to specific signals, for instance, from symbiotic and pathogenic microbes, and a range of abiotic stresses, such as nutrients and the mineral status of the soil, temperature, and water stress (157). Among the full range of phenolic structures in the rhizosphere, the flavonoids play specific benign roles in the management of microbial symbionts, attracting mutualist bacteria and triggering the germination of fungal spores. These microbes then colonize the roots, facilitating the uptake of nitrogen, water, nutrients, and minerals, in return for photosynthates, such as carbohydrate, from the plant (158,159). These relations can be quite specific; in the legume (Fabaceae) family, the plant grows root nodules for the bacteria to inhabit, with individual flavonoids managing relations with individual species of Rhizobium bacteria (148,157). Alternatively, they can be more general; for instance, ubiquitous flavonoids, such as querectin and rutin, manage the relation of the plant with the arbuscular mycorrhizal fungi that contribute to plant phosphorus uptake by colonizing the surface of roots in the vast majority of plants (148,159). Naturally, the protective reaction of the plant to infection by underground pathogenic fungi and bacteria is identical to that seen aboveground and often also involves the synthesis of phenolics.

At a greater distance from the plant, the soil still harbors a variety of phenolic secondary metabolites that were exuded by roots or leached from leaves and fallen plant tissue. For instance, flavonols and tannins protect against nematodes (148) and molluscs (160), whereas a number of phenolics in the soil inhibit the growth and germination of competitor plants. Many flavonoids exert these allelopathic properties by interfering with the signaling and transport of the hormone auxin in the roots of competitors or acting as prooxidants, among many other potential mechanisms of action.

In all of these many and varied interactions, individual phenolics are often multifunctional, multitalented compounds.

The plant signaling roles of polyphenols. Although a huge research effort has been directed toward understanding the effects of polyphenols on mammalian cellular signaling, we know very little about the potential endogenous signaling roles that polyphenols play in their home plant. Nevertheless, a number of strands of evidence suggest that flavonoids may play multiple “primary” plant signaling roles. For example, it is notable that these chemicals are often synthesized locally by specialized cells or tissues at specific times dictated by developmental processes or stressors. They are often then actively transported long distances in the plant, becoming “integral components of the plant signaling machinery,” functioning both as signaling molecules in their own right and by interfering with the activity of other signaling molecules (161). As an example, flavonoids play recently established cellular signaling roles in pollen germination and dormancy, the sanctioning of the transmembrane movement of the hormone auxin, the nodulation process that allows the colonization of root systems by symbiotic bacteria, and the lignification process, and they partake in intraplant communications (161,162). As an example of the latter, a recent study investigating gene expression showed that the allelopathic effects of juglone, a phenolic derivative that stu¨nt the growth of plants in the vicinity of walnut trees by preventing root elongation, are related in major part to the ability of juglone to interfere with MAPK cellular signal transduction and gene expression within the jasmonic acid, abscisic acid, and gibberellic acid hormonal pathways of the encroaching recipient plant (163). A putative wide-ranging signaling role for flavonoids is also strongly supported by their detection, along with their synthetic enzymes, in the nucleus of plant cells, suggesting that they may function in endogenous gene transcription (164,165).

Similarities in hormonal stress signaling between plants and animals. Both plants and animals have well-developed autocrine, paracrine, and endocrine hormonal systems. In plants, the typical net effect of hormonal signaling will include modulation of the synthesis of numerous secondary metabolites, including flavonoids and other polyphenols. The key hormones upregulating defensive reactions are the oxylin “jasmonates,” jasmonic acid, and its conjugated and hydroxylated derivatives, such as methyl-jasmonate and jasmonoyl-isoleucine. The jasmonates are formed from the eighteen-carbon PUFA α-linolenic acid when it is released from cellular glycerides by lipases in plant tissue as a consequence of biotic and abiotic stressors and subsequently metabolized via the octadecanoid pathway, which commences with oxidation by lipoxygenase enzymes (166,167). The jasmonate derivatives then travel to other cells and degrade cellular proteins [jasmonate ZIM (zinc-finger expressed in inflorescence meristem)-domain proteins] that are suppressing the transcription factors that dictate the many physical and chemical plant responses to stressors, unleashing the appropriate defensive reactions (168,169).

Therefore, although jasmonates are necessary for the induced synthesis of protective secondary metabolites, they represent only 1 strand of a complex interplay between hormones. These include salicylic acid, abscisic acid, auxin, and ethylene, as well as ubiquitous signaling molecules, such as NO. These chemicals are synthesized in varying quantities in response to differing stressors. For instance, jasmonic acid predominates in the response of a plant to insect herbivory and abiotic stressors, jasmonic acid and ethylene combined are synthesized in response to necrotrophic pathogen attack, and salicylic acid is induced after infestation with biotrophic pathogens (170). The synergistic and antagonistic crosstalk between these hormones and signaling molecules fine tunes the response of the plant to match the stressor (171), leading to complex stress-specific patterns of gene transcription (168,170). As an example, whereas abscisic acid tends to work synergistically with the jasmonates (167), salicylic acid has an antagonistic relationship, in which 1 of its functions is to downregulate the jasmonate pathways, in effect switching the response of the plant from a short-term synthesis of phytochemicals suited to

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abiotic stressors and herbivory and redirecting it toward a longer-term “immune” response that will be more suited to resisting biotrophic and viral pathogens. Naturally, the converse is also true, with the jasmonates working to down-regulate the salicylates (167,172) and salicylic acid modulating the gene expression associated with other hormones, such as auxins (175).

These plant hormonal systems bear some striking similarities to animal systems (139). The most straightforward example is the role that the “plant” hormone abscisic acid plays in the mammalian nervous system. This genetically conserved signaling molecule is synthesized and operates via the same signaling pathways and conserved genes across plants and animals (176,177). In both taxa, it guides the cellular reactions to abiotic stressors, such as heat and light. It also has wide-ranging functions within the mammalian immune system and modulates insulin release from the pancreas and the proliferation of stem cells. A number of these effects are related to its modulation, including upregulation, of the synthesis or function of PGs (176,178).

No less intriguingly, the oxylipin pathways that synthesize jasmonates in plants and the PGs in mammals are also genetically conserved orthologs (179). In mammals, this pathway leads to the synthesis of eicosanoids from the twenty-carbon PUFA arachidonic acid, which is released from cellular phospholipids by phospholipases and metabolized via pathways very similar to those seen in the formation of jasmonates in the plant, but in this case featuring both lipoxygenase and the COX-1 and COX-2 enzymes (180). In keeping with this shared heritage, the jasmonates in plants and the PGs in animals are closely related in structural (Fig. 2) and functional (179) terms. In animals, PGs and the COX enzymes contribute to the modulation of blood flow via the dilation or constriction of blood vessels and by determining the aggregation, or stickiness, of blood platelets, and they contract or relax bronchial and smooth muscle. Directly in keeping with the plant roles of jasmonates, they also govern a number of responses to stressors, including the regulation of inflammation and immune function, and the response to wounding (180,181). Therefore, the most striking difference in the response of the plant jasmonate and animal PG systems to stressors is primarily seen in the end product, with the animal response most often associated with inflammation or immune system activation and the plant response, although including many analogous cellular responses, also being typified by the additional synthesis of secondary metabolite chemicals (182).

A wealth of emerging evidence suggests that each of the key hormonal mediators of secondary metabolite synthesis in plants can have reciprocal effects within mammalian tissue. Modulation of endogenous abscisic acid function in humans is being investigated from a therapeutic point of view with respect to a variety of diseases (176), and both the jasmonates and salicylates exert multifarious, potentially beneficial effects on mammalian cancerous cells in a manner that resembles their innate activity in plant cells (182). The activity of salicylic acid in terms of inducing tumor cell apoptosis, in particular, resembles the hypersensitive response, or programmed cell death, that it coordinates in response to pathogens in plants (182). Salicylic acid itself, particularly in the structurally close form of acetylsalicylic acid, or aspirin, has also attracted a huge amount of research over many decades. Its activity in mammals systems can be seen as a direct extension of its role within plants (183), with many of its actions involving interactions with mammalian cellular processes that are directly conserved or very similar to those seen in plants. The most readily appreciable example is the inhibition of COX activity by acetylsalicylic acid, which leads to reduced PG synthesis, and the celebrated anti-inflammatory properties of aspirin (182). This can be seen as a direct reflection of the analogous antagonistic effect of salicylic acid within the jasmonate pathways that induce secondary metabolite synthesis in plants, in much the same way that the upregulation of PG function by abscisic acid in the animal mirrors its synergistic relation with the jasmonates in the plant (180).

**Is the modulation of brain function by polyphenols a consequence of unintended “cross-kingdom” signaling between plants and humans?**

Interference by the phenolic plant hormone salicylic acid in the synthesis of PGs is clearly an example of unintended

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**Figure 2** Conserved signaling molecules: the plant jasmonate hormones (e.g., jasmonic acid) and the orthologous mammalian eicosanoids/PGs (e.g., PGE₅) showing the analogous PUFA precursors. Reproduced from reference 139 with permission.
“cross-kingdom” signaling by the phytochemical. The concordance in hormonal signaling between plants and humans is not surprising given the similarities in cellular function exhibited by the two taxa. Both plants and humans inherited in excess of \~3000 genes from their last common ancestor (184,185). This shared genetic inheritance comprises a large core of common “housekeeping” genes that are essential for survival and that dictate a raft of processes, including central metabolism, genome replication and expression, and the many commonalities that we see in the molecular and physiologic properties of plant and animal cells (186). This does raise the question as to the extent to which this common genetic inheritance may underlie the mammalian physiologic effects of polyphenols.

The notion that some form of cross-kingdom signaling underpins the effects of polyphenols has been advanced previously (161). For instance, Howitz and Sinclair (187) proposed the concept of “xenohormesis” whereby animals and fungi read the chemical evidence of environmental stress from the plants on which they feed in the form of stress-induced secondary metabolites, such as polyphenols, which then trigger a hormetic response in the consuming organism (hormesis being the process by which a small amount of a potential stressor, such as a toxin, physical exercise, or starvation, enhances functioning by triggering an adaptive stress response). A favored example here is the ability of several polyphenols to interact in vitro with the mammalian sirtuin genes [silent information regulator two protein (SIRT or SRT)] that regulate a number of transcription factors (e.g., p53, NF-κB, PPARγ, and PPARγ coactivator 1-α) that play key roles in stress responses, cellular differentiation, and metabolism (188). Sirtuins ultimately modulate a range of critical metabolic and physiologic processes, including cellular metabolism, survival and aging, stress resistance, inflammation, immune function, and endothelial function. It is notable that much of the huge interest in the polyphenol resveratrol was sparked by the observation that it extends the lifespan of yeast and animals, such as the fruit fly (Drosophila melanogaster) and roundworm (Caenorhabditis elegans), in a manner similar to caloric restriction, apparently via a hormetic response mediated via the activation of sirtuin genes (189,190).

The notion of xenohormesis as an adaptive mechanism is intriguing, but as an explanatory tool of human/phytochemical interactions, it falls foul of the law of parsimony. It seems more likely that any beneficial effects are simply predicated on the similarities between signaling pathways in the taxa, with polyphenol/signaling interactions driven by the function the phytochemicals are trying to fulfill for their home plant, including their endogenous roles as signaling molecules within the plant itself. As described above, a wealth of evidence suggests that flavonoids owe their bioactivity in mammals to interactions with the transduction and signaling pathways that mediate cellular responses to stressors, such as the ubiquitous protein and lipid kinase signaling cascades (e.g., MAPK, PI3K/PKB, and TOR) (36,112,191). Many of the key components of cellular signaling are included in the common ancestral endowment of cellular housekeeping functions, and the same signaling cascades often play identical roles in mediating the cellular responses of plants and animals to stressors (192,193). Plants have a particularly rich complement of \( \geq 1000 \) kinase genes (194), and a striking 2.5% of the genome of the model plant Arabidopsis thaliana is given over solely to the \( \geq 600 \) genes that express the kinase subfamily of receptor-like kinases. These receptors bridge the cellular membrane and transduce extracellular signals into cellular secondary messenger MAPK signaling cascades. Phylogenetic evidence shows that the entire kinase superfamily and the specific receptor-like kinases evolved in a unicellular ancestor of both plants and animals. Beyond this point, the majority of plant receptor-like kinases remained specific to serine/threonine, whereas the majority of animal receptors evolved specificity to tyrosine (i.e., receptor tyrosine kinases) (195). Similarly, animals and plants also share the MAPK enzyme signaling pathways. Indeed, these are highly conserved across all eukaryotes (192,193), with plant MAPKs being most closely homologous to the ERK MAPK subfamily in mammals (196). In addition, plants also possess conserved PI3Ks and their interacting PKBs (197). The Arabidopsis genome also encodes 39 distinct, conserved “animal” AGC kinases (named after the member kinases: cAMP-dependent protein kinase A, cGMP-dependent protein kinase G, and phospholipid-dependent protein kinase C). These kinases modulate the activity of other intracellular second messengers, including cAMP, cGMP, and phospholipids, and make their own independent (to MAPK) contribution to stress signaling and engage in crosstalk interactions with the MAPK signaling pathways (194,198).

This extended protein kinase family plays analogous cellular transduction and signaling roles in plants and animals. One particularly pertinent example is the TOR kinase signaling pathway, a crucial growth-regulating cellular system that simultaneously collects information on stressors, nutrients, and internal energy states from multiple extracellular and intracellular inputs, including from the PI3K/PKB pathways. In benign conditions of nutrient and energy availability, TOR upregulates a vast range of energy-consuming activities, such as metabolism, cell proliferation, and the translation of mRNA, ultimately dictating a wealth of developmental and metabolic processes by regulating other key kinases (199,200). The TOR kinase pathways are, again, highly conserved, having originated before the last common eukaryotic ancestor, and both their function and core molecular components, including the upstream PI3K/PKB pathway, are shared across the cells of nearly all eukaryotic clades (201). For instance, in plants, TOR signaling pathways play a large part in regulating growth, with the TOR gene expressed most abundantly in rapidly growing and dividing tissues. As with mammalian cells, TOR also mediates plant cellular responses to stressors and directs autophagy, possibly via interactions with plant hormones, such as abscisic acid (202). This pathway comes sharply into focus for humans when we consider that, in mammals, TOR signaling
is aberrantly upregulated in cancer (203) and dysregulated in obesity and diabetes, contributing to insulin resistance and cardiovascular disease. It also plays a key role in the process of cellular ageing (200). Conversely, TOR inhibitors may provide novel treatments for these same diseases, and it has previously been observed that the life-extending properties of caloric restriction are mediated by reduced TOR signaling (200).

In mammals, the functioning of the TOR signaling pathway is also modulated by a wide range of flavonoids and other polyphenols. For instance, ubiquitous catechins, such as EGCG, and flavonols, such as quercetin, attenuate cell proliferation and tumorogenesis by inhibiting both PI3K/PKB and TOR signaling in mammalian cells, potentially by binding to the ATP binding sites of the kinase proteins (204,205). Similarly, quercetin, curcumin, and resveratrol were all shown to promote protective autophagy-mediated cell death in mammalian cancer cells via TOR inhibition (206). Resveratrol may also have its beneficial effects on cellular senescence, cell growth, glucose homeostasis, and cardiovascular function through its inhibition of TOR signaling via both sirtuin-dependent and -independent mechanisms. In the latter case, established mechanisms include modulation by resveratrol of PI3K/PKB signaling upstream of TOR and direct interactions with the TOR kinase itself (207,208). Similarly, anthocyanin-rich berries protect the hippocampus in irradiation models of aging by inhibiting TOR and attenuating inflammation, oxidative stress, and a loss of protective autophagy (209,210). Conversely, anthocyanin and flavanol supplementation in rodents activates TOR, promoting “growth” in terms of synaptic plasticity in cognition-relevant brain regions (112). This last finding suggests that flavonoids can exert bi-phasic, homeostatic effects within this single system.

Although the TOR and MAPK pathways and the other protein kinase pathways are heavily involved in directing gene transcription across taxa in response to a wide variety of stressors, the ultimate downstream products of this signaling effort differs between animals and plants. In animals, 1 consequence of activation will be the synthesis of COX and the PGs, whereas in the plant, the same signaling pathways will lead to the expression of lipoxygenase and the synthesis of jasmonates. In the case of plants, this, in turn, will ultimately lead to the synthesis of a raft of protective secondary metabolite chemicals (211,212), with crosstalk with other hormones shaping the specific response via their own interactions with the plant kinome (196,213,214).

In general, across taxa, stress/immune signaling and gene transcription is a complex affair that is typified by an abundance of feedback and feedforward loops that closely regulate the expression of signaling molecules and transcription factors. As an example, in plants, the expression of salicylic acid genes is essential for many stress responses, but an overaccumulation of salicylic acid is toxic to the cell, so feedback and feedforward loops between the components of pathways responsible for synthesizing salicylates, other hormones, and cellular factors carefully regulate its synthesis (175). Polyphenols and other phenolics represent the ultimate downstream products of a multitude of plant hormonal defense communications. They also travel within the plant, penetrate cells and cell nuclei (101), and may exert a raft of independent signaling properties. Therefore, they represent ideal candidates to both feedback within their own cellular synthetic pathways and interact with the cellular signaling pathways in neighboring and distant cells.

An interesting analogy can be drawn with the “vitamins”, which are typically plant-derived primary metabolite chemicals that play broadly analogous essential cellular roles when consumed by humans. The synthesis of several of these compounds cannot only be induced by a variety of stressors in the plant, but they can also self-regulate their own synthesis, preventing wasteful or damaging overexpression via direct feedback interactions within their own biosynthetic pathways (215). The possibility that polyphenols, and other secondary metabolites, interact with their own synthetic pathways in this manner has simply not been investigated in plants to date. However, it is clearly a possibility that the modulation of animal stress signaling pathways by polyphenols may simply echo the role of the phytochemicals within the genetically conserved or analogous signaling pathways within their own plant. In this context, it would make absolute sense for the polyphenol products of stress signaling to provide 1 of the many endogenous signals that contribute in the plant to the activity of the global stress, nutrition, and energy-sensing TOR kinase pathways. This activity may then be transferred directly after consumption into modulation of the conserved PI3K/PKB and TOR pathways in animals. Similarly, viewed in the context of the conserved nature of the jasmonate and PG synthetic pathways, the inhibition of the enzyme COX2 in animal tissue by a wide variety of flavonoids (114) can also be interpreted as an attempt by the flavonoids to inhibit lipoxygenase enzymes within the orthologous plant jasmonate pathways. This interaction within the plant may be part of the complex crosstalk between the antagonistic jasmonate and salicylate hormone systems (as suggested with regards salicylic acid itself) (180) or it may simply be feedback or feedforward within and across the jasmonate system.

It is also notable that the net effect of flavonoids within several animal signaling pathways is modulation of the synthesis of NO (114). This ubiquitous signaling molecule, which represents a key downstream product of signaling cascades in both taxa, plays diverse modulatory roles in both the synthesis and function of jasmonates, salicylates, and ethylene (216,217). Therefore, it is intrinsically tied to secondary metabolite synthesis pathways in plants. Any effects on NO synthesis in animal tissue may well also represent an attempt by the phytochemicals to interact with the various pathways dictating NO synthesis in their home plant.

Returning briefly to the yeast/animal sirtuin genes that fostered so much interest in the polyphenol resveratrol, it is also the case that, whereas mammals express 7 distinct, structurally similar sirtuin genes (SIRT1 to SIRT7), of which SIRT1 is the best characterized, plants also possess...
2 conserved sirtuin genes that are homologs of the human equivalents (188). These are expressed in the reproductive tissue (SRT1) and throughout plant tissue (SRT2), respectively (218). The specific roles of these sirtuins in plants are poorly understood, but evidence does suggest that they play roles in plant development via hormonal signaling and in the hypersensitive immune response (218). Intriguingly, the SRT2 plant homolog was shown to suppress salicylic acid–dependent defense against pathogens, suggesting that it may play a role in the mutually inhibitory crossstalk between salicylic acid and the jasmonates (219). Once again, this would seem to suggest that any effects of polyphenols on sirtuin-mediated stress responses in animals may simply reflect an echo of polyphenol/sirtuin interactions in the plant.

Finally, just to confirm that polyphenols do exert direct cross-kingdom signaling effects as a consequence of the role they are trying to play in their home plant, it is interesting to note that 1 of the few plant signaling roles of flavonoids that has attracted any research attention is their modulation of transmembrane auxin movement. Flavonoids accomplish this by binding to multidrug resistance permeability glycoproteins in cell membranes, with the consequence that they inhibit auxin efflux from the cell. The same transporters are genetically conserved in plants and animals, and they form the established target underlying the potential medicinal utility of flavonoids in terms of reducing drug efflux in mammalian multidrug resistant cells (162,220).

Is the modulation of brain function by polyphenols a consequence of intentional cross-kingdom signaling?

Although the examples immediately above suppose that any effects of polyphenols in mammals are simply a consequence of the close similarities in plant and animal signal transduction pathways, a second class of interactions would seem to be predicated on intentional cross-kingdom signaling by plants, albeit, in this case, that the consuming animals are not the intended target.

The phytoestrogens. The most obvious example here is the benign part that flavonoids play in the evolutionarily ancient symbiotic relations of the plant with both the bacteria and fungi that colonize the rhizosphere, facilitating the uptake of nitrogen, water, nutrients, and minerals. The most ancient of these relations, that between plants and arbuscular mycorrhizal fungi, is ubiquitous across all plant lineages, whereas the more specialized relations between nitrogen fixing rhizobial bacteria and plants arose at a later date. Both relations work via the same mechanisms. Flavonoids released into the rhizosphere are detected by microbial receptors, such as the bacterial nodulation D protein. The flavonoids either attract bacteria by chemotaxis or stimulate the germination of fungal spores in the soil. The plant then perceives the presence of the symbiotic fungus or bacteria through the chemical emission by the microbe of lipocholesteroligosaccharide nod or myc (standing for nodulation and mycorrhizal, respectively) factors that bind to and activate lysine motif receptor kinases in the plant (221). Several iterations of this autoregulatory feedback ultimately govern the population level of microbes and the amounts of hyphal branching within the root system. In the legume (Fabaceae) family, this process also results in the creation of specialized root nodules designed specifically to accommodate the bacterial colony (148,157). In a similar manner, a number of flavonoids are synthesized in response to the bacterial “quorum-sensing” signaling molecules that direct the population size and activity of bacteria in the rhizosphere. These flavonoids bind to bacterial receptors in a positive feedback mechanism that directs both bacterial behavior and flavonoid synthesis (159).

The most salient point here is that both the bacterial and fungal receptors involved in these processes are estrogen-like receptors that bear a striking similarity to mammalian ERα and ERβ in terms of the palette of chemicals and ligands that they recognize and to which they respond. They also exhibit the same ligand concentration-dependent activity, they co-occur with chaperone proteins in their unactivated state, and the nature of their gene transcription effects are similar (222,223). These microbial receptors also bind mammalian estrogens, such as 17β-estradiol (223). Indeed, it has been suggested that many of the proteins within the respective plant and microbe signaling pathways are orthologous (224) and that the bacterial estrogen-like receptor, the nodulation D protein, is a partial ortholog of the mammalian ER (225) (although this is disputed in reference 226). Certainly, the key structural elements that dictate the binding of phytoestrogens to mammalian ERs, for instance, the aromatic ring with a hydroxyl group corresponding to the C3 position in the 17β-estradiol ring system (61,223), also dictate the binding of the same compounds to microbial estrogen-like receptors (223). It is also notable that all of the key flavonoids that exert estrogenic effects in humans also take part in the rhizosphere plant/microbe estrogen-like receptor interactions described above (222,223,227). Therefore, the estrogenic effects of these phytoestrogens are predicated on an unintended transfer of the intended cross-kingdom plant/microbe signaling, predicated on the similarity in the cellular signal transduction equipment possessed by microbes and mammals.

Defense against microbial pathogens. Of course, the ability to disrupt quorum-sensing and estrogen-like receptor signaling in pathogenic microbes may be a useful defense mechanism (227), and it is notable that several antimicrobial phytoalexins with more restricted distributions, such as kievitone, phaseollin, and resveratrol, also have estrogenic properties (228). However, the TOR pathways may provide a better example of intentional cross-kingdom signaling in the defensive disruption of microbial signaling. The TOR kinase signaling pathway itself was originally identified in the 1990s when researchers were trying to discover the mechanism of action of a potent antifungal chemical produced by bacteria found 20 years previously in a soil sample from Easter Island (also known as Rapa Nui). They named...
the antifungal macrolide molecule rapamycin, and after many years of research, identified the targets of rapamycin as novel protein kinases that they named “target of rapamycin”, as well as a specific binding protein (FK binding protein 12) within the TOR pathway (229). To the fungus, the inhibition by rapamycin of the TOR pathway signals a lack of environmental nutrients and prevents protein synthesis and cellular proliferation, thereby inhibiting growth and handing an advantage to the rapamycin-synthesizing bacteria.

It also transpired that all of the components of the TOR pathway targeted by rapamycin are conserved in fungi, animals, and plants (201,230). If we accept that the bacteria evolved the synthesis of rapamycin as an antifungal strategy, then any effects of rapamycin in mammals would represent a cross-kingdom transfer of the intended cross-kingdom inhibition of TOR signaling in fungi. Rapamycin certainly has numerous useful medicinal applications for humans, all of which are predicated on TOR pathway inhibition. It started life in the late 1990s as an antifungal treatment, but it soon became apparent that its major use was as an immune suppressant and antiproliferative that could be used to prevent organ rejection after transplant surgery.

Recent research has also demonstrated that rapamycin has a number of properties in common with TOR-inhibiting polyphenols, such as EGCG, quercetin, and resveratrol, including the ability to increase longevity and protect against cancer in mammals (231). Conversely, the synthesis of the same polyphenols is upregulated by biotic stressors, including fungi, and they all exhibit antifungal properties (232,233). Unfortunately, as yet, we do not know whether these antifungal properties are predicated on interference with TOR signaling. However, this does raise the possibility that the cross-kingdom kinase signaling roles of many polyphenols may simply reflect an unintended transfer of their intended role as antimicrobials, in the same manner as rapamycin, due to the conserved nature of the TOR pathways.

Conclusions
The foregoing presents a prima facie case that the modulatory effects of polyphenols within human cellular signal transduction pathways, and therefore their beneficial effects on cardiovascular and brain function, are predicated on a cross-kingdom transfer of the signaling role that these phytochemicals play, either endogenously within their own plant or exogenously as they perform ecologic roles related to the management by the plant of its symbiotic and pathogenic microbial neighbors.

Because of a vast research effort over the past decades, we now know a great deal about the interactions of polyphenols within mammalian signal transduction pathways. In contrast we know very little about their interactions within the orthologous plant (and to a lesser extent microbe) signaling pathways. But why is this important? Elucidating the ecologic and endogenous signaling roles of phytochemicals is obviously of interest from a plant science perspective, but beyond that a fuller understanding of these endogenous signaling roles must also inform the analogous mammalian research. Refocusing just a small fraction of the huge research effort directed at polyphenol/mammal interactions toward the analogous systems in plants may well offer an exponential increase in our understanding of the potential of these phytochemicals regarding human health. Added to this, the plant research would be substantially cheaper and, in some cases, less ethically contentious.

It is also notable that the physiologic effects and mechanisms of action of the many metabolites created by the gut microbiota and phase I/II metabolism of polyphenols have been largely overlooked in favor of concentrating on the parent compounds. Given that many of these metabolites, including a number of ubiquitous simple phenolic acids, are found naturally in plants, it seems reasonable to suggest that plant models might make a reasonable starting point for rectifying the imbalance in this literature.

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Hypercholesterolemia Induces Adipose Dysfunction in Conditions of Obesity and Nonobesity

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ABSTRACT

It is well known that hypercholesterolemia can lead to atherosclerosis and coronary heart disease. Adipose tissue represents an active endocrine and metabolic site, which might be involved in the development of chronic disease. Because adipose tissue is a key site for cholesterol metabolism and the presence of hypercholesterolemia has been shown to induce adipocyte cholesterol overload, it is critical to investigate the role of hypercholesterolemia on normal adipose function. Studies in preadipocytes revealed that cholesterol accumulation can impair adipocyte differentiation and maturation by affecting multiple transcription factors. Hypercholesterolemia has been observed to cause adipocyte hypertrophy, adipose tissue inflammation, and disruption of endocrine function in animal studies. Moreover, these effects can also be observed in obesity-independent conditions as confirmed by clinical trials. In humans, hypercholesterolemia disrupts adipose hormone secretion of visfatin, leptin, and adiponectin, adipokines that play a central role in numerous metabolic pathways and regulate basic physiologic responses such as appetite and satiety. Remarkably, treatment with cholesterol-lowering drugs has been shown to restore adipose tissue endocrine function. In this review the role of hypercholesterolemia on adipose tissue differentiation and maturation, as well as on hormone secretion and physiologic outcomes, in obesity and non–obesity conditions is presented. Adv. Nutr. 5: 497–502, 2014.

Introduction

According to the WHO, coronary heart disease (CHD) has been the leading cause of death for the past decade and was responsible for 11.2% of all deaths in 2011. Hypercholesterolemia, or high blood cholesterol concentrations, refers to cholesterol carried by non-HDL lipoproteins and is 1 of the most recognized factors in the development of CHD. Thus, total plasma cholesterol concentrations ≥5.2 mmol/L or LDL-cholesterol concentrations ≥2.6 mmol/L are positively associated with the development of atherosclerosis and CHD. In adipose tissue, the presence of high circulating concentrations of LDL was shown to impair TG clearance and to generate other detrimental effects. However, the effects of hypercholesterolemia (HCE) in tissues other than blood as well as the systemic cross-talk between tissues are not completely understood.

Recent animal studies showed that, in liver, a major organ involved in cholesterol metabolism, HCE can cause hepatocyte dysfunction, fibrosis, and induction of the development of early stages of nonalcoholic steatohepatitis. Taking this into consideration, it is important to address the effects of HCE on other major organs of cholesterol metabolism and storage, such as adipose tissue.

In humans, adipocyte cholesterol concentration can reach up to 0.5% of total lipids, and adipose tissue constitutes the largest cholesterol pool in our body. It has been established that obesity leads to hypertrophied adipocytes due to excess TG and cholesterol accumulation. This, in turn, results in abnormal cellular cholesterol distribution. As a result, decreased plasma membrane (PM) cholesterol followed by increased fluidity has been observed in these cells. Overall, these features triggered by TG and cholesterol overload are hallmarks of dysfunctional adipocytes. Nevertheless, little attention has been paid to the obesity-independent effect of HCE on adipose function.

In recent animal studies in which weight and fat composition were not altered, HCE diets resulted in hypertrophied adipocytes and abnormal adipose function. In tissues other than blood as well as the systemic cross-talk between tissues are not completely understood. Recent animal studies showed that, in liver, a major organ involved in cholesterol metabolism, HCE can cause hepatocyte dysfunction, fibrosis, and induction of the development of early stages of nonalcoholic steatohepatitis. Taking this into consideration, it is important to address the effects of HCE on other major organs of cholesterol metabolism and storage, such as adipose tissue.

In humans, adipocyte cholesterol concentration can reach up to 0.5% of total lipids, and adipose tissue constitutes the largest cholesterol pool in our body. It has been established that obesity leads to hypertrophied adipocytes due to excess TG and cholesterol accumulation. This, in turn, results in abnormal cellular cholesterol distribution. As a result, decreased plasma membrane (PM) cholesterol followed by increased fluidity has been observed in these cells. Overall, these features triggered by TG and cholesterol overload are hallmarks of dysfunctional adipocytes. Nevertheless, little attention has been paid to the obesity-independent effect of HCE on adipose function.

In recent animal studies in which weight and fat composition were not altered, HCE diets resulted in hypertrophied adipocytes and abnormal adipose function.
It is estimated that >90% of Americans do not consume sufficient dietary vitamin E, as α-tocopherol, to meet estimated average requirements. What are the adverse consequences of inadequate dietary α-tocopherol intakes? This review discusses health aspects where inadequate vitamin E status is detrimental and additional vitamin E has reversed the symptoms. In general, plasma α-tocopherol concentrations <12 μmol/L are associated with increased infection, anemia, stunting of growth, and poor outcomes during pregnancy for both the infant and the mother. When low dietary amounts of α-tocopherol are consumed, tissue α-tocopherol needs exceed amounts available, leading to increased damage to target tissues. Seemingly, adequacy of human vitamin E status cannot be assessed from circulating α-tocopherol concentrations, but inadequacy can be determined from "low" values. Circulating α-tocopherol concentrations are very difficult to interpret because, as a person ages, plasma lipid concentrations also increase and these elevations in lipids increase the plasma carriers for α-tocopherol, leading to higher circulating α-tocopherol concentrations. However, abnormal lipoprotein metabolism does not necessarily increase α-tocopherol delivery to tissues. Additional biomarkers of inadequate vitamin E status are needed. Urinary excretion of the vitamin E metabolite α-carboxy-ethyl-hydroxychromanol may fulfill this biomarker role, but it has not been widely studied with regard to vitamin E status in humans or with regard to health benefits. This review evaluated the information available on the adverse consequences of inadequate α-tocopherol status and provides suggestions for avenues for research. Adv. Nutr. 5: 503–514, 2014.
deficiency in animals (6–8). The EAR for vitamin E was set in humans on the basis of vitamin E depletion and repletion studies in men with the use of erythrocyte hemolysis as the biomarker (4). The RDA of 15 mg α-tocopherol was extrapolated from that value (4). According to the IOM (4), only α-tocopherol meets human vitamin E requirements because it was the only form that was demonstrated to reverse vitamin E deficiency symptoms in humans, as well as being the only vitamin E form maintained in plasma and tissues, as discussed below.

Plants make 8 different forms of vitamin E, 4 (α-, β-, γ-, and δ-) tocopherols and 4 (α-, β-, γ-, and δ-) tocotrienols. The phytol tail of natural α-tocopherol is in the RRR-conformation, whereas chemically synthesized α-tocopherol (all racemic) contains 8 stereoisomers [RRR-, RSR-, RSS-, and RRS- (the 2R- forms) and SRR-, SRS-, SSR-, SSS-] (4). In the diet, α-tocopherol is found in foods such as nuts and seeds and in vegetable oils, such as wheat germ, sunflower seed, safflower, and olive. Judicious food choices allow consumption of 15 mg α-tocopherol daily (9). However, most Americans require supplements to attain these recommended α-tocopherol intakes (10). In a study assessing a biomarker of vitamin E status, Lebold et al. (11) found that individuals who are highly motivated and interested in their diets consumed nearly the recommended α-tocopherol amounts (4). However, surveys of vitamin E intakes of the general public found that 90% of men and 96% of women do not consume the EAR of 12 mg α-tocopherol per day (2). The 2010 Dietary Guidelines (12) did not emphasize that vitamin E is a relatively difficult nutrient to obtain from the diet. For example, a report from the USDA covering the period from 2000 to 2006 states that vitamin E intakes, measured as α-tocopherol equivalents (α-TEs), were “21.1 mg alpha-TE per capita per day in 2006, up from 19.5 mg alpha-TE per capita per day in 2000. The level of vitamin E has generally increased over the series with the highest level in 2006” (13). These intakes apparently exceed the RDA. This α-TE value, however, includes intakes of non–α-tocopherol forms, largely γ-tocopherol, in addition to α-tocopherol. The α-TEs were defined in the 1989 RDA (14) but were not used in the 2000 DRI for vitamin E (4); instead milligrams of 2R-α-tocopherol was defined as the vitamin E unit. α-TEs fell out of favor because non–α-tocopherols are rapidly metabolized, do not substitute for α-tocopherol, and cannot be metabolically converted to α-tocopherol by humans and therefore should not be included in measures of vitamin E intake (4). Thus, a key controversy to be addressed by the nutrition community is “What are the adverse consequences of inadequate dietary α-tocopherol intakes?”

α-Tocopherol deficiency and inadequacy

Vitamin E deficiency is seldom found in adults but is more frequently found in children, likely because they have limited stores and are growing rapidly, thereby allowing deficiency symptoms to be readily apparent. This section, therefore, emphasizes findings in children. It should be noted that there are some reports of vitamin E deficiency in adults. For example, after decades of inadequate vitamin E absorption due to short bowel syndrome, a 71-year-old man complained of neurologic abnormalities that were consistent with vitamin E deficiency and responded to vitamin E supplementation (15). Thus, vitamin E is required throughout the life span.

Deficiency symptoms in humans

In humans, severe vitamin E deficiency occurs as a result of genetic defects in the α-tocopherol transfer protein (α-TTP), causing the disorder ataxia with vitamin E deficiency (AVED) (16). The lack of functional α-TTP results in the rapid depletion of plasma α-tocopherol (17,18), thereby demonstrating that α-TTP is needed to maintain plasma α-tocopherol concentrations.

Fat malabsorption also leads to vitamin E deficiency. Examples of fat malabsorption include genetic defects in the microsomal TG transfer protein or in apoB (abeta- and hypobeta-lipoproteinemia, respectively) and fat-malabsorption syndromes, such as cholestatic liver disease or cystic fibrosis (19).

Human vitamin E deficiency symptoms include a progressive neurologic disorder, spinocerebellar ataxia, which occurs as a result of a dying back of peripheral nerves, specifically sensory neurons (20). As the vitamin E deficiency continues over time, the neurologic defects become so severe such that they result in ataxia (16). With progressing deficiency in humans, there is also muscle deterioration, and this deterioration can include the heart muscle. Vitamin E deficiency ultimately results in death. In severe vitamin E deficiency, cardiomyopathy was among the symptoms of a vitamin E–deficient child who died of hepatic and cardiac failure (21). Cardiomyopathy is also a symptom of vitamin E deficiency in some patients with AVED (16,20,22).

Vitamin E supplements in amounts well over 1000 mg/d have been prescribed for children with vitamin E deficiency. α-Tocopherol supplements are recommended because they prevent the further progression of the neurologic abnormalities, and in some cases reverse them. For example, when given before the onset of abnormalities, supplements prevented neurologic symptoms (23) and stopped the progression of myopathy in individuals with abetalipoproteinemia (24). Similarly, in children with AVED, vitamin E supplements improved symptoms and halted the disease progression (16).

Malnutrition. Vitamin E deficiency symptoms in humans have been sufficiently well characterized to allow detection of more subtle examples of vitamin E inadequacy. Examples of frank vitamin E deficiency due to low dietary intakes include children in India with severe malnutrition (25,26).

In addition to general malnutrition, severe vitamin E deficiency was recognized in those children because the specific neurologic abnormalities associated with vitamin E deficiency were detected. Vitamin E supplementation was initiated and was found to reverse the symptoms (25,26),
thereby confirming that the neurologic abnormalities were dependent on α-tocopherol status.

**Vitamin E inadequacy in children.** Assessing normal plasma α-tocopherol concentrations in children is complicated because α-tocopherol is transported in plasma lipoproteins and concentrations of cholesterol and lipoproteins, as well as of α-tocopherol, increase with age (27). An example in which circulating α-tocopherol concentrations did not reflect dietary intakes was illustrated in a study in which these values in adolescents and their parent or grandparent were compared. Although the estimates of mean ± SEM vitamin E intakes in adolescents (9.2 ± 0.2 mg α-tocopherol/d) were higher than those of the adults (8.4 ± 0.2 mg α-tocopherol/d), plasma α-tocopherol concentrations were lower in adolescents (17 ± 0.4 μmol/L, corrected for cholesterol concentrations) compared with adults (26 ± 0.6 μmol/L) (28). The values for plasma α-tocopherol concentrations in these adolescents were similar to those reported for healthy children in Tunisia (29) or Germany (30). These data emphasize the well-accepted finding that circulating α-tocopherol concentrations do not correlate very highly with dietary α-tocopherol intakes.

Given the close relation between circulating lipids and α-tocopherol, it is important to recognize that both variables may be decreased in malnutrition. Squali Houssaini et al. (31) studied control children compared with severely malnourished children in Morocco. They reported, “In severely malnourished children, albumin, cholesterol and low density lipoprotein (LDL) cholesterol, plasma selenium, vitamin E and zinc were low, whereas inflammatory proteins and triglycerides were high. These features worsened with essential fatty acid deficiency.” Their findings emphasize that malnutrition alters plasma lipid concentrations; thus, correction of plasma α-tocopherol for lipids may mask deficiency states. Decreased cholesterol concentrations were also observed in protein energy malnutrition (32). These cholesterol decreases were also associated with low circulating concentrations of α-tocopherol and increased inflammatory markers, such as IL-6. In contrast, Laryea et al. (33) suggested that the individuals they studied were active, normal Congolese village children and the low plasma α-tocopherol (mean ± SD: 7.3 ± 1.3 μmol/L) should be corrected for low lipids based on the observation that, when reported as tocopherol:lipid ratios, the vitamin E values were within the usual range for children. However, low plasma α-tocopherol concentrations (median: 7.33 μmol/L; range: 2.61–18.42 μmol/L) were also found in children with falciparum malaria infections compared with control children (median: 17.71 μmol/L; range: 6.48–28.08 μmol/L); both groups had similar α-tocopherol:cholesterol ratios [median (range): 4.61 (1.24–7.20) vs. 5.15 (1.80–8.92) μmol/mmol] because the children with malaria had depressed cholesterol concentrations (mean ± SD: 1.89 ± 0.62 vs. 3.47 ± 0.59 mmol/L in controls) (34). These data suggest that both malnutrition and infectious diseases can lower circulating cholesterol and its lipoprotein carriers.

Thus, correction of plasma α-tocopherol concentrations for lipids is not appropriate in cases in which circulating lipids are below normal concentrations.

Circulating α-tocopherol concentrations <12 μmol/L were defined by the IOM to be in the deficient/inadequate range for healthy adults (4). For comparison, European children in the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) study were reported to have mean ±SD circulating α-tocopherol concentrations of 23 ± 4.9 μmol/L (35), whereas pediatric reference intervals for circulating α-tocopherol from 1136 healthy U.S. children aged 7 to 17 y ranged from 11 to 30 μmol/L (36); and in U.S. children aged 7 mo to 9 y, values ranged from 12 to 40 μmol/L with a mean of ~20 μmol/L (37). Thus, the ranges of circulating α-tocopherol in healthy U.S. and European children were above the deficiency cutoff value of 12 μmol/L. However, there have been some reports of children in the United States with circulating α-tocopherol below this cutoff (38), suggesting low intakes. Indeed, some reports claim that dietary vitamin E intakes in U.S. children are generally below recommended values, except for those children taking supplements (39).

Taken together, these findings suggest that circulating α-tocopherol concentrations below the cutoff of 12 μmol/L are likely indicative of inadequacy if not frank vitamin E deficiency. Numerous reports worldwide have shown that such concentrations are frequently reported in children (Fig. 1). These low circulating α-tocopherol concentrations are caused by the combination of consumption of diets low in vitamin E, along with inadequate intakes of fat, protein, and calories. These latter dietary components are necessary for fat absorption and transport, which are required elements for vitamin E absorption and its lipoprotein transport, as reviewed elsewhere (40).

**Obesity and metabolic syndrome.** In contrast to malnourished children, many studies have shown that obese children...
do not have low plasma α-tocopherol concentrations; however, when their values were corrected for their circulating lipids, the α-tocopherol:lipid ratios were significantly lower than those in the control group because the obese children had elevated circulating cholesterol and TG concentrations (41–44). Vitamin E supplements in apparently adequately nourished obese children decrease oxidative stress markers (45), suggesting that obese children routinely consume inadequate amounts of antioxidants to prevent oxidative stress. Furthermore, it is likely that this increased oxidative stress is a consequence of chronic inflammation, which is seen secondary to obesity (46) and is a risk factor for other complications of obesity.

An extreme example is nonalcoholic fatty liver disease, which is a major cause of liver dysfunction and is increasing in children due to the increasing prevalence of obesity and type 2 diabetes. The severe negative effects associated with nonalcoholic fatty liver disease include progression to nonalcoholic steatohepatitis (NASH), liver cirrhosis, and ultimately liver cancer (47). Vitamin E supplementation decreases histologic evidence of NASH (48); therefore, supplementation has been tried in children with promising results (49). D’Adamo et al. (50) reported in obese children that 600 mg α-tocopherol daily doubled plasma concentrations from a mean (±SD) of 32.7 ± 1.5 μmol/L to 63 ± 14 μmol/L. The authors did not provide lipid-corrected values, but serum total cholesterol was, on average, 180 mg/dL (4.65 μmol/L) and TGs were 83 mg/dL (0.94 μmol/L). After 6 mo of vitamin E treatment, serum alanine aminotransferase decreased with vitamin E supplementation, indicating improved liver function. Supplementation also decreased urinary prostaglandin F2α, insulin, and fasting glucose concentrations, as well as their lipid profiles, and high-sensitivity C-reactive protein. It is unclear how many of these changes are due to the diet and behavior intervention rather than to vitamin E supplementation.

The findings in obese children raise the question as to whether the increased inflammation observed with obesity increases vitamin E requirements. Notably, interventions with vitamin E supplements in children (49) and in adults (48) with NASH had beneficial effects, especially with regard to serum alanine aminotransferase measures of liver dysfunction (51). In adults, Sanyal et al. (48) reported, “Vitamin E therapy, as compared with placebo, was associated with a significantly higher rate of improvement in nonalcoholic steatohepatitis (43% vs. 19%, P = 0.001)…” Taken together, these data suggest that obese children likely are consuming inadequate amounts of vitamin E, despite their apparently elevated circulating α-tocopherol concentrations.

To investigate whether antioxidant supplements could mitigate impaired inflammatory and antioxidant status, Murer et al. (45) studied overweight or obese children and adolescents (n = 44; mean ± SD age: 12.7 ± 1.5 y) participating in a lifestyle modification program, who were given daily antioxidants (vitamin E, 400 IU; vitamin C, 500 mg; selenium, 50 mg) or placebo for 4 mo. They then measured a number of variables, including the urinary vitamin E metabolite α-carboxy-ethyl-hydroxychromanol (α-CEHC). We previously proposed that the vitamin E metabolite could serve as biomarker of vitamin E adequacy because daily urinary α-CEHC excretion was reflective of adequacy when its excretion exceeded 1.39 μmol/g creatinine (11). Murer et al. (45) reported that the median urinary α-CEHC excretion in their obese and overweight children was low throughout the study in the placebo group [median (range) for baseline vs. postintervention:1.2 (0.01–2.9) vs. 1.2 (0.3–15.9) μmol/g creatinine], whereas in the antioxidant group it was low before supplementation but increased dramatically after antioxidant supplementation [1.8 (0.5–19.2) vs. 16.3 (0.01–81.2) μmol/g creatinine; P < 0.001 for intervention]. These data suggest that, despite apparently normal plasma α-tocopherol concentrations in the study participants, urinary α-CEHC excretion suggests inadequate vitamin E status. In support of this statement, vitamin E supplementation in these children also decreased F2-isoprostanes but not markers of inflammation (45).

The findings in obese participants emphasize that obesity does not necessarily reflect adequate micronutrient intakes, and vitamin E status may be inadequate for normal liver function in these individuals because they have increased oxidative stress. These findings are especially important because lipid peroxidation has been shown to cause dysregulation of liver lipoprotein secretion, which was prevented by increases in vitamin E intake in experimental animal studies (52,53). Taken together, these data emphasize the importance of adequate vitamin E status in obese individuals to maintain healthy liver function and potentially prevent the progression of fatty liver to more serious forms of the disease. They further raise the question of whether liver dysfunction is thus a symptom of vitamin E inadequacy.

**Circulating α-tocopherol concentrations as a biomarker of vitamin E status**

As is apparent from the previous discussion, circulating α-tocopherol concentrations are very difficult to interpret. In normal healthy adults who consume a variety of foods, including nuts, seeds, and whole grains, plasma α-tocopherol concentrations average ~20 μmol/L, whereas those individuals who consume supplements or fortified foods have concentrations that average ~30 μmol/L or more (11). However, as a person ages, plasma lipid concentrations also increase, and these increases in lipids also increase the plasma carriers for α-tocopherol, leading to higher circulating concentrations. However, abnormal lipoprotein metabolism does not necessarily increase α-tocopherol delivery to tissues.

The experimental findings in obese children described above highlight the difficulty in assessing vitamin E status by measuring only circulating α-tocopherol. Another example is in individuals with cholestatic liver disease, who have high circulating lipids. Their plasma α-tocopherol concentrations are apparently within normal ranges; however, their α-tocopherol to lipid ratios are low, and most important, tissue α-tocopherol concentrations are at deficient levels (54). Thus, if plasma lipids are elevated, then correction of
α-tocopherol for lipid concentrations is appropriate to assess adequacy. In this case, adequate values for α-tocopherol: lipid ratios should be similar to those in individuals with normal circulating lipid concentrations (55).

This close relation of plasma α-tocopherol to lipids has led some investigators, who evaluated poorly nourished children with low circulating lipids, to postulate on the basis of these ratios that the children’s α-tocopherol status was adequate. However, if both plasma lipids and α-tocopherol are abnormally low, then correction of circulating α-tocopherol concentrations for plasma lipids will yield a value indicating a normal α-tocopherol:lipid ratio. This assumption of adequate vitamin E status is likely invalid, because the low lipids reflect the inadequacy of the plasma carriers for delivery of vitamin E to tissues. Moreover, direct measurements of tissue α-tocopherol concentrations, or other surrogate markers of vitamin E status, have not been used to test this assumption. A normal circulating α-tocopherol:lipid ratio, which is caused by both low α-tocopherol and low lipids, reflects an adequate vitamin E status. The prevalence of stunting and anemia in malnourished children, who have limited intakes of both energy and micronutrients, suggests that these children lack important nutritional factors, including vitamin E (56).

Additional markers of inadequate vitamin E status are needed. Adipose tissue α-tocopherol concentrations have been used to assess vitamin E status (57–59). El-Sohemy et al. (58) reported that adipose tissue α-tocopherol concentrations were not well correlated with plasma α-tocopherol concentrations, but they did appear to reflect long-term vitamin E status. We found that in children suffering from severe burn injury, adipose tissue α-tocopherol concentrations rapidly (within 1 mo) become depleted, suggesting that this tissue can serve as an α-tocopherol storage site, releasing α-tocopherol upon increased metabolic demands (60). Additional studies are needed to evaluate intakes relative to tissue α-tocopherol concentrations and long-term health benefits.

Urinary α-CEHC may fulfill this biomarker role (11,61), but this marker of vitamin E status has not been widely studied with regard to vitamin E status in humans. Vitamin E metabolism is a hot topic and has been extensively studied with regard to non–α-tocopherol intakes in both humans and in experimental animals. Non–α-tocopherols are readily converted to their respective CEHC forms, even during α-tocopherol deficiency (62,63). Thus, hepatic vitamin E metabolism is a major regulator of the forms of vitamin E found in the body. The reader is directed to a recent review on this topic (64). Hypothetically, once liver α-tocopherol concentration reaches a threshold level, additional α-tocopherol will be metabolized; thus, plasma α-tocopherol reaches a “plateau,” whereas α-CEHC excretion increases exponentially (Fig. 2).

Anemia has traditionally been a marker of poor vitamin E status, but anemia in pregnant women in Bangladesh was not only associated with decreased plasma α-tocopherol concentrations but also with deficiencies of some other micronutrients (65), emphasizing that anemia is not necessarily caused by the lack of just 1 micronutrient. Thus, the multifactorial nature of nutritional status must be taken into account when evaluating adequacy, adding to the complexity of evaluating vitamin E status in free-living individuals.

**Consequences of low vitamin E status**

It is notoriously difficult to show adverse consequences of vitamin E deficiency in experimental animals, as well as in humans. In patients with cystic fibrosis, anemia and decreased erythrocyte survival (5) were widely accepted as signs of vitamin E deficiency until reports of neurologic abnormalities that responded to vitamin E supplementation were discovered (66–68). The neurologic abnormalities at the outset of vitamin E deficiency are so subtle that they are difficult to assess, but with progression of the deficiency they become readily demonstrable (69,70). This section of the review will therefore address health aspects in which inadequate vitamin E status is detrimental and where supplemental vitamin E has been shown to be beneficial for health, including pregnancy and neurologic diseases.

**Is vitamin E deficiency an important cause of spontaneous embryonic death?**

Vitamin E was discovered nearly 100 y ago because female rats fed a vitamin E–deficient diet resorbed their fetuses early in pregnancy (71); the cause of the embryonic failure has never been fully characterized. We investigated embryonic vitamin E deficiency in a vertebrate model, the zebrafish (Danio rerio), and discovered that α-tocopherol and α-TTP have critical roles in embryonic development. We based our research on the observation that α-TTP is expressed in the human yolk sac (72), that zebrafish embryos abundantly express α-TTP by 48 h postfertilization (hpf), and that α-TTP increases with oxidative stress in zebrafish embryos (73). We discovered that
α-tocopherol–deficient adult zebrafish could spawn and produce viable fertilized eggs, but within days the embryos suffered developmental impairment and increased mortality (74). The impaired brain formation in α-TTP knockdown zebrafish embryos raises the possibility that low vitamin E status has adverse events in early central nervous system development in other animals, including humans. Jishage et al. (75) showed that if the mother mouse did not express α-TTP and was not vitamin E supplemented, embryos (regardless of α-TTP status) developed neural tube defects and failed to come to term. Although the study by Jishage et al. focused on mouse maternal α-TTP deficiency, the embryonic phenotype and link to central nervous system development are similar to our findings in the zebrafish. In support of this notion, previous studies showed a clear association between maternal vitamin E status during gestation and cognitive function of the offspring in experimental animal models (76,77).

Importantly, we found that in the zebrafish embryo, α-TTP knockdown caused head malformation before 15 hpf (78). This phenomenon coincides with the timing for increased synthesis of highly peroxidizable lipids by the embryo, evidenced by increased gene expression in the head/brain of 2 FA elongase enzymes, elongation of very long-chain fatty acid (Elov4l)4 (79) and Elov5 (80). When we measured specific PUFA concentrations in zebrafish embryos between 24 and 72 hpf, we found that both α-tocopherol and DHA concentrations decreased in vitamin E–deficient embryos but not in control embryos. Moreover, arachidonic acid concentrations decreased 3 times faster in α-tocopherol–deficient embryos (21 pg/h) compared with vitamin E–sufficient E embryos (7 pg/h) (P < 0.0001) (81). At 36 hpf, vitamin E-deficient embryos contained double the 5-hydroxy-eicosatetraenoic acids and 7-hydroxy-DHA concentrations, whereas other detectable hydroxy-lipids remained unchanged (81). Thus, vitamin E deficiency during embryogenesis depleted both omega-3 and omega-6 FAs (DHA and arachidonic acid, respectively) and increased hydroxy-FAs derived from these PUFAs, suggesting that α-tocopherol is necessary to protect these critical FAs during development of the nervous system. Our studies show that the target zone that is most sensitive to α-tocopherol depletion is the head/brain/eye; without delivery of α-tocopherol, the brain fails to develop properly (78).

This absolute requirement for α-tocopherol by the zebrafish embryo takes place during a time analogous to the first 20 d of human embryonic gestation, a time during pregnancy before a woman knows she is pregnant. This time frame is 10–15 hpf for the zebrafish embryo (82), 9.5 d for rats (83), and 17–19 d for humans (84–86). Thus, the requirement for vitamin E very early in human pregnancy is analogous to situations of inadequate folic acid status.

The importance of α-tocopherol for preventing neural tube defects in humans can be surmised from studies in which multivitamins were compared with folic acid supplements. Specifically, folic acid supplements were not as effective in preventing neural tube defects as folic acid/multivitamin combinations, as shown in a review of 5 human trials (87). In a Hungarian trial to evaluate neural tube defects, the multivitamin contained 15 mg vitamin E along with other vitamins (88). The importance of vitamin E in preventing neural tube defects is emphasized by the findings from a study of neural tube defects and maternal micronutrient intakes, including 954 cases (300 with anencephaly, 654 with spina bifida) and 6268 controls (89). A decreased risk of spina bifida was associated with increased intakes of preconception supplements containing antioxidant vitamins E and C, as well as other micronutrients (89). The importance of vitamin E in the nervous system was also supported by a study in China that showed that higher maternal and cord blood α-tocopherol concentrations at birth were associated with improved cognitive function when the child was assessed at age 2 y (90). And conversely, low plasma α-tocopherol concentrations were associated with poorer cognitive function in patients with cystic fibrosis at diagnosis (91,92).

**Vitamin E in pregnancy.** The role of vitamin E in pregnancy is of increasing concern because it is clear that adequate nutritional status for the first 1000 d of life is necessary for subsequent adult health and well-being, given that stunting cannot be reversed after this critical window (93). Moreover, a study in Egypt emphasized that vitamin E is a key missing micronutrient in stunted children (56). The authors showed that 78.2% of stunted children were vitamin E deficient, with plasma α-tocopherol concentrations of 7.7 μmol/L compared with 14.1 μmol/L in control children (56). Fares et al. (94) reported that vitamin A, E, and D deficiencies were very common in very-low-birth-weight Tunisian neonates and were associated with pre-eclampsia (94). However, pre-eclampsia risk was not changed by vitamin E and C supplements in a number of studies in Western countries (95–99). The lack of benefit of vitamin E supplements in pre-eclampsia may be a result of the relative adequacy of vitamin E status of the women studied. For example, Poston et al. (99) reported that the circulating α-tocopherol:cholesterol ratios were >6 μmol/mmol in the placebo group and >9 μmol/mmol in the vitamin E and C supplement group; these ratios indicate that even the placebo group was well nourished with respect to vitamin E. Taken together, these data indicate that low vitamin E status may increase pre-eclampsia risk, but women with adequate vitamin E status do not benefit further from vitamin E supplements. The definitions of what is “low” and “adequate” vitamin E status are not clearly delineated and merit further research.

Worldwide, the adequacy of α-tocopherol status during pregnancy is unclear and not frequently measured, and thus the utility of vitamin E supplements in improving outcomes has been variable. In situations in which α-tocopherol status was documented to be low, vitamin E supplements had beneficial outcomes. For example, multivitamin supplements containing vitamin E reduced adverse pregnancy outcomes in HIV-positive women in Tanzania (100,101). However, by using a cutoff of <11.6 μmol/L for plasma α-tocopherol concentrations, the prevalence of low vitamin E...
status was 5.9% of nonpregnant women of reproductive age in the northern Persian Gulf region, leading the authors to conclude that most women had an adequate vitamin E status. Additionally, in a study in the United States (n = 9968; n = 4992 in the vitamin group and n = 4976 in the placebo group), where at baseline pregnant women were taking 22 IU vitamin E in a daily multivitamin (equal to the RDA), additional vitamin E supplements (400 IU) were not beneficial in reducing the risk of preterm births (102). By contrast, vitamin E supplements were associated with a decreased incidence of preterm births in a Hungarian population study (103). Although it is apparent that the vitamin E status of pregnant women must be adequate to successfully bear a child, these findings suggest that vitamin E supplements in excess of the RDA to adequately nourished women do not provide additional benefits.

**Neurologic disease and cognitive impairment with age.**

Given the importance of vitamin E in the developing nervous system and for the protection of peripheral nerves, as supported by studies in vitamin E–deficient humans and in experimental animals, it seems likely that vitamin E would also protect the nervous system with aging. There are some experimental data to support this hypothesis, especially with regard to Alzheimer disease. Vitamin E supplements were found to have benefit in slowing Alzheimer disease progression (104,105), but they did not seem to prevent Alzheimer disease occurrence (106). A recent meta-analysis found that patients with Alzheimer disease compared with cognitively intact elderly controls had significantly lower plasma α-tocopherol concentrations (P < 0.001) (107). Moreover, higher ventricular cerebrospinal fluid α-tocopherol concentrations, measured postmortem in 230 participants from the Religious Orders Study, were associated with a lower density of neuritic plaques and with higher performance on tests of perceptual speed measured before death (108). Furthermore, compared with cognitively normal individuals, patients with either Alzheimer disease or mild cognitive impairment had lower circulating concentrations of all forms of vitamin E and both disorders were associated with increased oxidized vitamin E (109).

In experimental vitamin E deficiency in mice, axonal degeneration was observed in the hippocampus, an important area for memory and cognition (110). The combination of vitamin E deficiency and α-TTP deficiency in mice caused atrophy and decreased branching of Purkinje neurons, which was associated with deficits in motor coordination and cognitive functions that were normalized upon vitamin E supplementation (111). Additionally in mice, impaired vitamin E delivery to the brain resulting from a knockout of the phospholipid transfer protein also resulted in increased memory impairment 1 wk after abeta25–35 peptide injection (112). This impairment could be prevented by vitamin E supplementation (112). These experimental findings are consistent with a report in elderly humans showing that a lifelong dietary pattern that results in nutrient intakes that provide increased circulating concentrations of vitamins B, C, D, and E is associated with a larger brain size (as assessed by MRI) and higher cognitive function (113).

Given the importance of vitamin E in protecting unsaturated FAs, it is not surprising that patients with Alzheimer disease have increased concentrations of circulating lipid peroxidation products (114). Importantly, phosphatidylycholine 16:0/22:6 (DHA-PC 38:6), which contains the highly oxidizable FA DHA, was identified as 1 of 10 phospholipids that were depleted in the plasma of human participants who went on to develop Alzheimer disease (115). By contrast, individuals who were in the top quartile of plasma DHA-PC concentrations among the Framingham Heart Study participants had a significant 47% reduction in the risk of developing all-cause dementia (116). Taken together, these findings suggest that vitamin E protects critical FAs in the brain from lipid peroxidation and that improved brain vitamin E status is protective for cognitive function. Interestingly, vitamin E supplements (300 mg daily for 615 d compared with 30 mg for 361 d) were found to double brain α-tocopherol concentrations in a study carried out in 2 terminally ill patients (117).

**Conclusions and Speculations**

This review evaluated the information available on the adverse consequences of inadequate α-tocopherol status. In general, plasma α-tocopherol concentrations <12 μmol/L are associated with increased infection, anemia, stunting of growth, and poor outcomes during pregnancy for both the infant and the mother. When low dietary amounts of α-tocopherol are consumed, tissue α-tocopherol needs exceed amounts available, leading to increased damage to target tissues. Hypothetically, these low α-tocopherol intakes in humans lead first to anemia because of the relatively rapid turnover of erythrocytes and their exposure to oxygen and their high iron contents. Further damage might be expected in other tissues with rapid turnover. Potentially, intestinal cells are spared because they are exposed to other dietary antioxidants, as well as to low oxygen concentrations. The nervous system is a special case because α-tocopherol is retained in the brain, likely as a result of brain expression of α-TTP (111). With continued extrahepatic tissue α-tocopherol depletion, peripheral nerves are at risk (5), likely due to their high PUFA contents compared with surrounding tissues. Sensory compared with motor neurons are likely more at risk because the information flow in sensory neurons is from the periphery to the brain, whereas in motor neurons the flow is in the opposite direction, potentially moving α-tocopherol toward the periphery. Severe, or perhaps chronic, vitamin E depletion ultimately decreases brain α-tocopherol, leading to damage and, in the elderly, cognitive impairment.

The adequacy of the middle range of α-tocopherol intakes is difficult to define. Plasma α-tocopherol concentrations between 12 and 20 μmol/L can be raised with increases in dietary intake, suggesting that hepatic α-TTP is not saturated. Studies in experimental animals suggested that hepatic α-TTP maintains circulating α-tocopherol, redistributing it among tissues.
and potentially allowing tissue α-tocopherol depletion (118). In this case, α-tocopherol returning from the periphery to the liver is not metabolized but is salvaged by hepatic α-TTP and returned to the plasma (119), where it could be taken up by tissues with lipoprotein receptors. This process tends to increase circulating α-tocopherol concentrations and normalize them at the expense of depletion of tissue α-tocopherol.

Hepatic α-tocopherol trafficking, disposition, and metabolism are not well understood or characterized. The well-known lack of correlation between dietary vitamin E intakes and circulating α-tocopherol [for examples, see (58,120,121)] in this middle range of intakes speaks to the efficiency of the regulatory controls governing circulating α-tocopherol concentrations. These processes serve to protect circulating lipids, which are readily oxidized and potentially exposed to higher oxygen concentrations, as well as reactive oxygen species and free metals. Here the special case of fatty liver disease is of interest because the progression of this disorder to more serious forms of the disease is dependent on oxidative damage to lipids (122), suggesting that inadequate vitamin E intakes may promote disease progression.

Supplements providing vitamin E intakes in excess of 100-fold dietary intakes increase plasma concentrations by ~2- to 4-fold above baseline values (123–126). The limitation on plasma concentrations appears to be a result of increased hepatic vitamin E metabolism and excretion, as discussed previously (40). Intakes of 12–15 mg α-tocopherol/d are sufficient in normal healthy adult individuals to provide adequate vitamin E status on the basis of the health benefits associated with these intakes (127).

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References


In humans, recent trials demonstrated that individuals with HCE and altered plasma lipid profile present disrupted adipokine secretion as well as elevated proinflammatory markers and other features related to adipose tissue dysfunction (14). Moreover, these effects were attenuated by using plasma cholesterol-lowering drugs such as 3 hydroxy-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (15) and ezetimibe, which binds to Niemann-Pick C1-like 1, inhibiting cholesterol absorption (16).

Cholesterol overload can also affect the expression of sterol regulatory element–binding proteins (SREBPs) through negative feedback (17). Reduced expression of SREBPs will result in reduced peroxisome PPARγ2 expression and a subsequent reduction of the downstream genes involved in adipocyte development (18–20). This pathway was confirmed by use of a PPARγ2 agonist, which recovered the adipocytic differentiation capacity of mouse adipose–derived stromal cells (mASCs) (17).

The purpose of this review is to put in perspective the new evidence on the obesity-independent effect of HCE in adipose tissue and contrast this information with the better recognized role of obesity on adipocyte dysfunction.

**Current Status of Knowledge**

**Obesity-dependent effects of cholesterol in the adipocyte.** Adipocytes have the unique characteristic of containing very low amounts of cholesterol in the esterified form (6). This differs from several cell types, including hepatocytes, adrenal cells, foam macrophages, and others (5,21). In fact, in only <6% of adipocytes is cholesterol stored in the esterified form (5). For this reason, it is important to study the dynamics of free cholesterol in the adipocyte.

In obesity, adipocyte TGs and cholesterol overload are common features, which usually translate into hypertrophied adipocytes (7–9). During obesity, cells present enlargement of PMs, generating PM cholesterol depletion and results in the activation of SREBP-2 (11). This transcription factor induces the production of intracellular cholesterol resulting in cholesterol overaccumulation in the lipid droplet, generating cholesterol imbalance, which leads to adipocyte stress (22). When PM cholesterol is depleted by the action of methyl-β-cyclodextrin (Chol: MbCD; cholesterol acceptor), this process results in activation of SREBP-2 (23) and insulin resistance (11). It is possible that this transcription factor plays a main role in the observed increased expression of adipocyte-derived secretion products including angiotensinogen, TNF-α, and IL-6 (9). Last, intracellular cholesterol accumulation by itself was shown to be toxic to several tissue cell types, including hepatocytes, smooth muscle cells, and cardiomyocytes (24,25).

**Cholesterol transport in the adipocyte.** Even though adipose tissue contains the largest cholesterol pool in the body (6), cholesterol synthesis in the adipocyte is very low compared with other cell types. Radiolabeled acetate precursor experiments have shown that cholesterol synthesis in adipose tissue only accounts for 4% of what is produced in the liver (26). Therefore, most of the cholesterol in the adipocyte is delivered by lipoprotein-mediated mechanisms. Interestingly, cholesterol can be delivered into the adipocyte through HDL scavenger receptor Bi (SR-Bi)–dependent (27,28) or –dependent mechanisms (29) and LDL receptor and oxidized-LDL scavenger receptor pathways (30,31). HDL-cholesterol removal by SR-B1 takes place in the caveolae, a specific plasma membrane lipid raft where SR-B1 binds HDL and extracts cholesterol ester (CE). Once in caveolae, CE can be internalized into an intracellular membrane compartment or taken up by HDL (32). In the SR-B1–independent mechanism, CE is transferred from HDL to a specific plasma membrane compartment via CE transfer protein (CETP), then apoE is secreted into this compartment and acquires CE. Finally, apoE loaded with CE is directed to the extracellular matrix and recaptured by the LDL receptor–related 1 protein (28). Another cholesterol source for the adipocyte is ox-LDL (30). This lipoprotein enters the adipocyte through the cluster of differentiation (CD) 63, SR-B1, or ox-LDL receptor 1 and is further proteolytically degraded (30). Taking into consideration the fact that most of the cholesterol derived in this tissue is from lipoproteins, it is certain that circulating lipoprotein concentrations have a strong effect on adipocyte cholesterol status.

**Obesity-independent effects of cholesterol in the adipocyte.** Hypercholesterolemia not only was correlated with adipocyte cholesterol but it also was shown to regulate adipocyte development (10–13,16). VLDL, LDL, and HDL were demonstrated to induce differentiation of 3T3-L1 cells into adipocytes, with LDL being the strongest stimulant (33–35). Hypercholesterolemia also leads to increased formation of ox-LDL, a lipoprotein that was shown to strongly inhibit adipocyte differentiation (36). For example, Chen et al. (37) cultured and differentiated 3T3-L1 cells into mature adipocytes by adding ox-LDL and evaluated the effects of this lipoprotein on adipokine secretion and endoplasmic reticulum (ER) stress markers. Interestingly, ox-LDL incubation led to a greater accumulation of cholesterol in 3T3-L1–derived adipocytes. This, in turn, resulted in elevated mRNA and protein levels of ER stressor markers: glucose-regulated protein 78 and CCAAT/enhancer binding protein (C/EBP) homologous protein. ER stress is followed by the unfolded protein response and leads to activation of both c-Jun N-terminal kinases and IκB kinase, and these proteins are mediators of inflammatory cytokine production. Cytokines, such as TNF-α, can cause neutrophil, macrophage, T cell, and other immune cell infiltration (38). Last, this process translates into increased secretion of visfatin and resistin (adipokines associated with increased risk of CHD and type 2 diabetes) (37).

Resistin is known to degrade LDL receptors in the liver and as a consequence increase plasma concentrations of LDL (39). However, whether this detrimental feedback cycle
between increased ox-LDL in circulation and resistin secretion from the adipose tissue occurs during physiologic conditions has yet to be elucidated. On the other hand, visfatin was shown to bind to the insulin receptor in the liver and induce insulin resistance (40). In addition, visfatin was also associated with increased atherosclerotic plaque rupture and secretion of proinflammatory cytokines IL-1β, TNF-α, and IL-6 in CD14+ monocytes (40).

In another important in vitro study, mASCs were treated with Chol:MbCD to mimic cholesterol overload. It was found that Chol:MbCD increased esterified cholesterol and had low effect in free cholesterol intracellular concentrations. This resulted in increased proliferation and reduced differentiation into mature adipocytes (17). In addition, plasma and protein concentrations of SREBP, a key transcription factor for adipocyte differentiation (41), and its downstream PPARγ2, a main transcriptional regulator of several adipocyte lipid storage genes (19), were upregulated. A summary of the obesity-dependent and obesity-independent effects of cholesterol in adipocytes is presented in Fig. 1.

**Adipose tissue responses to cholesterol challenges: animal experiments.** In past years, several studies elucidating the effects of diet-induced obesity and dyslipidemia have provided insight into the importance of cholesterol balance in adipose tissue. Nonetheless, it is remarkable that the effects of diet-induced HCE in adipose tissue were not investigated until recently. It was in 1979 when Krause et al. (42) found that adipocyte cholesterol in rats was elevated with HCE diets, and this finding was corroborated by multiple animal studies (13,43). The obese LDL receptor−deficient mouse is an animal model that develops insulin resistance and atherosclerosis when fed high-fat, high-carbohydrate diabetogenic diets (DD) (43). However, it was observed that the addition of 0.15% cholesterol into the DD resulted in more extensive atherosclerosis lesions than when mice were fed the same diet without this cholesterol challenge (13). Moreover, when compared with diabetogenic mice, the epididymal adipose tissue from mice fed the high-cholesterol DD presented hypertrophied adipocytes, increased concentrations of proinflammatory cytokines TNF-α and monocyte chemoattractant protein 1 (MCP-1) and significant increases in macrophage infiltration (13). Even though the cholesterol effect in this model was evident, the use of a DD could represent a confounding factor for the effect of cholesterol in this study. Nevertheless, this study provides strong evidence of obesity-independent physiologic changes induced by HCE in adipose tissue.

Guinea pigs share a very similar lipoprotein and cholesterol metabolism profile with humans, which makes them an excellent model to study diet-induced atherosclerosis (5,21,44). Obesity-independent, diet-induced HCE effects were tested (13). Cholesterol accumulation was greater in adipose tissue in guinea pigs fed a high-cholesterol diet (HCD). In addition, increases in proinflammatory cytokine (IL-2, TNF-α, MCP-1) concentrations in this tissue were observed. In agreement with these results, adipocytes from HCE guinea pigs presented increased macrophage infiltration, hyperplasia, and increased necrosis.

Last, in another study that evaluated the effects of HCE on systemic inflammation, pigs were fed an HCD (5% cholesterol) and an HCD with the addition of atorvastatin (HMG-CoA reductase inhibitor). The HCD resulted in the development of HCE with 20% higher concentrations of serum total cholesterol and LDL cholesterol. Following this trend, subcutaneous abdominal white adipose tissue (WAT) of these pigs presented increased infiltration of activated T lymphocytes and increased concentrations of proinflammatory cytokines (TNF-α, IFN-γ); and in agreement with other studies (4,42), they also presented hypertrophied adipocytes (12). Strikingly, when atorvastatin was added to the HCD, HCE was reduced and the detrimental effects generated by this condition in the subcutaneous abdominal WAT were attenuated (12). This was followed by decreases in lymphocyte infiltration in the subcutaneous abdominal WAT, decreased concentrations of TNF-α and IFN-γ, and reduced adipocyte hypertrophy. It is remarkable that these pigs presented only mild HCE with only 20% higher cholesterol plasma concentrations than normocholesterolemic pigs and still developed localized inflammatory responses in several tissues including WAT (12). These results emphasize how significant this condition is for the proper function and development of adipose tissue.

**Cross-talk between adipose tissue and other organs.** It is important to mention that in most of these animal studies,
adipose tissue inflammation and malfunction are associated with the development of atherosclerosis and liver disease. Currently, it is not clear which tissue is the first to trigger the detrimental proinflammatory feedback-loop cross-talk between tissues affected by HCE. However, the evidence points to the liver as the main organ involved in lipoprotein metabolism regulation (45). Hypercholesterolemic guinea pigs were shown to develop features of nonalcoholic fatty liver disease before showing any evidence of atherosclerosis (5). In LDL receptor–deficient mice, a high-fat HCD resulted in hepatocyte inflammation within 7 d (46). Hepatic serum amyloid A (SAA) and C-reactive protein (CRP) plasma concentrations were increased in several animal models fed HCE diets (13,45). SAA enhances lipoprotein-proteoglycan binding in vascular tissue, which can potentially be atherogenic (47). In addition, secretion of many proinflammatory cytokines is observed in liver during HCE states (12,13). This may contribute to the chronic low-grade systemic inflammation feedback between this tissue and adipose depots, exacerbating the development of CHD.

**Human adipose tissue response to HCE.** Much evidence from familial HCE trials in humans has validated the strong effects of this condition on the development of atherosclerosis and systemic inflammation (48–52). Adipose has been shown to be an exceptionally active metabolic and endocrine tissue involved in several pathologies (53–55). Because adipose tissue stores most of the cholesterol in our body, it seems logical to evaluate the effects of HCE in this tissue and its role in the development of atherosclerosis. Nonetheless, few human studies investigating the effects of HCE in adipose tissue have been conducted; therefore, this will be the focus of the following section.

An elegant study by Veilleux et al. (56) in 2013 investigated the relation between adipocyte hypertrophy and metabolic disorder in women. The authors reported that participants (n = 207) who presented hypertrophied omental adipocytes also had a higher total-to-HDL-cholesterol ratio. Notably, this was independent of fat composition and distribution.

Patients with CHD present altered adipokine secretion, increased concentrations of proinflammatory cytokines, and macrophage infiltration in multiple types of adipose tissue such as epicardial and omental (9,57). Although the relation of these anomalies with HCE is not completely clear, statins were shown to decrease and modulate these detrimental effects (57).

Moreover, a recent study in patients with isolated HCE may have provided stunning information about the impact of HCE on adipose tissue (14). In this clinical trial, 49 participants with isolated HCE were separated into 3 groups: placebo, statin, and statin + ezetimibe. CRP, FFAs, TNF-α, and adipokines (visfatin, leptin, and adiponectin) were measured in plasma after 12 wk of therapy. As expected, the combined therapy exerted a greater reduction in HCE. This, in turn, was followed by a decrease in the inflammatory biomarkers TNF-α and CRP. Unexpectedly, cholesterol-lowering therapy resulted in increased or normalization of adiponectin. This adipokine regulates many metabolic processes such as FA and glucose metabolism, and it is only secreted by adipose tissue (39). Concomitantly, leptin and visfatin were reduced by the statin + ezetimibe treatment. Recent findings have exposed visfatin as a proatherogenic adipose hormone (40). In addition, visfatin was recently linked with the progression of type 2 diabetes (58). Finally, leptin is a pivotal adipokine that regulates energy balance, appetite, and hunger. High leptin concentrations are common in leptin-resistant patients (59). The fact that cholesterol-lowering drugs had such a strong effect on adipose hormone secretion opens a new window on the importance of HCE in human adipose function.

**Conclusions**

Because HCE is commonly accompanied by obesity and hypertriglyceridemia, most of the studies on cholesterol accumulation in adipose tissue that used in vivo models involve the development of obesity. For this reason, it has beenhard to separate adipocyte dysfunction generated by the development of obesity and TG overload from the effect of cholesterol in these cells. Even under physiologic conditions, it is likely that these 2 factors are presented together; therefore, it is important to study their independent effects on adipocyte function. Strong evidence from in vitro models uncovered obesity-independent effects of cholesterol in adipocyte development and functionality. Small numbers of animal studies on this topic have also been performed. Still, enough evidence has pointed to adipose tissue as a main organ affected by HCE and to be pivotal in the development of atherosclerosis. It is now known that HCE generates cholesterol overload in adipocytes from multiple adipose tissue regions, thus indicating the importance of evaluating the effects of HCE in human adipose tissue. Clinical trials have supported that HCE without obesity or hypertriglyceridemia can impair adipose tissue endocrine function. It is of high importance to determine the main mechanisms by which adipose cholesterol accumulation impairs adipocyte function and development. Future research needs to focus on the effects of isolated HCE in adipose tissue hypertrophy and adipokine secretion. This will provide an understanding of the clinical and physiologic implications of these conditions on the development of chronic low-grade metabolic diseases in which adipocyte function is pivotal.

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**References**


responsible for the initial scientific and public interest in tomatoes as a health-promoting food. However, advances in lycopene science have revealed that the biologic function of lycopene is through a variety of plausible mechanisms because its metabolic products impact a variety of cellular processes (6,7).

There is a strong relation between tomato intake and blood concentrations of lycopene (8–10). Epidemiologic investigations have shown an inverse relation between plasma, serum, and tissue concentrations of lycopene and risk of CVD (11–15). These data led to the conjecture that lycopene is the primary reason that tomatoes are associated with favorable reductions in CVD and other health outcomes. Correspondingly, efforts to increase lycopene consumption have ranged from breeding tomatoes with higher amounts of lycopene to extracting and concentrating lycopene to be sold as a dietary supplement.

Considering the background and efforts to increase lycopene consumption, a major question that remains to be answered is whether delivering lycopene or tomato extracts rich in lycopene through a capsule is as effective as or more effective than consuming tomatoes, the primary food source of lycopene and other key nutrients. The purpose of the current review aimed to address this question relative to CVD risk by examining the available clinical trials assessing the efficacy of lycopene delivered through consumption of tomato products versus lycopene as a dietary supplement on CVD risk factors. Clinical trials were identified in Medline with PubMed searches that included the key words tomato and, or lycopene and lipids, cholesterol, blood pressure (BP), oxidative stress, antioxidant, inflammation, endothelial function, and flow mediated dilation (FMD). Clinical trials were cross-referenced against review article citations where appropriate.

The Tomato
Tomatoes are the edible fruits from the plant Solanumlycopersicum, commonly known as a tomato plant. The tomato belongs to the nightshade family. Historically, tomatoes were found in Mexico, but with the Spanish colonization of the Americas, the species and its use spread around the world. The tomato is consumed raw and as a processed ingredient in many dishes, sauces, salads, and drinks.

Tomatoes contain high quantities of vitamin C, vitamin A, fiber, and potassium along with a variety of other nutrients in lesser amounts. Potassium and fiber are 2 nutrients that have been targeted in prescriptive diets for BP and lipid management. Tomatoes also contain components to favor reduction-oxidation (redox) balance (e.g., lycopene, vitamin C, polyphenols, and phenolic acids) that reduce the risk of cellular oxidative damage, modulating cellular signaling pathways involved in inflammation and endothelial function among other cellular events. Lycopene is one of the most biologically active plant-derived compounds, and tomatoes and tomato-based foods are the richest sources of lycopene in the American diet (2). Therefore, the package of nutrients and bioactive components that tomatoes deliver suggests an important protective role of tomatoes in a heart-healthy diet.

Lycopene: Sources, Chemistry, Bioavailability, and Transport
As a supplement, lycopene is usually packaged in dark bottles to protect from light exposure, which could lead to degradation and loss of bioactivity. Lycopene is sold as a pure extract or as part of a tomato extract, such as Lyc-O-Mato (LycRed Group). In foods, lycopene is found in watermelon and red grapefruit; however, tomatoes and tomato products represent >85% of all the dietary sources of lycopene consumed in the North American diet (16).

Lycopene is a natural red pigment synthesized by plants and microorganisms but not by animals. Lycopene is an acyclic isomer of β-carotene with no provitamin A activity. Lycopene is a highly unsaturated hydrocarbon containing 11 conjugated and 2 unconjugated double bonds (17). Because of the high number of conjugated dienes within lycopene, its potency as an effective singlet oxygen quencher is about twice that of β-carotene and ~10 times that of vitamin E, although lycopene circulates at much lower concentrations than vitamin E, which may impact its role biologically as a radical quenching antioxidant (18,19).

Lycopene from plant sources exists predominantly as an all-trans isomer; however, the more bioavailable form is cis-lycopene (20). The cis-isomer geometry allows for more efficient incorporation of lycopene into mixed micelles in the lumen and of the small intestine, into chylomicrons in the enterocyte, and into VLDLs by the liver (17,21). The trans-cis isomerization occurs readily under acid conditions (22), such as in gastric juices, as well as with exposure to light and thermal energy. Cooking and processing (i.e., thermal energy) converts some of the trans-lycopene to cis-lycopene but also releases lycopene from the cell structure matrix, increasing its bioaccessibility (23,24). Accordingly, lycopene bioavailability is greater from tomato paste and tomato purée than from raw tomatoes (25–27). Lycopene bioavailability from supplements does not appear to differ from processed tomato paste when consumed with a meal (28).

Dietary fat also enhances the bioavailability of lycopene (29,30) by providing stimuli for bile secretion for assembly of micelles and incorporation of lipids, lycopene, and other lipophilic components. Dietary lycopene bioavailability is therefore greater after cooking or processing tomatoes when tomato products are consumed with oil and other dietary fats as a source of dietary lipids (25–27,29–31).

Lycopene bioavailability in supplemental form has not been as extensively studied and remains an important area for research. Cohn et al. (28) compared the bioavailability of lycopene from tomato juice, tomato paste, and a lycopene tablet, each consumed with a standardized meal. Bioavailability and plasma kinetic profiles of lycopene tablets and tomato paste were not different, although both resulted in greater lycopene bioavailability than the tomato juice, reinforcing the importance of processing and lipids for...
Comparison of Dietary Intake and Physical Activity between Women with and without Polycystic Ovary Syndrome: A Review

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ABSTRACT
Polycystic ovary syndrome (PCOS) is a prevalent endocrine disorder affecting women of reproductive age worldwide. In addition to deleterious effects on fertility imparted by PCOS, women with PCOS are at increased risk of obesity, diabetes, cardiovascular disease, depression, and certain cancers. Hormonal and metabolic aberrations in PCOS have the potential to influence dietary intake and physical activity levels. There are emerging global data that women with PCOS have different baseline dietary energy intakes compared with women without PCOS. These alterations in diet may exacerbate clinical symptoms and compound risk of chronic disease in patients. Few studies have compared baseline physical activity levels between women with and without PCOS. Although comparisons between studies are confounded by several factors, the data point to no differences in activity levels among PCOS and non-PCOS groups. This review provides an assessment of the current literature on baseline dietary intake and physical activity levels in women with PCOS. Future recommendations to strengthen research in this area are provided, given the implications to aid in the development of effective nutrition-focused interventions for PCOS. Adv. Nutr. 5: 486–496, 2014.

Introduction
As a leading cause of anovulatory infertility and a risk factor for endometrial dysfunction and uterine cancer, polycystic ovary syndrome (PCOS) represents a serious health concern for women across the life span (1,2). PCOS is characterized by a heterogeneous collection of symptoms: infrequent or absent menstrual cycles, biochemical or clinical evidence of androgen excess, and polycystic ovarian morphology (3,4). PCOS occurs in a striking proportion of women of reproductive age, ranging from 6% to 15% worldwide, depending on the diagnostic criteria used (3,5,6). PCOS should be regarded as a broad-spectrum disorder because its consequences for patients extend beyond impairments of the reproductive system to include serious metabolic (i.e., metabolic syndrome, type 2 diabetes, and cardiovascular disease) and psychological sequelae (i.e., depression, anxiety, poor self-esteem, and reduced quality of life) (3,7,8).

Researchers have established that up to 80% of the PCOS population is overweight or obese with obesity prevalence rates, dependent on the ethnicity and geographical location (3,9). Although PCOS can manifest in both normal weight and overweight women, some evidence supports that increased central adiposity is present across all BMI categories (10–12). It is debatable as to whether women with PCOS have a unique predisposition to obesity or whether obesity drives development of PCOS (13). Data supporting lower basal metabolic rate (14) and postprandial thermogenesis (15) in individuals with PCOS compared with age- and weight-matched controls may account for a higher prevalence of obesity among the PCOS population. However, reports on differences in basal metabolic rate among women with or without PCOS are inconsistent (16). There is also the potential for appetite circuits to be affected by the abnormal hormone profile in PCOS. Testosterone replacement was shown to increase meal frequency in male rodents (17), whereas anti-androgenic pharmaceutical therapy was found to reduce meal-related hunger in women with bulimia (18). The anti-androgenic finding may be particularly relevant because women with PCOS exhibit appetite indications similar to those in women with bulimia (19). Women with PCOS also demonstrated smaller reductions in postprandial ghrelin (i.e., an orexigenic hormone) and lower postprandial cholecystokinin concentrations (i.e., an anorexigenic hormone) compared with age- and/or weight-matched controls

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Collectively, these findings are consistent with the hypothesis that women with PCOS have lower perceived satiety and greater appetite compared with women without PCOS. These findings are tempered by other studies that showed no differences or a blunted response in ghrelin concentrations among women with or without PCOS (22,23). Last, experimental and clinical evidence supports that testosterone promotes abdominal fat deposition in women (11,24,25). Increased abdominal adiposity has been linked to elevated leptin secretion and leptin resistance, which may result in impaired satiety and increased energy intake (26).

Irrespective of whether PCOS causes obesity or a reverse causation exists, it is recognized that obesity, particularly abdominal obesity, worsens clinical and metabolic features of PCOS (3).

Lifestyle intervention is recommended as a first-line treatment in overweight and obese women with PCOS (27). Uncontrolled trials involving hypocaloric diets with physical activity (1200 kcal/d) and low-carbohydrate, ketogenic diets (<20 g carbohydrate/d, unlimited consumption of high-biologic-value protein and dairy) support improvements in hyperandrogenism, frequency of menses, ovulation, pregnancy rates, insulin resistance, and lipid profile when accompanied by modest weight reductions for women with PCOS (28,29). Randomized controlled trials with reduced-energy diets also support improvements in hyperandrogenism and insulin resistance in women with PCOS. Yet, data on ovulation and other reproductive outcomes are less clear (30–32). There are limited data on the feasibility or effectiveness of long-term weight-loss interventions for this population. Moreover, only a few studies examined diet alterations to improve cardiometabolic risk factors in normal weight women with PCOS (33,34). Understanding the baseline dietary intake and physical activity levels of the PCOS population is essential to aid in the development of effective lifestyle interventions in free-living settings. The primary aim of this review was to examine the current literature on baseline dietary intake and physical activity habits in women with PCOS. Furthermore, this review provides recommendations to strengthen future studies in this area of research.

Studies were identified by searching the electronic databases PubMed, CINAHL, and PsycINFO for studies published after 1990 and before January 2014. A search was performed by using a combination of keywords relevant to PCOS and diet, lifestyle, and nutrition assessment methods. Ten studies from various countries were included in the review based on a Population, Intervention, Comparison, Outcome framework established a priori by the authors. In short, studies included for review were limited to original research articles in which 1) the primary objective was to assess baseline diet and physical activity levels between adult women with and without PCOS, 2) enrollment exceeded 10 participants in each study arm, and 3) diet and physical activity were assessed in a free-living sample. A description and the main findings of each study included for review are summarized in Table 1.

**Current Status of Knowledge**

**Comparison of dietary intake between women with and without PCOS.** Two studies compared baseline dietary intake between women with and without PCOS by using case-control study designs in the United States (35,36). Wright et al. (36) assessed dietary intake by using FFQs in mostly middle-aged women undergoing the perimenopausal transition. This was evidenced by the number of women in both control and PCOS groups who reported the absence of menses for 12 mo. By contrast, Douglas et al. (35) assessed the food records of reproductive-aged women who were ~20 y younger than the sample used by Wright et al. Both dietary assessment methods used by these studies have been commonly used to assess dietary intake (37,38), yet each has distinct strengths and weaknesses. Whereas diet records over several days are expected to reflect usual intake and have less reliance on participant memory, this approach may have limited accuracy because participants are aware that their dietary intake would be scrutinized on specific days. This may result in atypical dietary intake and provide misleading dietary information (38,39). The FFQ is an appropriate measure to assess usual dietary composition over a longer period of time; however, the accuracy of the data can be limited by the respondents’ abilities to recall their diet habits (38). It is also impossible to discern whether a PCOS diagnosis may have been a catalyst to altered dietary intake due to the study design.

When the data were pooled without regard to body composition, both Wright et al. (36) and Douglas et al. (35) noted that the PCOS group consumed more servings of white bread compared with the control group. When dietary intake was assessed with respect to BMI categories, Wright et al. (36) reported that normal weight women with PCOS (BMI <25 kg/m²) consumed significantly lower total energy diets compared with BMI-matched women without PCOS (~400 fewer kcal). This may be attributed to the lower reported intakes of carbohydrates (~43 g), protein (~15 g), total fat (~19 g), saturated fat (~5 g), monounsaturated fat (~7 g), polyunsaturated fat (~6 g), and cholesterol (~60 mg) by the normal weight PCOS group compared with controls. An examination of food servings also revealed that normal weight women with PCOS consumed less bread, cereal, rice, pasta, and meat products compared with BMI-matched controls. This may be considered clinically significant as it provided an energy difference of ≥250 kcal/d between the 2 groups. These findings led Wright et al. to hypothesize that women with PCOS within a normal weight range restricted their daily energy intake to a clinically significant margin to offset...
<table>
<thead>
<tr>
<th>Study (reference)</th>
<th>Sample, assessments used</th>
<th>Outcomes</th>
<th>Limitations</th>
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| Wright et al. 2004 (36) | Groups: n = 84, PCOS; n = 79, controls  
Age: 46.7 ± 58 y, PCOS; 48.2 ± 57 y, controls  
BMI (kg/m²): 32.1 ± 9.3, PCOS; 29.0 ± 6.0, controls  
Location: Pittsburgh, PA  
Race: Caucasian: 83%, PCOS; 90%, controls;  
non-Caucasian: 13%, PCOS; 10%, controls  
PCOS definition: oligoamenorrhea plus either hirsutism, hyperandrogenism and/or elevated LH/FSH  
No specific exclusion criteria were applied  
Assessments: FFQ, physical activity questionnaire | No differences in daily food and nutrient intake or physical activity between PCOS and control groups  
Lower nutrient intake in normal weight PCOS (n = 21) vs. normal weight control (n = 33) groups*: total energy/d, CHO (g/d), protein (g/d), fat (g/d), SFAs (g/d), MUFAs (g/d), PUFAs (g/d), cholesterol (mg/d)  
Lower bread, cereal, rice, pasta, meat, fish, poultry, egg intake in normal weight PCOS vs. normal weight control groups (servings)*  
Lower milk product intake in overweight PCOS (n = 15) vs. overweight control (n = 19) groups (servings)*  
Lower meat, fish, poultry, egg intake in obese PCOS (n = 48) vs. obese control (n = 27) groups (servings)*  
Higher carbohydrate and lower fat intakes in PCOS group vs. Reaven study recommendations (49)  
Details on physical activity assessment tool not reported | Diagnostic criteria used yielded a heterogeneous PCOS group  
Population studied used medications known to influence endocrine profile (e.g., oral contraceptive, antandrogens)  
No reported exclusion criteria on medications that may influence weight, appetite  
Older, potentially perimenopausal, populations studied; heterogeneous control group used with 41% reporting oligoamenorrhea; low generalizability to younger women with PCOS  
Power analysis not provided for post hoc comparisons among BMI-matched groups  
Did not report energy expenditure or energy balance  
Did not compare with U.S. DRI |
| Douglas et al. 2006 (35) | Groups n = 30, PCOS; n = 27, controls  
Age: 28.9 ± 63 y, PCOS; 28.9 ± 6.5 y, controls  
BMI (kg/m²): 29.1 ± 48, PCOS; 29.7 ± 48, controls  
Location: Birmingham, AL  
Race: Caucasian: 83%, PCOS; 85%, controls;  
black: 13%, PCOS; 11%, controls other: 4%, PCOS; 4% controls  
PCOS definition: oligoamenorrhea plus hirsutism and/or hyperandrogenism  
Exclusion criteria: diabetes, use of insulin sensitzers or glucose-lowering drugs and adherence to a modified diet  
Assessments: 4-d food records (Wed/Thu/Sat/Sun) | No differences in nutrient intake between PCOS and control groups  
Greater white bread intake in PCOS vs. control groups (servings)* | Population studied used drugs known to influence endocrine profile  
No reported exclusion criteria on other medications that may influence weight, appetite  
Comprehensive dietary intake not collected on all days of week  
Overall study groups not matched for BMI  
Power analysis not provided  
Data on physical activity not collected  
Did not compare with U.S. DRI |
| Álvarez-Blanco et al. 2011 (42) | Groups: n = 22, PCOS; n = 59, controls  
Age: 26.3 ± 7.6 y, PCOS; 32.2 ± 7.5 y, controls  
BMI (kg/m²): 35.2 ± 67, PCOS; 32.2 ± 61, controls  
Location: Madrid, Spain  
Race not reported  
PCOS definition: oligoamenorrhea plus hirsutism and/or hyperandrogenism  
Exclusion criteria: use of hormonal contraception and medications that interfere with metabolism, hypocaloric dieting, implausible energy intake, supplement use  
Assessments: FFQ, exercise habits assessed using interview | No differences in nutrient intake and physical activity between PCOS and control groups  
PCOS group intake vs. U.S. dietary recommended intake*:  
Above: total fat (g/d), SFAs (% of energy/d), MUFAs (% of energy/d), dietary cholesterol (mg/d), sodium (mg/d), vitamin C (mg/d), vitamin D (µg/d), calcium (mg/d), magnesium (mg/d)  
Below: fiber (g/d), potassium (mg/d), vitamin E (mg/d) | Study groups not matched for age  
Power analysis not provided  
Details on physical activity assessment tool not reported  
Did not report energy expenditure or energy balance  
Did not compare intake with EFSA-recommended intake, which is established for European countries |
<table>
<thead>
<tr>
<th>Study (reference)</th>
<th>Sample, assessments used</th>
<th>Outcomes</th>
<th>Limitations</th>
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<tbody>
<tr>
<td><strong>Barr et al. 2011</strong>&lt;sup&gt;(43)&lt;/sup&gt;</td>
<td><strong>Group</strong> n = 198, PCOS</td>
<td>Greater daily nutrient intake in PCOS vs. controls (national survey reference)*: total energy/d, CHO (g/d), protein (g/d), fat (g/d), fat (% of energy/d), SFAs (g/d), MUFA (g/d), PUFAs (g/d), total sugar (g/d), fiber (g/d)</td>
<td>Diagnostic criteria for PCOS not provided, heterogeneous PCOS group studied</td>
</tr>
<tr>
<td>Age: 32.6 ± 63 y</td>
<td></td>
<td>Lower daily nutrient intake in PCOS vs. controls (national survey reference)*: CHO (% of energy/d)</td>
<td>Recruitment based on self-reported diagnosis of PCOS</td>
</tr>
<tr>
<td>BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;): 27.4 ± 7.3</td>
<td></td>
<td>Lower daily glycemic index in normal weight (n = 80) vs. overweight PCOS (n = 100) groups* PCOS group intake vs. UK recommended intake*: Above: total energy/d, protein (g/d), fat (g/d), SFAs (g/d), MUFA (g/d), PUFAs (g/d)</td>
<td>Reference population may contain women with PCOS</td>
</tr>
<tr>
<td>Location: London, UK</td>
<td></td>
<td>Below: CHO (g/d), fiber (g/d)</td>
<td>No reported exclusion criteria on medications that may influence endocrine profile</td>
</tr>
<tr>
<td>Race: Caucasian: 97%, PCOS; unknown: 3%, PCOS</td>
<td></td>
<td>Greater activity in moderate-intensity physical activity (min/d) in normal weight (n = 80) vs. overweight (n = 100) PCOS groups*</td>
<td>Older, potentially perimenopausal, women included</td>
</tr>
<tr>
<td>Exclusion criteria: pregnancy, breastfeeding, eating disorders, and use of weight-loss medications</td>
<td></td>
<td>Did not report energy expenditure or energy balance</td>
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<tr>
<td>Assessments: 7-d food and activity record</td>
<td></td>
<td><strong>Toscani et al. 2011</strong>&lt;sup&gt;(41)&lt;/sup&gt;</td>
<td><strong>Groups</strong>: n = 43, PCOS; n = 37, controls</td>
</tr>
<tr>
<td><strong>Age</strong>: 22.7 ± 5.6 y, PCOS; 29.7 ± 4.9 y, controls</td>
<td></td>
<td>PCOS group intake vs. U.S. recommended intake*: below: fiber (g/d), MUFA (% of energy/d), PUFAs (% of energy/d)</td>
<td>Study groups not matched for age</td>
</tr>
<tr>
<td>BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;): 30.9 ± 5.5, PCOS; 29.7 ± 5.2, controls</td>
<td></td>
<td>No associations between androgen status and nutrients</td>
<td>No reported exclusion criteria on medications that may influence weight and appetite</td>
</tr>
<tr>
<td>Location: Porto Alegre, Brazil</td>
<td></td>
<td></td>
<td>High reporting bias because participants may alter diet before scheduled visit</td>
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<tr>
<td>Race: Caucasian: 90%, PCOS; 74%, controls;</td>
<td></td>
<td></td>
<td>Data on physical activity not collected</td>
</tr>
<tr>
<td>African-European: 10%, PCOS; 26%, controls</td>
<td></td>
<td></td>
<td>Comparisons with U.S. recommended intake may not be appropriate for Brazilian populations</td>
</tr>
<tr>
<td>PCOS definition: oligoamenorrhea plus either hirsutism and/or hyperandrogenism</td>
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<tr>
<td>Exclusion criteria: medications known to interfere with hormone concentrations, BMI &gt;40 kg/m&lt;sup&gt;2&lt;/sup&gt; and diabetes</td>
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<td>Assessments: 24-h dietary recall</td>
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<td><strong>Tsai et al. 2012</strong>&lt;sup&gt;(47)&lt;/sup&gt;</td>
<td><strong>Groups</strong>: n = 45, PCOS; n = 161, controls</td>
<td>Greater daily nutrient intake in PCOS vs. control groups*: fat (% of energy/d)</td>
<td>Diagnostic criteria used yielded heterogeneous PCOS group</td>
</tr>
<tr>
<td>Age: 32.7 ± 42 y, PCOS; 34.7 ± 3.6 y, controls</td>
<td></td>
<td>Lower daily nutrient intake in PCOS vs. control groups*: total energy/d, CHO (g/d), CHO (% of energy/d)</td>
<td>PCOS group comprised infertile women with various etiologies including unexplained infertility</td>
</tr>
<tr>
<td>BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;): 23.0 ± 4.4, PCOS; 21.3 ± 2.9, controls</td>
<td></td>
<td>Positive associations among hormones and nutrients in PCOS*: FSH and CHO (g/d), FSH and CHO (% of energy/d)</td>
<td>Study groups not matched for BMI</td>
</tr>
<tr>
<td>Location: Taipei, Taiwan</td>
<td></td>
<td>No differences in daily nutrient intake between hyperandrogenic (n = 21) and nonandrogenic (n = 24) PCOS groups</td>
<td>No reported exclusion criteria on medications that may influence weight and appetite</td>
</tr>
<tr>
<td>Race not reported</td>
<td></td>
<td></td>
<td>Power analysis not provided for post hoc comparisons between PCOS phenotypes</td>
</tr>
<tr>
<td>PCOS definition: 2 of 3 symptoms: 1) oligomenorrhea, 2) hirsutism and/or hyperandrogenemia, 3) polycystic ovaries</td>
<td></td>
<td></td>
<td>Data on physical activity not collected</td>
</tr>
<tr>
<td>Exclusion criterion: hormonal therapy</td>
<td></td>
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<tr>
<td>Study (reference)</td>
<td>Sample, assessments used</td>
<td>Outcomes</td>
<td>Limitations</td>
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</tbody>
</table>
| Altieri et al. 2013 (40) | Groups: n = 100, PCOS; n = 100, controls  
Age: 27.7 ± 5.2 y, PCOS; 28.4 ± 5.8 y, controls  
BMI (kg/m²): 34.7 ± 5.5, PCOS; 34.8 ± 5.4, controls  
Location: Bologna, Italy  
Race not reported  
PCOS definition: 2 of 3 symptoms: 1) oligoamenorrhea, 2) hirsutism and/or hyperandrogenemia, 3) polycystic ovaries  
Exclusion criteria: endocrine or metabolic disorders; medications that influence appetite, reproduction, glucose, or lipid concentrations; psychoactive drugs; eating disorders; intensive lifestyle interventions  
Assessments: 7-d food records | Greater daily nutrient intake in PCOS vs. control groups*: fiber (g/d)  
Lower daily nutrient intake in PCOS vs. control groups*: lipids (% of energy/d)  
Greater starchy sweets (g/d), cheese (g/d), oil (g/d) in PCOS vs. control groups*  
Lower cooking fats (g/d) in PCOS vs. control groups*  
Positive associations between hormones and nutrients in PCOS*: A4 and total energy, A4 and protein (g/d), A4 and cholesterol (mg/d)  
Negative associations between hormones and nutrients in PCOS*: SHBG and total energy/d, SHBG and CHO (% of energy/d), SHBG and oligosaccharides (g/d) | Diagnostic criteria used yielded a heterogeneous PCOS group  
Data on physical activity not collected  
Did not compare intake with EFSA-recommended intake, which is established for European countries |
| Moran et al. 2013 (44) | Groups: n = 409, PCOS; n = 7057, controls  
Age³: 33.5 ± 1.4 y, PCOS; 33.7 ± 1.5 y, controls  
BMI (kg/m²)³: 29.3 ± 7.5, PCOS; 25.6 ± 5.8, controls  
Location: Australia (national survey)  
Race not reported  
PCOS definition not provided  
No specific exclusion criteria were applied  
Assessments: FFQ, physical activity 1-wk recall | Greater daily nutrient intake in PCOS vs. control groups*: total energy/d, fiber (g/d), folate (µg/d), iron (mg/d), magnesium (mg/d), phosphorus (mg/d), vitamin E (µg/d), sodium (mg/d)³, zinc (mg/d)³, calcium (mg/d)³, potassium (mg/d)³, niacin (mg/d)³  
Lower daily nutrient intake in PCOS vs. control groups*: SFAs (% of energy/d), glycemic index, retinol (µg/d)  
PCOS group reported higher diet quality than control group  
PCOS group intake vs. U.S. DRI*: above: SFAs (% of energy)  
No differences in self-reported physical activity between PCOS and control groups  
PCOS group reported greater amount of sitting time compared with controls³  
PCOS group reported higher energy intake/d and glycemic index diet between classic PCOS (n = 39) and control (n = 44) groups*  
Higher glycemic index diet between classic PCOS (n = 39) and ovulatory PCOS (n = 22) groups*  
No differences in total energy intake and glycemic index diet between ovulatory PCOS (n = 22) and control (n = 44) groups  
No differences in physical activity between PCOS and control groups | Recruitment based on self-reported diagnosis of PCOS  
Control group may contain undiagnosed women with PCOS  
No reported exclusion criteria on medications that may influence weight, appetite, or reproduction  
Study groups not matched for age or BMI  
PCOS group reported greater amount of sitting time compared with controls³  |
| Graff et al. 2013 (46) | Groups: n = 61, PCOS; n = 44, controls  
Age: 22.7 ± 6.2 y, PCOS; 25.0 ± 6.3 y, controls  
BMI (kg/m²): 28.9 ± 5.6, PCOS; 27.1 ± 5.7, controls  
Location: Porto Alegre, Brazil  
Race: Caucasian: 88% of sample; African-European: 12% of sample  
PCOS definition: 1) classic PCOS: oligoamenorrhea, hirsutism and/or hyperandrogenemia with or without polycystic ovaries; 2) ovulatory PCOS: hirsutism and polycystic ovaries in the presence of regular menstrual cycles and normal androgens  
Exclusion criteria: diabetes, medications that alter hormone concentrations, pregnancy, BMI ≥40 kg/m²  
Assessments: FFQ, 6-d pedometer use | Greater daily nutrient intake in PCOS vs. control groups*: total energy/d, glycemic index³, glycemic load³, sodium (mg/d)³  
Greater energy intake/d and glycemic index diet between classic PCOS (n = 39) and control (n = 44) groups*  
Higher glycemic index diet between classic PCOS (n = 39) and ovulatory PCOS (n = 22) groups*  
No differences in total energy intake and glycemic index diet between ovulatory PCOS (n = 22) and control (n = 44) groups  
No differences in physical activity between PCOS and control groups | Diagnostic criteria used yielded a heterogeneous PCOS group  
Included both adolescents and adults with PCOS  
No reported exclusion criteria on medications that may influence weight and appetite  
Power analysis not provided for post hoc comparisons between PCOS phenotypes  
Pedometer may not comprehensively capture physical activity data  
No differences in physical activity between PCOS and control groups  |

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<table>
<thead>
<tr>
<th>Study (reference)</th>
<th>Sample, assessments used</th>
<th>Outcomes</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ahmadi et al. (45)</td>
<td>Groups: n = 65, PCOS; n = 65, controls</td>
<td>Greater daily nutrient intake in PCOS vs. control groups*: Power analysis not provided for post hoc comparisons</td>
<td>No differences in daily nutrient intake between normal weight (n = 49) and overweight (n = 16) PCOS groups Did not report energy expenditure or energy balance</td>
</tr>
</tbody>
</table>

Baseline diet and activity in PCOS

Ahmadi et al. noted no differences in overall macronutrient and energy intake among overweight and obese women with PCOS compared with their respective BMI-matched controls. Overweight women with PCOS reported lower intakes of milk products compared with overweight controls, whereas obese women with PCOS reported consuming more servings of meat, fish, poultry, and eggs than the obese controls. Most of these differences were less than 1 serving apart. This may or may not be considered clinically significant, depending on the type of protein consumed.

By using the 7-d food records from a large cohort of women (n = 198) with a self-reported PCOS diagnosis, Barr et al. (43) reported that women with PCOS in the United Kingdom had higher total energy intakes (~350 kcal) compared with a reference population. They noted that women with PCOS consumed higher amounts of total carbohydrates (229.0 vs. 198.0 g), protein (78.0 vs. 66.3 g), dietary fat (85.0 vs. 61.1 g), saturated fat (26.5 vs. 22.2 g), monounsaturated fat (29.7 vs. 21.7 g), polyunsaturated fat (16.2 vs. 12.6 g), sugar (102.0 vs. 87.4 g), and dietary fiber (16.5 vs. 13.0 grams) compared with a reference population (43). On the basis of these results, it can be recommended that sugar intake should be monitored when conducting dietary assessments in patients with PCOS in the United Kingdom. Barr et al. also reported that overweight women with PCOS consumed higher-glycemic-index diets compared with normal weight women with PCOS. These findings were consistent with reports from Australia involving a cohort of women (n = 409) with a self-reported diagnosis of PCOS (44). Moran et al. (44) noted a small, but statistically significant difference in total daily energy intake (~50 kcal) between women with PCOS and controls on the basis of a validated FFQ. The PCOS group consumed higher amounts of iron (12.3 vs. 11.6 mg), magnesium (272 vs. 258 mg), phosphorus (1471 vs. 1401 mg), and vitamin E (5.9 vs. 5.6 mg) when adjusted for total daily energy intake and lower amounts of saturated fat (15.1% vs. 15.4% of energy) and retinol (295 vs. 311 µg). Although the studies by Barr et al. (43) and Moran et al. (44) represent the largest studies that assessed baseline dietary intake in PCOS to date, both were limited by their reliance on a self-reported diagnosis of PCOS. It is possible that the control populations contained women with PCOS and/or other endocrine issues because Barr et al. did not exclude PCOS features from their control population survey. Moran et al. used a diagnostic question within a survey that restricted PCOS diagnosis and treatment to within 3 y. This may have classified women with PCOS who were diagnosed earlier in their lives or not seeking treatment as controls. Collectively, there is the potential for differences in dietary intake between groups to be underestimated by these studies.

Higher energy diets were also reported in Iranian (45) and Brazilian (46) women with PCOS. Ahmadi et al. (45) compared the 3-d, 24-h dietary recalls of Iranian women with and without PCOS and noted that overall daily energy intake was higher (~300 kcal) in women with PCOS. Iranian
women with PCOS also reported higher total fat (~2% kcal), polyunsaturated fat (0.6 g), and saturated fat (0.8 g) intakes compared with controls. This was contradicted by Altieri et al. (40), who reported that the Italian PCOS group consumed a lower-fat diet (~1% kcal) compared with healthy controls. The 24-h dietary recall used by Ahmadi et al. has similar disadvantages to a FFQ because it relies on participant memory. However, it is a convenient method that can provide accurate dietary information when collected by a trained interviewer using standardized approaches. Similar to Ahmadi et al., Graff et al. (46) reported that Brazilian women with PCOS had higher total daily energy intakes (~250 kcal) compared with controls with the use of an FFQ. Brazilian women with PCOS reported consuming a higher-glycemic-index (2 units), glycemic load (~33 units), and sodium (~430 mg) diet. However, these differences disappeared after adjusting for age and BMI. Graff et al. (46) recognized the heterogeneous composition of their PCOS population and performed an assessment of dietary intake on the basis of PCOS phenotypes. They found that women with a classical form of PCOS (i.e., chronic anovulation and hyperandrogenism), but not those with ovulatory PCOS (i.e., hyperandrogenism, polycystic ovaries but regular menstrual cycles), had significantly higher total daily energy intake compared with controls. These differences became negligible after adjusting for age and BMI.

Last, Tsai et al. (47) investigated baseline dietary intake in Taiwanese women with PCOS by using 3-d food records. Taiwanese women with PCOS reported lower total daily energy intakes (110 kcal) compared with infertile women without PCOS. The PCOS group consumed more total dietary fat (~3% of energy) but lower amounts of total daily carbohydrates (~4% of energy; 30 g), which likely accounted for the energy difference between groups. Comparing the results of this study with others is challenging because groups were not matched for BMI and their control population comprised infertile women (including those with unexplained infertility).

Comparison with national dietary guidelines. Six of the 10 studies compared nutrient intake in women with PCOS with established dietary guidelines (35,36,41–43,48). Wright et al. (36) noted that women with PCOS in the United States had slightly higher carbohydrate and lower fat intakes compared with the dietary recommendations for insulin-resistant individuals established by Reaven (49) (i.e., diet consisting of 45% carbohydrates, 15% protein, 10% polyunsaturated fat, 20% monounsaturated fat, and <10% saturated fat). The Reaven recommendations may not be an optimal comparator for this population because certain PCOS phenotypes may not be prone to insulin resistance (50) and the low carbohydrate recommendation may be difficult to achieve in a free-living setting. When compared with the 2010 Dietary Guidelines for Americans (51), women with PCOS in the United States consumed excessive saturated fat (12% of total daily energy intake vs. <10% of total daily energy intake). The PCOS group consumed amounts within the Acceptable Macronutrient Distribution Ranges for carbohydrate and protein (51), which was similar to the results of the U.S. study conducted by Douglas et al. (35). Douglas et al. (35) determined that the PCOS group consumed more than the recommended amount of saturated fat as established by the National Cholesterol Education Program (<7% kcal/d). The PCOS group also exceeded American Heart Association recommendations for sodium (~2400 mg/d) and did not meet dietary fiber recommendations (25–30 g/d). When compared with the 2010 Dietary Guidelines, their reported values are consistent with the conclusion that American women with PCOS consume excessive sodium and insufficient fiber in their diets (35,51). When the PCOS group was stratified by BMI, normal weight and obese women with PCOS exceeded dietary fat Acceptable Macronutrient Distribution Range recommendations by 2% and 5%, respectively, whereas overweight women with PCOS consumed within the normal range (36). This emphasizes the importance of accounting for BMI when assessing baseline nutrient intake within the PCOS population.

Barr et al. (43) used the UK’s Reference Nutrient Intake (RNI) guidelines to determine whether their PCOS group met dietary guidelines. On the basis of the results, women with PCOS exceeded the reference intakes for fat (i.e., total fat, saturated fat, polyunsaturated fat) and mean dietary glycemic index but did not meet fiber recommendations. The reported values also indicated that women with PCOS in the United Kingdom consumed more protein and but did not meet carbohydrate recommendations. The RNI established in the United Kingdom may not be an appropriate measure to determine nutrient adequacy (52). There is significant potential to overestimate the percentage of women with PCOS who are not meeting dietary guidelines because the RNI values are defined as nutrient intakes required to meet the recommendations for 97.5% of a national population.

Alvarez-Blasco et al. (42), Toscani et al. (41), and Moran et al. (44) used DRIs established in the United States to assess nutrient intake in Spanish, Brazilian, and Australian populations, respectively (Table 1). The dietary recommendations designed to meet the needs of the American population may not be a useful reference for countries that have different dietary patterns, food environment, and cultural beliefs and the potential for genetic variations in metabolism. The European Food Safety Association has established dietary reference values for the intake of carbohydrates, fats, and water that are likely more appropriate for European countries, including Spain (53). Similarly, the Australian National Health and Medical Research Council and the New Zealand Ministry of Health have established nutrient reference values specifically for the Australian and New Zealand populations (54). To the best of our knowledge, there are no established South American nutrient value recommendations.

When comparing the dietary intake results with the corresponding national dietary guidelines, we concluded that Spanish, British, and Australian women with PCOS exceeded the recommended intakes for total, saturated, and/or
monounsaturated fats when compared with women without PCOS (42–44). Álvarez-Blasco et al. (42) reported that women with PCOS in Spain exceeded the U.S. recommended dietary cholesterol intake, while not meeting the fiber, potassium, and vitamin E recommendations. Both Spanish (42) and Italian (40) women with PCOS consumed excessive total fat but inadequate fiber when compared with European Food Safety Association recommendations. Similarly, Australian women with PCOS had inadequate fiber and vitamin E intakes compared with the Australian nutrient reference values (44). The global data indicate that women with PCOS exceed total fat and saturated fat recommendations, while not meeting recommended amounts of dietary fiber in their diet. Women without PCOS included in these studies appear to have similar results when comparing nutrient intake to national nutrient reference values across countries. Meeting nutrient recommendations may be a key public health issue for clinicians and researchers to resolve across both PCOS and non-PCOS populations.

**Biomarkers and diet.** Two of the studies included in this review determined associations between biochemical markers and nutrients (Table 1). Tsai et al. (47) reported a positive association between carbohydrate intake (g and % of energy) and follicle-stimulating hormone. Follicle-stimulating hormone is a hormone that promotes follicular growth in the ovaries and is typically lower compared with its companion gonadotropin (luteinizing hormone) in a subset of women with PCOS (55,56). Altieri et al. (40) observed positive associations of total energy, protein (g), and cholesterol intakes with androstenedione (a precursor of testosterone). Although androstenedione is not a diagnostic marker of PCOS, a subgroup of women with PCOS exhibit elevated concentrations of this androgen (57). Collectively, these data are consistent with the hypothesis that PCOS symptoms may be related to dietary intake. Further research is needed to corroborate these findings and to determine the physiologic mechanisms behind these associations.

**Physical activity.** Six of the 10 studies performed an evaluation of physical activity levels in women with PCOS (36,42–46). By using a validated physical activity questionnaire, Wright et al. (36) did not detect any differences in self-reported physical activity levels between American women with PCOS and healthy controls. Both the PCOS and control groups reported similar amounts of time engaged in various activities, including vigorous, moderate, and light activity, as well as sleeping or reclining, for typical weekdays or weekend days. These findings were consistent with those of Álvarez-Blasco et al. (42), Ahmadi et al. (45), and Graff et al. (46), who also noted no differences in overall physical activity among Spanish, Iranian, and Brazilian women with or without PCOS. Wright et al. (36) noted that women with PCOS reported greater sitting time. Unlike the findings in an Australian cohort (44), this difference did not reach significance ($P = 0.064$). Wright et al. (36) did not detect differences in physical activity among PCOS and control groups when the data were analyzed by BMI categories (i.e., normal weight, overweight, and obese).

By using 7-d activity records, Barr et al. (43) showed that the majority of women with PCOS (74%) in their UK study reported achieving 30 min of daily moderate-intensity activity. This was consistent with the UK national recommendations for healthy living (58). Approximately half of the overweight and obese women with PCOS did not achieve the recommended 60 min of daily moderate-intensity activity (43). The authors admit that a self-selected sample might not have yielded a representative sample of women with PCOS because their approach may have overestimated physical activity due to the inclusion of highly motivated individuals. This study did not include comparisons with healthy age- and BMI-matched controls. Rather, Barr et al. (43) examined the potential for differences in physical activity among normal weight and overweight women with PCOS. They noted that normal weight women with PCOS reported longer durations of moderate-intensity physical activity compared with overweight and obese women with PCOS of the same age. Coupled with their findings of lower-glycemic-index diets in normal weight women with PCOS, this study supported that diet and physical activity behaviors were associated with BMI among women with PCOS.

The emerging data on baseline physical activity levels in women with PCOS are challenging to interpret because none of the studies used the same physical activity evaluation tool. The limitations for the methods used merit consideration. Wright et al. (36) used the Paffenbarger Physical Activity Questionnaire, which has been primarily validated in mixed-gender populations (59). It is uncertain whether this tool provides an accurate measure of physical activity for pre- and perimenopausal women with PCOS. Álvarez-Blasco et al. (42) and Ahmadi et al. (45) evaluated physical activity on the basis of an assessment of exercise habits by using interview questions. The validity of this approach is uncertain because the details regarding the validation of their interview tools were not provided. The 7-d activity records used by Barr et al. may be considered a more accurate quantification of physical activity since because is minimal dependence on memory, in contrast to the 7-d self-recall implemented in the Australian study (43). However, 7-d activity records place greater burden on participants, which can impact the reporting accuracy. Graff et al. (46) was the only research group to eliminate recall bias and use pedometers, which objectively quantified 6 d of baseline physical activity among participants. Graff et al. reported no difference in physical activity levels between women with or without PCOS. Although these data are strengthened by their inclusion of an objective measure of physical activity, we are unaware of any validation study on the pedometer model that was used. Moreover, pedometers may have low accuracy when assessing energy expenditure (60). Future studies would benefit from a combination of objective and subjective instruments in the situation that the objective tool may malfunction in the field. Information on perceptions of physical activity

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may also have relevance when used in conjunction with objective measures.

**Summary and Future Recommendations**

It is important to recognize that studies assessing diet and physical activity of women with PCOS used broad definitions for PCOS. This creates a challenge when interpreting the literature because the PCOS group comprise of several distinct clinical phenotypes. Most research groups used criteria supported by the American Society for Reproductive Medicine and the European Society of Human Reproduction and Embryology, known as the Rotterdam criteria (61), which yield heterogeneous PCOS phenotypes. Hormonal and metabolic differences exist among these clinical phenotypes, which may serve as confounding factors when examining lifestyle variables (62,63). As Graff et al. (46) demonstrated, there may be distinct differences in dietary intake among clinical phenotypes of PCOS. This is consistent with repeated reports that women with milder variants of PCOS have improved metabolic status and different health risks compared with those with more severe phenotypes (64,65). Researchers must establish a clear distinction between PCOS status to provide an accurate comparison of lifestyle habits between women with and without clinical variants of PCOS.

Energy balance is an important determinant of weight that has not been adequately explored in women with PCOS. Few studies performed concomitant assessments of physical activity when examining dietary intake in women with PCOS. Future studies would be strengthened by the addition of objective tools to measure physical activity (e.g., accelerometers), which can provide an unbiased account of energy expenditure. Although there are emerging data on the associations between biochemical markers and dietary intake, more of these analyses are needed in PCOS populations to develop hypotheses related to potential predictors of dietary intake and physical activity in women with PCOS. Dietary interventions featuring weight loss were shown to have a positive effect on reproductive outcomes (27,30,31,48). However, methods to maintain weight loss should be further examined. Researchers should consider the interaction between environmental influences, personal beliefs, and biological variables in women with PCOS to fully understand drivers of diet and physical activity behaviors. Experts have suggested that depression and/or low self-esteem place women with PCOS at higher risk of emotional eating and decreased exercise, which contribute to a long-term positive energy balance and weight gain (66). By examining and understanding these associations, it may be possible to identify potential key intervention targets with a high likelihood for success in the PCOS population. The roles of race and ethnicity also merit further consideration. There is existing evidence supporting racial disparities in reproductive function among women with PCOS (67,68). Because only a few studies disclosed the race of their participants, we were unable to draw any conclusions regarding any potential influence of race on dietary intake or physical activity.

**Conclusions**

This review is the first to our knowledge to summarize the literature on baseline dietary intake and physical activity in women with and without PCOS and to provide recommendations to strengthen research within this area. There are emerging global data that women with PCOS have different baseline dietary intakes compared with women without PCOS. Although the limited number of studies in the United States suggest that dietary intake is similar to that of women without PCOS irrespective of BMI (35,36), both studies recommend that diet and its effect on metabolic outcomes be more thoroughly examined in this population. These recommendations were based on the observation that differences existed in the consumption of certain foods among women with PCOS (e.g., high glycemic index), despite similarities in overall energy or nutrient intake. Moreover, notable differences in dietary intake were evident in women with PCOS when BMI was taken into consideration. Internationally, most studies indicate higher energy intakes in women with PCOS, with excessive saturated fat and inadequate fiber consumption. However, there appears to be no significant differences in self-reported physical activity between women with and without PCOS. The use of objective tools may be the next step to determine energy expenditure in this population. Moving forward, we recommend that researchers incorporate life stage and clinical phenotypes into their analysis when examining baseline dietary intake and physical activity in the PCOS population. Larger sample sizes with sufficient power to discern the impact of BMI and clinical phenotype will also serve to strengthen future studies.

**Acknowledgments**

All authors have read and approved the final manuscript.

**References**


57. Georgopoulos NA, Papadakis E, Armeni AK, Katsikis I, Roupas ND, Panidis D. Elevated serum androstenedione is associated with a more severe phenotype in women with polycystic ovary syndrome (PCOS). Hormones (Athens) 2014;13:213–21.


enhanced bioavailability of lycopene. Because tomatoes also contain other lipid-soluble components, such as phytoene, phytofluene, ζ-carotene, γ-carotene, β-carotene, eurorsporene, and lutein, consuming processed tomatoes under these conditions would enhance absorption of several other nutrients in addition to lycopene. Hence, a distinct advantage of consuming processed tomato products over a lycopene supplement is the inclusion of additional nutrients and bioactive components in the diet—consistent with dietary recommendations to eat more fruits and vegetables. Therefore, an important unanswered question that this review aims to address is whether there is convincing scientific justification to recommend lycopene supplementation over tomato intake for reducing CVD risk.

Cardiovascular Risk Factors

Traditional risk factors for CVD include cigarette smoking, elevated BP, elevated total cholesterol (TC) and LDL cholesterol, low HDL cholesterol, type 2 diabetes, and advanced age (32,33). Others risk factors include obesity, family history, being male, and sedentary lifestyle, although these are not a focus of this review. Although these risk factors for CVD predict up to 50% of CVD (34), it has become increasingly important to shift prevention efforts toward other risk factors, including biomarkers and intermediate markers, associated with CVD morbidity and mortality.

In this review, selected traditional (lipids and BP) and emerging (oxidative stress, inflammation, and endothelial function) risk factors will be discussed. These risk factors or markers were chosen because of the existing mechanistic evidence for a role of lycopene or tomato intake in modulating these risk factors, as well as because of sufficient clinical trial evidence to review and attempt to make conclusions. The prevailing hypothesis for lycopene’s bioactivity in the body is through its ability to act as an antioxidant. Although the “oxidative stress hypothesis” has evolved over the years, oxidative stress and inflammation are paramount in the process of CVD development, along with endothelial dysfunction and elevated BP. Therefore, oxidative stress will be reviewed first, followed by inflammation, endothelial function, BP, and finally lipid metabolism.

Oxidative Stress: Tomatoes versus Lycopene

Oxidative stress. An increase in oxidative stress resulting in oxidative damage has been implicated in the initiation, progression, and complication of CVDs (35–38). Oxidative stress is characterized by an imbalance between reactive oxygen species (ROS) and antioxidant defenses (39,40). Within the vessel wall, different oxidants can originate from cellular and extracellular sources, and from enzymatic and nonenzymatic pathways (41). Overproduction of ROS [or reactive nitrogen species (RNS)] occurs with aging and under various environmental challenges, such as smoking, pollution, and consumption of the Western diet (42–45). Unmanaged, ROS/RNS can cause damage to cellular components such as DNA, proteins, and lipids, resulting in impaired cellular function or mutation and/or cell death. Examples of ROS causing damaging effects within the human body include singlet oxygen (1O2), superoxide (O2−), peroxyl radicals (ROO•), hydroxyl radical (HO•), and peroxynitrite (ONOO−) (46,47). The sources of ROS in the vessel wall include excessive stimulation of NAD(P)H oxidase or from sources such as mitochondrial electron transport chain, xanthine oxidase, and uncoupled endothelial NO synthase (46,48–52). Another source of oxidation is myeloperoxidase, which is secreted by phagocytes, including neutrophils, monocytes, and macrophages. Myeloperoxidase is an enzyme that generates hypochlorous acid (HOCl). Chlorinated biomolecules are considered to be specific markers of oxidation reactions catalyzed by myeloperoxidase (53). This system can give rise to various products, some of which are relevant to LDL oxidation. For example, the generation of free amino acid or protein-bound tyrosyl radicals may, in turn, participate in secondary oxidation reactions, including LDL oxidation (53,54). Oxidized LDLs are integral to the conversion of macrophages into foam cells in the vessel wall: the foundation for plaque formation.

Balancing oxidative “stress.” A paradox in metabolism is that, whereas oxygen is required for life, it is also a highly reactive molecule that can cause damage to living organisms when excessive amounts of ROS are generated or not removed or neutralized before they cause damage. The goal of the antioxidant defenses system is to strike a “balance” to achieve levels for optimal cellular function. Superoxide (O2−) is among the most commonly generated ROS, which is generally dismutated immediately by superoxide dismutase (SOD) to hydrogen peroxide (H2O2). H2O2 is further degraded into H2O by catalase or several types of peroxides, including glutathione peroxidases and peroxiredoxin (thioredoxin peroxidases) (55). In addition to enzymatic defenses, cells also rely on nonenzymatic molecules such as ascorbic acid, carotenoids, glutathione, or polyphenols to achieve cellular redox balance. Several nonenzymatic antioxidant molecules are obtained from the diet, particularly from a diet rich in fruits and vegetables.

Lycopene is an example of a lipid-soluble (hydrophobic) compound with in vitro antioxidant properties that can be obtained from food or supplements. In general, lipid-soluble antioxidants protect cell membranes from lipid peroxidation, whereas water-soluble antioxidant molecules react with oxidants in the hydrophilic cell cytosol and blood plasma. Lycopene is lipophilic and affects lipid metabolism (56) and lipid oxidation, which are involved in atherosclerotic processes. Several investigations have studied the role of lycopene in the protection of lipids from oxidation (lipid peroxidation) and, in particular, LDLs from oxidation.

Oxidized LDLs. Oxidized LDLs are highly atherogenic (57,58). Oxidized LDLs, caused partially by the end products of lipid peroxidation, stimulate cholesterol accumulation and foam cell formation giving rise to fatty streaks and plaques in the arterial wall. Additionally, oxidized LDLs stimulate the synthesis of adhesion molecules by endothelial cells and
induces an array of proinflammatory events, initiating as well as advancing progression and complication of vascular disease. Hence, minimizing oxidation of LDL could have significant preventative and therapeutic implications for reducing CVD risk, including risk of life-threatening events.

Ten clinical trials have reported on the effect of supplementation with lycopene (Table 1), and 17 clinical trials reported on tomato and tomato-based products as a source of lycopene (Table 2) on LDL oxidation. Lycopene supplementation ranged from 5 h (postprandial evaluation) to 12 wk of treatment. Lycopene doses ranged from 6.5 mg/d lycopene (for 8 wk) to 75 mg/d (for 1 wk). Lyc-O-Mato, which is a tomato lycopene complex containing several phytonutrients including phytoene, phytofluene, β-carotene, tocopherols, and phytosterols, in addition to lycopene, was used most frequently as the lycopene supplement. Other lycopene supplements have included extracts from tomato, synthetic lycopene beadlets, or tomato oleoresin (see Table 1). Overall, of 10 studies reporting changes in LDL oxidation, 4 reported beneficial outcomes (59–62), 1 at 5 h after consumption of lycopene with a fatty meal (61), and 3 others after 1, 3, or 8 wk of supplementation. The 1- and 3-wk interventions were effective but also included coadministration with fish oil (62) or used a relatively high dose (75 mg/d for 1 wk) (59), which may have accounted for faster improvements. Engelhard et al. (60) reported decreases in LDL oxidation after 8 wk of treatment using 15 mg/d lycopene supplementation; however, in a dose-response study (0–30 mg/d, including an intermediate dose of 15 mg lycopene) for 8 wk, changes in LDL oxidation status were not observed (63). Longer duration trials at similar daily dosages did not improve LDL oxidation status.

One distinct difference among trials was the heterogeneity among study populations selected; Engelhard et al. (60) recruited participants with stage 1 hypertension, whereas other studies generally included healthy individuals of varying ages with differences in body weight ranging from healthy weight to obese. There were no other apparent associations between body weight (as assessed by BMI) and changes in LDL oxidation after follow-up.

In comparison to lycopene supplementation, which showed improvements in measures of LDL oxidation status in 40% (4 of 10) of the reported studies, consumption of tomatoes and/or tomato products resulted in the following:

1) significant improvements in LDL oxidation status in 7 of 17 studies (an eighth study reported a trend for improvement, \( P = 0.08 \)), 2) mixed results in 2 of 17 studies [one study showed improvements in nonsmokers, but not in smokers (64); and the second study conducted in smokers only showed improvements on one out of two measures of LDL oxidation (65)], and 3) 7 of the 17 studies reported no observable differences in LDL oxidation status after tomato/tomato product intervention. Interventions ranged in duration [6 h (postprandial) to 12 wk], the tomato products consumed (paste, soup, juice, sauce, purée, ketchup), and dose of lycopene from varying tomato sources (from 8 to 50 mg/d).

Participants were generally healthy with the exception of 1 study that recruited individuals with type 2 diabetes (65) and 1 other study included renal transplant patients (66). Two studies included smokers (64,67), in whom tomato juice consumption did not improve resistance to LDL oxidation, even when directly compared with nonsmokers in the same study (64,67). One interpretation of the results is that a greater amount of tomato products delivering a higher dose of antioxidants may be required to overcome the stress imposed by smoking, because addition of vitamin C, vitamin E, and β-carotene to tomato juice resulted in improvements in the resistance of LDLs to oxidation (67).

The evaluation of protective activity for tomato products in nonsmokers who were otherwise healthy indicated significant improvements in oxidized LDL status in 7 of 13 studies (59,64,68–72), with an eighth study reporting nonsignificant \( P = 0.08 \) improvements in LDL oxidation status (73).

There was no clear “optimal” dose or amount of tomato product to consume. After standardizing treatment interventions on the basis of lycopene content of products, improvement in resistance to LDL oxidation was observed after consuming a variety of tomato products in amounts that delivered as low as an average of 8 mg of lycopene/d for 3 wk (72), incorporating 100 g raw tomato and 15 g tomato paste twice per week, respectively, and 60 g tomato sauce thrice per week to 50.4 mg of lycopene/d for 1 wk (59), 126 g spaghetti sauce (39.2 mg lycopene), or 540 mL tomato juice (50.4 mg lycopene). By comparison, Thies et al. (74) provided a variety of commercially available tomato products to disease-free middle-aged adults delivering up to 50 mg lycopene/d for 12 wk but did not observe improved LDL oxidation status.

Overall, there seems to be a modest benefit in consuming tomato products versus taking a lycopene supplement daily on measures of oxidized LDLs; however, the benefit appears to be confined to relatively healthy, nonsmoking individuals. Research remains sparse among “at risk” individuals, and particularly among the growing population of those with metabolic syndrome and diabetes. One of 2 studies reported improved LDL oxidation status in individuals with type 2 diabetes after consumption of tomato juice (65). Future studies are required to examine dose/amount effects and requirements for decreasing oxidation of LDLs in individuals with CVD risk factors.

**Oxidative stress and antioxidant capacity.** The development of reliable methods for assessing oxidative stress status and quantifying antioxidant capacity (AOX) of foods/supplements remains a subject of interest and intense research in hopes to match the AOX of foods with biologic AOX in vivo. AOX is the ability of a compound to reduce pro-oxidants (75). Methods for measuring AOX of a food or biologic sample have been classified as inhibition methods involving reactive species (76). The most commonly used methods include oxygen radical absorbance capacity (ORAC), ferric reducing ability of plasma [also known as ferric-reducing antioxidant power (FRAP)],...
<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>First author</th>
<th>n</th>
<th>Participants</th>
<th>Study design</th>
<th>Length of Treatment</th>
<th>Lycopene source</th>
<th>Treatments</th>
<th>Lycopene dose</th>
<th>LDL oxidation</th>
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<tr>
<td>(59) 1998</td>
<td>Agarwal</td>
<td>19 M, F OW 25–40 y</td>
<td>Crossover 4 treatments 1 wk each</td>
<td>Lyc-O-Mato</td>
<td>Placebo</td>
<td>75 ✔ ↓</td>
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<td></td>
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<td></td>
<td></td>
<td>Lyc-O-Mato</td>
<td>Spaghetti sauce</td>
<td>392 ✔ ↓</td>
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<td></td>
<td>Tomato juice</td>
<td>504 ✔ ↓</td>
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<td>(139) 2000</td>
<td>Carroll</td>
<td>51 M, F 60–83 y</td>
<td>Parallel 3 treatments 12 wk</td>
<td>Lyc-O-Pen</td>
<td>Placebo</td>
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<td>Lycopene</td>
<td>133 —</td>
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<tr>
<td>(61) 2000</td>
<td>Fuhrman</td>
<td>4 30–45 y</td>
<td>Postprandial 1 treatment BC 0–5 h</td>
<td>Tomato oleoresin</td>
<td>Lycopene with fatty meal, 1200 kcal</td>
<td>30 ↓ at 5 h</td>
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<td>(140) 2001</td>
<td>Hininger</td>
<td>175 M</td>
<td>Parallel 4 treatments 12 wk</td>
<td>Tomato extract</td>
<td>Placebo</td>
<td>0 —</td>
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<td>Lycopene</td>
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<td>β-Carotene</td>
<td>0 —</td>
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<td>(141) 2002</td>
<td>Olmedilla</td>
<td>400 M, F HW 25–45 y</td>
<td>Parallel 100 treatments Sequential n=100/treatment 20 wk</td>
<td>Tomato extract</td>
<td>Vitamin E</td>
<td>0 —</td>
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<td>Lycopene (or other carotenoids)</td>
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<td>Vitamin E + lycopene (or other treatments)</td>
<td>133 —</td>
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<td>(62) 2003</td>
<td>Kiokias</td>
<td>32 M, F HW 32 ± 11 y</td>
<td>Crossover 2 treatments 3 wk each</td>
<td>Lyc-O-Mato</td>
<td>Fish oil</td>
<td>0 ↓</td>
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<td></td>
<td>Fish oil + carotenoid extract with Lyc-O-Mato</td>
<td>45 ↓</td>
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<tr>
<td>(60) 2006</td>
<td>Engelhard</td>
<td>31 Grade 1 hypertension 30–70 y No medications</td>
<td>Double-blind Sequential 2 treatments 16 wk: 4 wk placebo, 8 wk treatment, 4 wk placebo</td>
<td>Lyc-O-Mato</td>
<td>Placebo</td>
<td>0 ↓</td>
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<td>Lyc-O-Mato with meals</td>
<td>15 ↓</td>
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<tr>
<td>(63) 2008</td>
<td>Devanraj</td>
<td>77 M, F OW &gt;40 y</td>
<td>Parallel 4 treatments 8 wk</td>
<td>Lycopene, all trans</td>
<td>Placebo</td>
<td>0 —</td>
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<td>Lycopene</td>
<td>65 —</td>
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<td>Dose-response</td>
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<td>30 —</td>
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<td>(108) 2009</td>
<td>Markovits</td>
<td>16 OB, HW</td>
<td>1 treatment BC 4 wk</td>
<td>Lyc-O-Mato</td>
<td>Lycopene</td>
<td>30 —</td>
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<td>(74) 2012</td>
<td>Thies</td>
<td>225 M, F OW, OB 40–65 y</td>
<td>Parallel 3 txt 12 wk 4-wk run-in</td>
<td>Lycopene</td>
<td>Low Tomato, LT</td>
<td>0 —</td>
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<td>LT + Lycopene</td>
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<td>High Tomato</td>
<td>32–50 —</td>
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1 BC, baseline control; HW, healthy weight for height based on standard BMI criteria; OB, obese BMI; OW, overweight BMI; ↓, decrease or reduction; —, neutral or no effect compared to control arm, P < 0.05 (or baseline in 1-arm trials, P < 0.05).
<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
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<th>LDL oxidation</th>
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<tr>
<td>(59)</td>
<td>1998</td>
<td>Agarwal M, F OW 25–40 y</td>
<td>19</td>
<td>Healthy M, F Crossover 4 treatments</td>
<td>1 wk each Tomato juice</td>
<td>Placebo 0 mg</td>
<td>Lyc-O-Mato 1.2 g</td>
<td>Spaghetti sauce 126 g</td>
<td>Tomato juice 540 mL</td>
<td>237 mL</td>
<td>75</td>
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<tr>
<td>(67)</td>
<td>1998</td>
<td>Steinberg M, F HW, OW 28 ± 3 y</td>
<td>39</td>
<td>Smokers M, F Crossover 2 treatments</td>
<td>4 wk Tomato juice</td>
<td>Tomato juice 237 mL</td>
<td>Tomato juice + vitamins C and E and β-carotene</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<tr>
<td>(142)</td>
<td>1999</td>
<td>Dugas Healthy, 22–45 y</td>
<td>2</td>
<td>2 treatments LDL enrichment study</td>
<td>3 wk Tomato juice</td>
<td>Tomato juice 12 ounces</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<td>(66)</td>
<td>1999</td>
<td>Sutherland Renal transplant recipients</td>
<td>15</td>
<td>M, F Crossover 4 treatments</td>
<td>4 wk Tomato juice</td>
<td>Synthetic orange drink</td>
<td>Tomato juice Depletion 0 g</td>
<td>Tomato juice 330 mL</td>
<td>Carrot juice 330 mL</td>
<td>Dried spinach 10 g</td>
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<tr>
<td>(68)</td>
<td>2000</td>
<td>Bub Healthy M</td>
<td>23</td>
<td>2 treatments 4 treatments</td>
<td>2 wk each Tomato juice</td>
<td>Depletion 0 g</td>
<td>Tomato juice 330 mL</td>
<td>Carrot juice 330 mL</td>
<td>Dried spinach 10 g</td>
<td>Spinach/mango 300 g (2.1)</td>
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<tr>
<td>(64)</td>
<td>2000</td>
<td>Chopra Healthy F Smokers Nonsmokers 24–52 y</td>
<td>34</td>
<td>Healthy F Smokers Nonsmokers 24–52 y</td>
<td>7 d each Tomato/watermelon</td>
<td>Depletion 0 g</td>
<td>Tomato juice 320 mL</td>
<td>—</td>
<td>—</td>
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<td>(65)</td>
<td>2000</td>
<td>Upritchard Diabetes (T2D)</td>
<td>57</td>
<td>Diabetes (T2D) M, F 50–75 y</td>
<td>4 wk Tomato juice</td>
<td>Depletion 0 g</td>
<td>Tomato juice 300 mL</td>
<td>Vitamin E 800 IU</td>
<td>Vitamin C 500 mg</td>
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<td>(70)</td>
<td>2003</td>
<td>Hadley Healthy M, F</td>
<td>60</td>
<td>Healthy M, F &gt;40 y</td>
<td>15 d Tomato soup</td>
<td>Depletion 0 g</td>
<td>Tomato soup (condensed)</td>
<td>Tomato soup (RTE) 320 mL</td>
<td>V8 juice 340 mL</td>
<td>—</td>
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<td>(72)</td>
<td>2003</td>
<td>Visioli Healthy F HW 22–38 y</td>
<td>12</td>
<td>Healthy F HW 22–38 y</td>
<td>3 wk Tomato Raw</td>
<td>Depletion 0 g</td>
<td>Tomato Raw 100 g X 2 wk</td>
<td>Sauce 60 g X 3 wk</td>
<td>Paste 15 g X 2 wk</td>
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<td>(73)</td>
<td>2004</td>
<td>Briviba Healthy M</td>
<td>22</td>
<td>Healthy M Crossover 2 treatments</td>
<td>2 wk each 2-wk carotenoid depletion run-in, 2-wk washout Tomato juice</td>
<td>Carrot juice 330 mL</td>
<td>—</td>
<td>—</td>
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<tr>
<td>(86) 2004 Rao</td>
<td>17</td>
<td>Healthy M, F</td>
<td>1 treatments BC</td>
<td>4 wk</td>
<td>Tomato products</td>
<td>Tomato products, sauce, paste, soup, juice, purée, ketchup</td>
<td>30–454 mL</td>
<td>29–33</td>
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<td>(77) 2005 Bub</td>
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<td>Crossover 2 treatments</td>
<td>2 wk each</td>
<td>Tomato juice</td>
<td>Carrot juice, Tomato juice</td>
<td>330 mL, 330 mL</td>
<td>0</td>
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<td>(71) 2007 Silaste</td>
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<td>3 wk</td>
<td>Tomato juice</td>
<td>Low-tomato diet, Tomato juice, ketchup with all main meals</td>
<td>None 400 mL, 30 g</td>
<td>27</td>
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<td>(131) 2011 Shidfar</td>
<td>32</td>
<td>Diabetes (T2D) M 40–60 y</td>
<td>1 treatment BC</td>
<td>8 wk</td>
<td>Tomato, raw</td>
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<td>200 g at lunch</td>
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<td>(69) 2012 Burton-Freeman</td>
<td>25</td>
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<td>(74) 2012 Thies</td>
<td>225</td>
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1. BC: baseline control; HW: healthy weight for height based on standard BMI criteria; NI: not indicated; OB: obese BMI; OW: overweight BMI; RTE: ready to eat; T2D: type 2 diabetes; ↓: decrease or reduction; =: neutral or no effect compared with control arm, P < 0.05 (or baseline in 1-arm trials, P < 0.05).
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<td>Rao 19</td>
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1. AOX, antioxidant capacity; BC, baseline control; Cu, Zn-SOD, superoxide dismutase with copper or zinc; F$_2$ iso U, F$_2$ isoprostane urine; GPx, glutathione peroxidase; GSH, glutathione; GSSG, glutathione disulfide; HFm, high-fat meal; HNE, 4-hydroxynonenal or 4-hydroxy-2-nonenal; HW, healthy weight for height based on standard BMI criteria; LFm, low-fat meal; MDA, malonaldehyde; Ni, not indicated; OB, obese BMI; OW, overweight BMI; Ox, oxidative; PON-1, paraoxonase 1; Se-GSH-Px, selenium cofactor for glutathione peroxidase; SH, thiol; SOO, superoxide dismutase; TEAC, Trolox equivalent antioxidant capacity; TRAP, total peroxyl radical trapping; 8-OH-dG, 8-hydroxydeoxyguanosine; ↓, decrease or reduction; ↑, increase; =, neutral or no effect compared with control arm. $P < 0.05$ (or baseline in 1-arm trials, $P < 0.05$).
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<td>N</td>
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<td>Rehman 5 Healthy M, F (1:4) HW 27 ± 7 y</td>
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<td>1 dose</td>
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<td>Riso 10 Healthy F</td>
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<td>Visioli 12 Healthy F HW 22–38 y</td>
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1 AOX, antioxidant capacity; BC, baseline control; bw, body weight; CAT, catalase; CHD, coronary heart disease; Cu, Zn-SOD, superoxide dismutase with copper or zinc; F2 Iso, F2 isoprostane; FRAP, ferric-reducing antioxidant power; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, glutathione; GSSG, glutathione disulfide; HETE, hydroxyeicosatetraenoic acid; HNE, 4-hydroxy-2-nonenal; HW, healthy weight for height based on standard BMI criteria; MDA, malondialdehyde; Ni, not indicated; OB, obese BMI; ORAC, oxygen radical absorbance capacity; OW, overweight BMI; Ox, oxidative; P, plasma; PCO, protein carbonyl content; PON-1, paraoxonase 1; Px, peroxide; Q/R, glutathione/arginine; Se-GSH-Px, selenium cofactor for glutathione peroxidase; SOD, superoxide dismutase; Suppl, supplement; T2D, type 2 diabetes; TAC, total antioxidant capacity; TEAC, Trolox equivalent antioxidant capacity; tGSH, total glutathione; TJ, tomato juice; TRAP, total peroxyl radical trapping; U, Urine; 8-OH-dG, 8-hydroxydeoxyguanosine; ↓, decrease or reduction; ↑, increase; =, neutral or no effect compared to control arm, P < 0.05 (or baseline in 1-arm trials, P < 0.05).
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<td></td>
<td>LFm: breakfast, 1110 kcal, 243 g carbohydrate</td>
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</tr>
<tr>
<td>(108)</td>
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<td>Markovits</td>
<td>16</td>
<td>OB, HW</td>
<td>1 treatment</td>
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<td>Lyc-O-Mato</td>
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<td>30 mg/d</td>
<td>CRP (↓)</td>
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<td>CRP (↓)</td>
</tr>
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<tr>
<td>(74)</td>
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<td>Thies</td>
<td>225</td>
<td>M, F</td>
<td>Parallel</td>
<td>12 wk</td>
<td>Lycopene extract</td>
<td>Low tomato</td>
<td>0 mg/d</td>
<td>CRP (↓)</td>
</tr>
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<td></td>
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<td></td>
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<td>HW, OW, OB</td>
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<td></td>
<td></td>
<td>40–65 y</td>
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<td>CRP (↓)</td>
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<tr>
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<td>McEneny</td>
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<td>M, F</td>
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<td>Lycopene extract</td>
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<td>SAA (↓) (serum)</td>
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<td></td>
<td>HW, OW, OB</td>
<td>3 treatments</td>
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<td></td>
<td></td>
<td></td>
<td>40–65 y</td>
<td></td>
<td></td>
<td></td>
<td>Low tomato</td>
<td>0 mg/d</td>
<td>SAA (↓) (HDL)</td>
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</table>

1 BC, baseline control; CRP, C-reactive protein; HFm, high-fat meal; hsCRP, high-sensitivity C-reactive protein; HW, healthy weight for height based on standard BMI criteria; LFm, low-fat meal; OB, obese BMI; OW, overweight BMI; SAA, serum amyloid A; ↓, decrease or reduction; =, neutral or no effect compared with control arm, *P* < 0.05 (or baseline in 1-arm trials, *P* < 0.05).
<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>First author</th>
<th>n</th>
<th>Participant characteristics</th>
<th>Study design</th>
<th>Length of treatment</th>
<th>Tomato source</th>
<th>Treatments</th>
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<th>Lycopene dose</th>
<th>Inflammation findings</th>
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<td>Diabetes (T2D) M, F</td>
<td>Parallel</td>
<td>4 wk</td>
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<td>Placebo</td>
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<td>50s and 60s</td>
<td>4 treatments</td>
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<td>Vitamin C</td>
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<td>Watzl</td>
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<td>Crossover</td>
<td>2 wk</td>
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<td>Carrot juice</td>
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<td>IL-2 (†) with treatment, (——) b/n treatment</td>
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<td></td>
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<td></td>
<td>HW</td>
<td>2 treatments</td>
<td>2-wk run-in, 2-wk washout, 2-wk run-out</td>
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<td>300 mL</td>
<td>37</td>
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</tr>
<tr>
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<tr>
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<td>2006</td>
<td>Sánchez-Moreno</td>
<td>12</td>
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<td>1 treatment</td>
<td>14 d</td>
<td>Tomato soup</td>
<td>Tomato-based soup</td>
<td>500 mL, 250 mL x2/d</td>
<td>Ni</td>
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<td></td>
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<tr>
<td>(126)</td>
<td>2007</td>
<td>Blum</td>
<td>103 (1:1)</td>
<td>Healthy M, F</td>
<td>Parallel</td>
<td>1 mo</td>
<td>Tomato</td>
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<td>0</td>
<td>CRP (—)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OW, OB</td>
<td>2 treatments</td>
<td></td>
<td></td>
<td>Yes tomatoes</td>
<td>300 g</td>
<td>30</td>
<td></td>
</tr>
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<td></td>
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<td>46 ± 14 y</td>
<td>BC</td>
<td></td>
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<td>Usual diet</td>
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<td>Jacob</td>
<td>24</td>
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<td>2 wk</td>
<td>Tomato juice</td>
<td>Tomato juice</td>
<td>250 mL x2/d</td>
<td>21</td>
<td>IL-1β (—)</td>
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<td>250 mL x2/d</td>
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<td>19–27 y</td>
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<td>Burton-Freeman</td>
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<td>Crossover</td>
<td>2 wk</td>
<td>Tomato paste</td>
<td>Control meal</td>
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<td>0</td>
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<tr>
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<td></td>
<td>HW</td>
<td>Postprandial</td>
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<td>27</td>
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<td>2 treatments</td>
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<td>AM, PM</td>
<td></td>
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<tr>
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<td>2012</td>
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<td>225</td>
<td>Healthy F</td>
<td>Parallel</td>
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<td>Tomato products</td>
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<td>M, F (1:1.5)</td>
<td>3 treatments</td>
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<td>10</td>
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<td>HW, OW, OB</td>
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<td>Carotenoid depletion</td>
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<td>Tomato juice</td>
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<td>Tomato juice</td>
<td>Tomato juice</td>
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<tr>
<td>(110)</td>
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<td>Ghavipour</td>
<td>106</td>
<td>Healthy F</td>
<td>Parallel</td>
<td>20 d</td>
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<td>OW, OB</td>
<td>2 treatments</td>
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<td>110 mL X3</td>
<td>0</td>
<td>37</td>
<td></td>
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<td></td>
<td>20–40 y</td>
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<td></td>
<td></td>
<td>110 mL X3 with</td>
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</tbody>
</table>

(Continued)
Trolox equivalent antioxidant capacity (TEAC), and total peroxyl radical-trapping antioxidant parameter (TRAP). The ORAC and FRAP methods are probably the most established measures of AOX but still have their limitations. Antioxidant reactions are typically chain reactions that involve initiation, propagation, branching, and termination of free radicals. From a mechanistic standpoint, each AOX assay provides somewhat different information relative to their action in the sense of antioxidants inhibiting formation of ROS (initiation) or impacting propagation and branching steps and thereby breaking radical chain sequences, and unfortunately, often yield incongruent results. None of the assays provide specific insight to protection from damage, such as to lipids, DNA, or protein or upregulating gene expression of endogenous antioxidant defenses. Therefore, studies using these assay methodologies for AOX status to illustrate the associated health effects of tomato intake or lycopene supplementation should be interpreted with caution.

Comparing nonspecific plasma or serum AOX results after lycopene supplementation and tomato product consumption revealed no improvements in AOX after lycopene supplementation (0 of 4 indicated improvements) by using ORAC, TRAP, or TEAC methods. After tomato product consumption, only 2 studies out of 10 showed improvements in plasma or serum AOX. Changes in nonspecific measures of AOX do not always correlate with specific indicators of stress or damage to biologic components. For example, Kiokias and Gordon (62) reported no change in ORAC after a 3-wk intervention with fish oil plus lycopene supplement but did report decreases in LDL oxidation and DNA damage. Similarly, a decrease in lipid oxidation products was observed without change in AOX of plasma (68,72,77,78); and conversely, with increased AOX (i.e., TRAP) from tomato juice consumption (79), no change in lipid peroxidation (i.e., TBARS) was evident. Consequently, the utility of these nonspecific measures of oxidative status has been questioned.

Specific oxidative stress and damage indicators. When ROS are produced at a rate that exceeds antioxidant defense capabilities, oxidative stress can result. Sequentially, these free radicals start chain reactions that can damage major constituents in the cells, such as protein, lipid, and DNA resulting in functional impairments, mutations, and possibly cell death. Measuring specific changes in cellular components may be a better indicator of protection by dietary constituents, including lycopene and tomatoes.

As shown in Table 3, lycopene supplementation had a consistent lack of effect on changes in lipid peroxidation (i.e., TBARS, malondialdehyde, F2-isoprostanes) or protein oxidation (i.e., protein thiols), with only 2 trials reporting decreased serum TBARS or malondialdehyde (80,81). Notably, lycopene supplementation reduced DNA oxidative damage in 5 of 8 studies (62,63,82–84), although responses were inconsistent relative to the use of the comet assay with and without peroxide/oxidative stress induction (82,83,85).
TABLE 7  Clinical trials examining lycopene supplementation on markers of endothelial function

<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>First author</th>
<th>Participant characteristics</th>
<th>Study design</th>
<th>Length of treatment</th>
<th>Lycopene source</th>
<th>Treatments</th>
<th>Lycopene dose</th>
<th>FMD findings</th>
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</thead>
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<tr>
<td>(107)</td>
<td>2008</td>
<td>Dennis</td>
<td>M, F (2:1)</td>
<td>Postprandial</td>
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<td>Lycopene</td>
<td>Challenge meals</td>
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<td>siCAM (—), sVCAM (—)</td>
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<td></td>
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<td>HW, OW 18–26 y</td>
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<td>LFM: breakfast</td>
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<td>1110 kcal, 245 g carbohydrate</td>
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<tr>
<td>(84)</td>
<td>2011</td>
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<td>8 wk each</td>
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<td>ENDO-PAT (†)</td>
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<td>3 treatments</td>
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<td>Lycopene</td>
<td>6</td>
<td>sVCAM (—)</td>
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<td>Lycopene</td>
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<td>sICAM (—)</td>
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<td>M, F</td>
<td>Parallel</td>
<td>12 wk</td>
<td>Lycopene extract</td>
<td>Low tomato</td>
<td>0</td>
<td>ICAM-1 (—)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HW, OW, OB 40–65 y</td>
<td>3 treatments</td>
<td></td>
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<td>Low tomato +</td>
<td>10</td>
<td></td>
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<td></td>
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<td></td>
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<td>High tomato</td>
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</table>

1 BC, baseline control; ENDO-PAT, endo-peripheral arterial tonometry; FMD, flow-mediated dilation; HFM, high-fat meal; HW, healthy weight for height based on standard BMI criteria; ICAM-1, intracellular adhesion molecule 1; LFM, low-fat meal; OB, obese BMI; OW, overweight BMI; siCAM, soluble intracellular adhesion molecule; sVCAM, soluble vascular cellular adhesion molecule; †, decrease or reduction; ††, increase — neutral or no effect compared with control arm, P < 0.05 (or baseline in 1-arm trials, P < 0.05).

On the other hand, 10 of 17 studies testing tomato products reported decreases in endpoints related to lipid peroxidation. One study also evaluated protein oxidation and found an increase in protein thiols (86). Four of 6 studies (87–90) also showed significant decreases in DNA oxidation, with a fifth showing a marginal effect (P = 0.07) (91) (Table 4). Of the 6 studies measuring changes in endogenous antioxidant defenses, only 2 (92,93) reported increased antioxidant enzymes along with decreases in malondialdehyde.

Collectively, there are stronger data supporting the consumption of tomato products to reduce oxidative stress and damage markers. Lycopene also appears to have a beneficial effect on oxidative damage but not as strong. Concluding, there are stronger data supporting the consumption of tomato products to reduce oxidative stress and damage markers. Lycopene also appears to have a beneficial effect on oxidative damage but not as strong.

**Inflammation: Tomatoes versus Lycopene**

Over the past 2 decades substantial advances in basic and clinical science have illuminated the role of inflammation and the underlying cellular and molecular mechanisms that contribute to atherogenesis (57,58,94–97). Inflammation and oxidative stress are intimately linked. Changes in cellular redox status impact cellular processes, including NF-κB, a central signaling molecule in the induction of inflammation. NF-κB is a transcription factor that stimulates the encoding of a number of genes including those responsible for production of cytokines, chemokines, immunoreceptors, cell adhesion molecules, and acute phase proteins (98). Under conditions of oxidative stress, NF-κB is activated, resulting in an inflammatory response. Other important mediators of inflammation include pattern recognition receptors, such as toll-like receptors (TLRs), and kinases such as MAPK and c-Jun N-terminal kinase. The inflammatory response can be triggered by stimuli such as endotoxin (LPS from bacteria), viruses, and changes in amounts of ROS, FAs, cytokines, growth factors, and carcinogens.

Lycopene is a potent lipophilic antioxidant in vitro. Given the links between inflammation, oxidative stress, and obesity (a low-grade chronic inflammatory state) and chronic disease, investigations to determine the anti-inflammatory effects of lycopene or tomatoes have increased. Animal studies suggest a role for tomatoes and lycopene in reducing inflammation (99–102). C-reactive protein (CRP), an acute phase protein synthesized and secreted by the liver, is a commonly used marker of inflammation associated with CVD (103,104). The CDC and the American Heart Association have established clinical cutoffs for CRP: low risk, <1 mg/L; intermediate risk, 1–3 mg/L; and high risk, >3 mg/L (104). Changes in select cytokines, chemokines, immunoreceptors, cell adhesion molecules, and/or acute phase proteins are also used to assess inflammation status in response to intervention (105,106).

Changes in inflammatory status are an important component of determining CVD risk. Seven studies testing lycopene supplements reported changes in inflammatory biomarkers (Table 5). Of the 7 studies, 4 reported results for CRP: only 1 study reported a decrease in CRP (84), whereas the 3 others indicated no change in CRP (74,107,108). Changes in other inflammatory endpoints, such as IL-6, serum amyloid A (SAA), and TNF-α, were inconsistent (i.e., unchanged in some investigations and decreased in others) (see Table 5).

Nine investigations examined the effect of tomato intake on changes in inflammatory biomarkers (Table 6). Of the 9 studies, 5 studies reported at least 1 marker [e.g., IL-6, TNF-α, CRP, monocyte chemoattractant protein 1 (MCP-1)] consistent with improved inflammation status (69,78,109–111). CRP was assessed in 5 studies. Although 1 study reported a decrease in CRP (78), 4 others measuring CRP...
<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>First author</th>
<th>n</th>
<th>Participant characteristics</th>
<th>Study design</th>
<th>Length of treatment</th>
<th>Tomato source</th>
<th>Treatments</th>
<th>Daily dose</th>
<th>Lycopene dose</th>
<th>FMD findings</th>
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<td>(65)</td>
<td>2000</td>
<td>Upritchard</td>
<td>57</td>
<td>Diabetes (T2D) M, F</td>
<td>Parallel</td>
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<td>Placebo</td>
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<td>0 mg/d</td>
<td>ICAM-1 (—)</td>
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<tr>
<td></td>
<td></td>
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<td></td>
<td>50–60 y</td>
<td>4 treatments</td>
<td>run-in</td>
<td>Tomato juice</td>
<td>Tomato juice</td>
<td>500 mL (250 mL ×2)</td>
<td></td>
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</tr>
<tr>
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<td></td>
<td></td>
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<td>Vitamin E</td>
<td></td>
<td>0 mg</td>
<td>VCAM-1 (—)</td>
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<tr>
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<td></td>
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<td></td>
<td>Vitamin C</td>
<td></td>
<td>0 mg</td>
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<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>500 mg</td>
<td>0 mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(126)</td>
<td>2007</td>
<td>Blum</td>
<td>103</td>
<td>Healthy M, F OW, OB</td>
<td>Parallel</td>
<td>1 mo 2 treatments BC</td>
<td>Tomato</td>
<td>No tomatoes</td>
<td>0 g</td>
<td>0 mg/d</td>
<td>sICAM (†) (P = 0.07)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>46 ± 14 y</td>
<td>2 treatments</td>
<td></td>
<td>Yes tomatoes</td>
<td>300 g</td>
<td></td>
<td>sVCAM (—)</td>
<td></td>
</tr>
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<td></td>
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<td>Usual diet</td>
<td></td>
<td></td>
<td></td>
<td>E-selectin (—)</td>
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<tr>
<td>(148)</td>
<td>2011</td>
<td>Stangl</td>
<td>19</td>
<td>Postmenopausal F</td>
<td>Crossover</td>
<td>24 h 7 d</td>
<td>Tomato purée</td>
<td>No tomato</td>
<td>0 g</td>
<td>0 mg/d</td>
<td>FMD (—)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2 treatments</td>
<td></td>
<td>Tomato purée</td>
<td>purée with</td>
<td>70 g</td>
<td></td>
<td></td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4 wk</td>
<td></td>
<td></td>
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<tr>
<td>(69)</td>
<td>2012</td>
<td>Burton-Freeman</td>
<td>25</td>
<td>Healthy M, F HW</td>
<td>Crossover</td>
<td>1 d 6 h</td>
<td>Tomato paste</td>
<td>Control meal</td>
<td>0 g</td>
<td>0 mg/d</td>
<td>FMD (—)</td>
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<td></td>
<td></td>
<td></td>
<td>2 treatments</td>
<td></td>
<td>Tomato meal</td>
<td></td>
<td>94 g</td>
<td></td>
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<tr>
<td>(74)</td>
<td>2012</td>
<td>Thies</td>
<td>225</td>
<td>M, F (1:1.5) HW, OW, OB</td>
<td>Parallel</td>
<td>12 wk 4-wk run-in</td>
<td>Tomato products</td>
<td>Low tomato</td>
<td>Limited</td>
<td>0 mg/d</td>
<td>ICAM-1 (—)</td>
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<tr>
<td></td>
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<td></td>
<td>40–65 y</td>
<td>3 treatments</td>
<td></td>
<td>Low tomato + lycopene</td>
<td></td>
<td>10 mg capsule</td>
<td></td>
<td>PWV (—) (arterial stiffness)</td>
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<td>High tomato</td>
<td>Points system</td>
<td>32–50</td>
<td></td>
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<tr>
<td>(125)</td>
<td>2012</td>
<td>Xaplanteris</td>
<td>19</td>
<td>M, F 39 ± 13 y</td>
<td>Crossover</td>
<td>14 d 2-wk run-in/</td>
<td>Tomato paste</td>
<td>No tomato</td>
<td>0 g</td>
<td>0 mg/d</td>
<td>FMD (†)</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>2 treatments</td>
<td>wash-out</td>
<td>Tomato paste</td>
<td>Yes tomato</td>
<td>70 g</td>
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</table>

1 BC, baseline control; FMD, flow-mediated dilation; HW, healthy weight for height based on standard BMI criteria; ICAM-1, intracellular adhesion molecule 1; OB, obese BMI; OW, overweight BMI; PWV, pulse wave velocity; sICAM, soluble intracellular adhesion molecule; sVCAM, soluble vascular cellular adhesion molecule; T2D, type 2 diabetes; †, increase; —, neutral or no effect compared with control arm, P < 0.05 (or baseline in 1-arm trials, P < 0.05).
### TABLE 9  Clinical trials examining lycopene supplementation on blood pressure

<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>First author</th>
<th>n</th>
<th>Participant characteristics</th>
<th>Study design</th>
<th>Length of treatment</th>
<th>Lycopene source</th>
<th>Treatments</th>
<th>Lycopene dose</th>
<th>mg/d</th>
<th>Blood pressure findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>(60)</td>
<td>2006</td>
<td>Engelhard</td>
<td>31</td>
<td>Grade 1 hypertension 30–70 y No medications</td>
<td>Sequential, 2 treatments</td>
<td>16 wk: 4 wk placebo, 8 wk treatment, 4 wk placebo</td>
<td>Lyc-O-Mato</td>
<td>Placebo Lyc-O-Mato with meals</td>
<td>15</td>
<td>SBP (↓) DBP (↓)</td>
<td></td>
</tr>
<tr>
<td>(129)</td>
<td>2009</td>
<td>Paran</td>
<td>50</td>
<td>Grade 1 hypertension 46–66 y</td>
<td>Crossover 2 treatments Double-blind</td>
<td>6 wk each</td>
<td>Lyc-O-Mato</td>
<td>Placebo Lyc-O-Mato with meals</td>
<td>15</td>
<td>SBP (↓) DBP (↓)</td>
<td></td>
</tr>
<tr>
<td>(130)</td>
<td>2009</td>
<td>Ried</td>
<td>36</td>
<td>Prehypertension HW, OW 50 ± 12 y</td>
<td>Parallel -1 Crossover -2/ active treatment only 3 treatments</td>
<td>8 wk each</td>
<td>Lyc-O-Mato</td>
<td>Placebo Tomato extract Dark chocolate</td>
<td>15</td>
<td>SBP (↓) DBP (↓)</td>
<td></td>
</tr>
<tr>
<td>(84)</td>
<td>2011</td>
<td>Kim</td>
<td>126</td>
<td>Healthy M</td>
<td>Parallel 3 treatments</td>
<td>8 wk each</td>
<td>Lycopene</td>
<td>Placebo Lycopene Lycopene</td>
<td>0</td>
<td>SBP (↓)</td>
<td></td>
</tr>
<tr>
<td>(74)</td>
<td>2012</td>
<td>Thies</td>
<td>225</td>
<td>M,F (1:1.5) HW, OW, OB 40–65 y</td>
<td>Parallel 3 treatments</td>
<td>12 wk</td>
<td>Lycopene extract</td>
<td>Low tomato Low tomato + lycopene High tomato</td>
<td>10</td>
<td>SBP (↓) DBP (↓)</td>
<td></td>
</tr>
</tbody>
</table>

ACE, angiotensin converting enzyme; CCB, calcium channel blocker; DBP, diastolic blood pressure; HW, healthy weight for height based on standard BMI criteria; OB, obese BMI; OW, overweight BMI; SBP, systolic blood pressure; ↓, decrease or reduction; —, neutral or no effect compared with control arm, P < 0.05 (or baseline in 1-arm trials, P < 0.05).
reported no effect on CRP (65,69,74,110). Heterogeneity across these studies in terms of sample size (up to \( n = 225 \)), age of people studied, duration, and the dose and amount of tomato products consumed may account, at least in part, for the lack of consistency in results. Whether changes in other markers of inflammation are advantageous to overall health remains under intense investigation.

Overall, improvements in inflammatory markers such as CRP after lycopene supplementation were inconsistent. Studies using tomato products were also inconsistent in their findings for CRP, but tomato intake may provide some advantage showing improvements in other inflammatory markers (78,109,110) although their clinical utility remains undetermined. Collectively, the data are underwhelming for the anti-inflammatory effects of lycopene or tomato products; however, additional investigations are expected to yield new data on this topic.

Of note, on the basis of preclinical data and at least 1 trial, there are documented effects of tomato products on inflammation during the postprandial state (69). Future investigations may initially focus on understanding the shorter-term postprandial effect, and then move to longer duration trials thereafter. Addressing oxidative and inflammatory fluctuations and their derived insults during postprandial metabolism may be at the heart of innovation in effective food-based therapeutic strategies.

Endothelial Function: Tomatoes and Lycopene

The vascular endothelium is a critical regulator of vascular homeostasis. The endothelium has multiple functions as a semiselective barrier between the vessel lumen and surrounding tissues, regulating thrombosis and fibrinolysis, angiogenesis, and synthesis and secretion of vasodilating (e.g., NO) and vasoconstricting (e.g., endothelin-1) factors (34,112,113). Loss of proper endothelial function is a hallmark of vascular diseases and is often regarded as a key early event in the atherosclerotic process. A key mechanism of endothelial dysfunction is the lack of bioavailable NO (112). The prevailing wisdom for a lack of available endothelium-derived NO is an increase in ROS. Residual ROS, particularly \( \text{O}_2^• \) from exhausted defenses, reacts with NO to produce peroxynitrite (ONOO\(^•\)), a harmful RNS in NO-producing endothelial cells (114,115). Rapid degradation of NO by \( \text{O}_2^• \) is 1 of the most widely accepted mechanisms involved in the alteration of the eNOS/NO signaling pathway, resulting in impaired endothelial function.

Endothelial Function: Tomatoes and Lycopene is examined in the table and studies on blood pressure. Table 10 provides a summary of clinical trials examining tomato and tomato products on blood pressure.

**Table 10: Clinical trials examining tomato and tomato products on blood pressure**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>First author</th>
<th>n</th>
<th>Participant characteristics</th>
<th>Study design</th>
<th>Length of treatment</th>
<th>Tomato source</th>
<th>Treatments</th>
<th>Daily dose</th>
<th>Lycopene dose</th>
<th>Effects on blood pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>(65)</td>
<td>2000</td>
<td>Upritchard</td>
<td>57</td>
<td>Diabetes (T2D) M, F &lt;75 y</td>
<td>Parallel</td>
<td>4 wk</td>
<td>Tomato juice</td>
<td>Placebo</td>
<td>Capsule</td>
<td>0</td>
<td>SBP (−)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>50s and 60s</td>
<td>4 treatments</td>
<td></td>
<td></td>
<td>Vitamin E</td>
<td>800 IU</td>
<td>44</td>
<td>DBP (−)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Vitamin C</td>
<td>500 mg</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
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<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(131)</td>
<td>2011</td>
<td>Shidfar</td>
<td>32</td>
<td>Diabetes (T2D) M, F HW, OW</td>
<td>Parallel</td>
<td>8 wk</td>
<td>Tomato, raw</td>
<td>Raw tomato</td>
<td>200 g</td>
<td>SBP (−)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>40–60 y</td>
<td>1 treatment</td>
<td></td>
<td></td>
<td>At lunch</td>
<td></td>
<td>DBP (−)</td>
<td></td>
</tr>
<tr>
<td>(74)</td>
<td>2012</td>
<td>Thies</td>
<td>225</td>
<td>M, F HW, OW, OB 40–65 y</td>
<td>Parallel</td>
<td>12 wk</td>
<td>Tomato products</td>
<td>Low tomato</td>
<td>Limited</td>
<td>0</td>
<td>SBP (−)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3 treatments</td>
<td></td>
<td></td>
<td>Low tomato + lycopene</td>
<td>10 mg</td>
<td>DBP (−)</td>
<td></td>
</tr>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>High tomato</td>
<td>Points system</td>
<td>32–50</td>
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</tr>
</tbody>
</table>

1 BC, baseline control; DBP, diastolic blood pressure; HW, healthy weight for height based on standard BMI criteria; OB, obese BMI; OW, overweight BMI; SBP, systolic blood pressure; T2D, type 2 diabetes; Y, decrease or reduction; —, neutral or no effect compared with control arm, \( P < 0.05 \) (or baseline in 1-arm trials, \( P < 0.005 \)).
TABLE 11  Clinical trials examining lycopene supplementation on lipids

<table>
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<tr>
<th>Reference</th>
<th>Year</th>
<th>First author</th>
<th>n</th>
<th>Participant characteristics</th>
<th>Study design</th>
<th>Length of treatment</th>
<th>Lycopene source</th>
<th>Treatments</th>
<th>Lycopene dose</th>
<th>Lipid findings</th>
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<tbody>
<tr>
<td>(59)</td>
<td>1998</td>
<td>Agarwal</td>
<td>19</td>
<td>M, F</td>
<td>Crossover</td>
<td>1 wk each</td>
<td>Lyc-O-Mato</td>
<td>Placebo</td>
<td>0</td>
<td>TC (—)</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>4 treatments</td>
<td></td>
<td></td>
<td>Lyc-O-Mato</td>
<td>75</td>
<td>TG (—)</td>
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<td></td>
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<td></td>
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<td>Spaghetti sauce</td>
<td>39.2</td>
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<td>Tomato juice</td>
<td>50.4</td>
<td>HDL (—)</td>
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<tr>
<td>(143)</td>
<td>1999</td>
<td>Böhm</td>
<td>22</td>
<td>F</td>
<td>Parallel</td>
<td>6 wk</td>
<td>Lyc-O-Mato</td>
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<td>5</td>
<td>TC (—)</td>
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<td>3 treatments</td>
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<td>Tomato</td>
<td>5</td>
<td>TG (—)</td>
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<td>Tomato juice with dinner</td>
<td>5</td>
<td>HDL (—)</td>
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<td>(62)</td>
<td>2003</td>
<td>Kiokias</td>
<td>32</td>
<td>M, F</td>
<td>Crossover</td>
<td>3 wk each</td>
<td>Lyc-O-Mato</td>
<td>Fish oil</td>
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<td>Tomato juice with dinner</td>
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<td>Red tomato paste</td>
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<td>TG (—)</td>
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<td>Grade1 hypertension</td>
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<td>30–70 y</td>
<td>2 treatments</td>
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<td>15</td>
<td>TG (—)</td>
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<td>(80)</td>
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<td>Misra</td>
<td>41</td>
<td>Healthy postmenopausal F</td>
<td>Parallel</td>
<td>6 mo</td>
<td>LycorRed</td>
<td>HRT</td>
<td>0</td>
<td>TC (—)</td>
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<td></td>
<td></td>
<td>2 treatments</td>
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<td>LycorRed</td>
<td>4</td>
<td>TG (†)</td>
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<tr>
<td>(79)</td>
<td>2007</td>
<td>Shen</td>
<td>24</td>
<td>Volunteers, Unde</td>
<td>Parallel</td>
<td>6 wk</td>
<td>Lycopene, food-grade</td>
<td>Lycopene</td>
<td>TC (—)</td>
<td>40</td>
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<td>fined</td>
<td>3 treatments</td>
<td></td>
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<td>Tomatoes, small raw</td>
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<td>LDL (—)</td>
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<td>BC</td>
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<td>Tomato juice</td>
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<td>HDL (—)</td>
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<td>Lunch and dinner</td>
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<td>HDL (—)</td>
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<tr>
<td>(107)</td>
<td>2008</td>
<td>Denniss</td>
<td>27</td>
<td>M, F (2:1)</td>
<td>Postprandial</td>
<td>1 wk</td>
<td>Lyc-O-Mato</td>
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<tr>
<td>(63)</td>
<td>2008</td>
<td>Devaraj</td>
<td>77</td>
<td>M, F</td>
<td>Parallel</td>
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<td>(136)</td>
<td>2010</td>
<td>Talvas</td>
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<td>16</td>
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(Continued)
Cell culture experiments established that lycopene can protect NO from $O_2^-$ destruction (117) and attenuate cytokine-induced endothelial cell adhesion molecule expression and leukocyte-endothelial interactions (118). Moreover, in mice, tomato supplementation protected against endothelial vaso-motor dysfunction developed in response to a 4-mo atherogenic high-fat diet (119). Emerging evidence suggests a significant role of dietary factors in modulating endothelial function both negatively (120–122) and positively (123,124). Western high-fat, high-carbohydrate diets increase markers of endothelial dysfunction, and consumption of certain plant components can reduce markers of dysfunction.

Few studies examined endothelial function in response to lycopene supplementation (Table 7; $n = 3$) or tomato consumption (Table 8; $n = 6$). Of the 3 trials testing lycopene supplements (74,84,107), only Kim et al. (84) reported a positive outcome among 126 individuals divided into 3 treatments (~42/group) for 8 wk who consumed either 0, 6, or 15 mg lycopene/d. Effects were most apparent for 15 mg/d, which corresponded with improvements in microvascular function as measured by endo-peripheral arterial tonometry as well as decreased concentrations of soluble intracellular adhesion molecule (sICAM) and soluble vascular adhesion molecule (sVCAM). A concomitant decrease in systolic BP was also observed, along with improvements in inflammatory and oxidative stress status.

For tomato intake, only 1 of 6 studies reported a positive increase in endothelial function as measured by FMD (~3.3% increase) (125). The increase in FMD was shown after a 2-wk washout (baseline) followed by 14 d of inclusion of 70 g tomato paste added into participants’ usual diet versus no added tomato paste to the diet.

Adhesion molecules are another indicator of endothelial disruption, although they are also considered markers of inflammation that are associated with the vascular endothelium. A marginally significant ($P = 0.07$) improvement in adhesion molecules (sICAMs) was observed after 1 mo of adding tomatoes (compared with no tomatoes) to the diet (126); however, other markers (sVCAM and E-selectin) were unchanged. Overall, few investigations have addressed the effects of lycopene or tomato intake on endothelial function.

**BP: Tomatoes and Lycopene**

Normal BP is continuously regulated by the autonomic nervous system through an extensive network of receptors, hormones, and nerves. The endothelium is critical in the maintenance of normal BP. Likewise, endothelial dysfunction is recognized for its role in hypertension and vascular disease. Central to the function of the endothelium is sufficient NO bioavailability, a master molecule known for its important relaxant properties and influence on BP (127,128). Oxidative stress conditions decrease the bioavailability of NO. Therefore, consuming antioxidants has potentially beneficial effects on BP control and CVD risk.

To date, 5 lycopene supplement and 3 tomato studies (Tables 9 and 10, respectively) have tested their effects on
<table>
<thead>
<tr>
<th>Reference</th>
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<th>n</th>
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<th>Study design</th>
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<td>(59)</td>
<td>1998</td>
<td>Agarwal</td>
<td>19</td>
<td>Healthy M, F, OW 25–40 y</td>
<td>Crossover</td>
<td>1 wk each, 4 treatments</td>
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<td>Placebo</td>
<td>0 mg</td>
<td>TC (−)</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Spaghetti sauce</td>
<td>Lyc-O-Mato</td>
<td>75 mg</td>
<td>TG (−)</td>
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<td></td>
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<td></td>
<td></td>
<td>Parallel</td>
<td>4 wk, 4 treatments</td>
<td>Spaghetti sauce</td>
<td>Tomato juice</td>
<td>39.2 mg</td>
<td>LDL (−)</td>
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<td></td>
<td></td>
<td>Tomato juice</td>
<td>50.4 mg</td>
<td>HDL (−)</td>
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<tr>
<td>(65)</td>
<td>2000</td>
<td>Upritchard</td>
<td>57</td>
<td>Diabetes (T2D), M/F &lt;75 y</td>
<td>Parallel</td>
<td>4 wk, 4-wk placebo run-in</td>
<td>Tomato juice</td>
<td>Placebo</td>
<td>0 mg</td>
<td>TC (−)</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>Tomato juice</td>
<td>0 mg</td>
<td>TC (−)</td>
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<td></td>
<td></td>
<td>Parallel</td>
<td>15 d, 3 treatments</td>
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<td>44 mg</td>
<td>TC (−)</td>
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<td>V8 juice</td>
<td>Vitamin E</td>
<td>0 mg</td>
<td>TC (−)</td>
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<td></td>
<td></td>
<td></td>
<td>Vitamin C</td>
<td>0 mg</td>
<td>TC (−)</td>
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<tr>
<td>(70)</td>
<td>2003</td>
<td>Hadley</td>
<td>60</td>
<td>Healthy M, F, &gt;40 y</td>
<td>Parallel</td>
<td>15 d, 3 treatments</td>
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<td>Tomato soup (condensed)</td>
<td>35 mg</td>
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<td>V8 juice</td>
<td>Tomato soup (RTE)</td>
<td>23 mg</td>
<td>TG (−)</td>
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<td>(135)</td>
<td>2004</td>
<td>Collins</td>
<td>10</td>
<td>Healthy M, F, HW, OW, OB 35–68 y</td>
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<td>No tomatoes</td>
<td>0 mg</td>
<td>TC (−)</td>
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<td></td>
<td></td>
<td>Tomato juice</td>
<td>0 mg</td>
<td>TC (−)</td>
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<td></td>
<td>Parallel</td>
<td>1 mo, 2 treatments</td>
<td>Tomato soup</td>
<td>No tomatoes</td>
<td>30 mg</td>
<td>TC (−)</td>
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<td></td>
<td>Tomato soup</td>
<td>Yes tomatoes</td>
<td>30 mg</td>
<td>TC (−)</td>
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<td></td>
<td></td>
<td>Small raw tomatoes</td>
<td>0 mg</td>
<td>TC (−)</td>
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<tr>
<td>(133)</td>
<td>2006</td>
<td>Blum</td>
<td>98</td>
<td>Healthy M, F, OW, OB 46 ± 14 y</td>
<td>Parallel</td>
<td>1 mo, 2 treatments</td>
<td>Tomato soup</td>
<td>Usual diet</td>
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<td>TC (−)</td>
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<td>Tomato soup</td>
<td>Yes tomatoes</td>
<td>30 mg</td>
<td>TC (−)</td>
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<td>(92)</td>
<td>2006</td>
<td>Bose</td>
<td>90</td>
<td>Healthy and diabetes (T2D)</td>
<td>Crossover</td>
<td>60 d, (n = 30 of T2D only)</td>
<td>Tomatoes (cooked)</td>
<td>Tomatoes (cooked)</td>
<td>200 g in Suppl group (n = 30)</td>
<td>TC (−)</td>
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<td>(n = 30)</td>
<td>(n = 30 of T2D only)</td>
<td>TC (−)</td>
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<td>Parallel</td>
<td>7 d, 1 treatment</td>
<td>Tomato juice</td>
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<td>~18 mg/70 kg</td>
<td>TC/HDL (−)</td>
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<td>(n = 30 of T2D only)</td>
<td>TC (−)</td>
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<td>(134)</td>
<td>2006</td>
<td>Madrid</td>
<td>17</td>
<td>Healthy M, F</td>
<td>1 treatment</td>
<td>7 d, 1 treatment</td>
<td>Tomato juice</td>
<td>Tomato juice</td>
<td>~25 g</td>
<td>TC (−)</td>
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<td>(n = 30)</td>
<td>(n = 30 of T2D only)</td>
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<tr>
<td>(93)</td>
<td>2007</td>
<td>Bose</td>
<td>80</td>
<td>Healthy and CHD</td>
<td>1 treatment</td>
<td>60 d, 1 treatment</td>
<td>Tomato (cooked)</td>
<td>Tomatoes (cooked)</td>
<td>~40 mg</td>
<td>TC (−)</td>
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<td>(n = 30 of T2D only)</td>
<td>TC (−)</td>
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<tr>
<td>(79)</td>
<td>2007</td>
<td>Shen</td>
<td>24</td>
<td>Unspecified, HW 18–23 y</td>
<td>Parallel</td>
<td>6 wk, 3 treatments</td>
<td>Tomato juice</td>
<td>Small raw tomatoes</td>
<td>~40 mg</td>
<td>TC (−)</td>
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<td></td>
<td>Tomato juice</td>
<td>Food-grade lycopene</td>
<td>~40 mg</td>
<td>TC (−)</td>
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<td></td>
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<td></td>
<td>Lunch and dinner</td>
<td>~40 mg</td>
<td>TC (−)</td>
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<th>Treatments</th>
<th>Lycopene dose</th>
<th>Lipids</th>
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<tr>
<td>(71) 2007 Silaste</td>
<td>21</td>
<td>Healthy M,F</td>
<td>20–49 y</td>
<td>Sequential 2 treatments BC</td>
<td>3 wk</td>
<td>2-wk baseline, 3-wk low-tomato diet</td>
<td>Tomato juice</td>
<td>Low tomato diet</td>
<td>Ketchup</td>
<td>High tomato diet</td>
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<td>(78) 2008 Jacob</td>
<td>24</td>
<td>Healthy M,F</td>
<td>19–27 y</td>
<td>Parallel 2 treatments</td>
<td>2 wk</td>
<td>2-wk run-in</td>
<td>Tomato juice</td>
<td>Tomato juice</td>
<td>21 mg</td>
<td>TC (↑)</td>
</tr>
<tr>
<td>(136) 2010 Talvas</td>
<td>30</td>
<td>Healthy OW</td>
<td>50–70 y</td>
<td>Crossover 2 treatments</td>
<td>1 wk each</td>
<td>Tomato paste</td>
<td>Yellow tomato</td>
<td>0 mg</td>
<td>TC (↑)</td>
<td></td>
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<tr>
<td>(131) 2011 Shidfar</td>
<td>32</td>
<td>Diabetes (T2D) M</td>
<td>40–60 y</td>
<td>1 treatment BC</td>
<td>8 wk</td>
<td>2-wk non-tomato run-in</td>
<td>Tomato, raw</td>
<td>Raw tomato</td>
<td>apoB (↑)</td>
<td>apoA-1 (↑)</td>
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<tr>
<td>(74) 2012 Thies</td>
<td>225</td>
<td>M,F</td>
<td>HW, OW, OB</td>
<td>Parallel 3 treatments</td>
<td>12 wk</td>
<td>4-wk run-in</td>
<td>Tomato products</td>
<td>Low tomato</td>
<td>0 mg</td>
<td>TC (↑)</td>
</tr>
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</table>

1 BC, baseline control; HW, healthy weight for height based on standard BMI criteria; OB, obese BMI; OW, overweight BMI; Suppl, supplement; T, tomato T2D, type 2 diabetes; TJ, tomato juice; RTE, ready to eat; TC, total cholesterol; ↓, decrease or reduction; ↑, increase; —, neutral or no effect compared with control arm, P < 0.05 (or baseline in 1-arm trials, P < 0.05).
BP. In 3 of 5 lycopene supplement studies, lycopene at 15 mg/d for 6–8 wk decreased systolic BP (60,84,129) and decreased diastolic BP (60,129). The benefits were apparent in individuals with stage 1 hypertension (60,129) who were otherwise healthy (84). Two other studies reported no differences in BP after lycopene supplementation at similar doses and duration (74,130).

Among 3 studies, tomato products decreased BP in individuals with type 2 diabetes after 8 wk of consumption of 200 g raw tomatoes (131); however, no changes were observed in a second investigation in individuals with type 2 diabetes or in those who were relatively healthy after consumption of 500 mL tomato juice or a high-tomato diet for 4 or 12 wk, respectively (65,74). It is not clear whether tomato type, intervention duration, sample size, amount consumed, or starting BP influenced the results. Overall, the data for tomato products suggest that a higher initial BP results in more favorable declines in BP (60,129,132), such that those with prehypertension or hypertension may be better indicated for improvements in BP.

In summary, there is promising evidence for a role for both lycopene supplementation and tomato products for improvements in systolic and diastolic BP. To date, data are more supportive for lycopene supplementation than tomato intake for reducing BP. Additional research is needed to determine the mechanisms through which lycopene and tomato intake affects BP control. Tomatoes provide several other essential nutrients and phytochemicals that support healthy BP, including potassium and fiber.

**Lipids: Tomatoes and Lycopene**

Elevated TC, elevated LDL cholesterol, and lower HDL cholesterol are widely recognized as risk factors for CVD. The relation between lycopene and tomato product ingestion and cholesterol are widely recognized as risk factors for CVD. The relationship between lycopene and tomato product ingestion and cholesterol metabolism was recently reviewed (56). Briefer, possible mechanisms implicated in cholesterol reduction by lycopene and tomato derivatives include decreased cholesterol synthesis through inhibition of 3-hydroxy-3-methylglutaryl-CoA reductase activity and expression, modulation of LDL receptors, and inhibition of acetyl-coA acetyltransferase activity (56). Although animal and cell culture studies have revealed favorable effects of lycopene and tomato feeding on lipid and cholesterol metabolism, the literature is inconsistent. Of 11 clinical trials testing the effects of lycopene supplementation (Table 11), only 1 showed improvements in TC, LDL cholesterol, and HDL cholesterol, although an increase in TGs was also reported in these postmenopausal women (80). Another study reported improvements in TGs but not in TC, LDL cholesterol, or HDL cholesterol when combined with fish oil (62). One study measured LDL particle size, finding increased size after lycopene supplementation (84), which is favorable for CVD risk reduction on the basis of findings that smaller, dense LDLs are more atherogenic (57,58). In contrast, among 14 clinical trials that provided tomato products for 1 to 12 wk (Table 12), TC decreased in only 3 studies (70,71,78), LDLs decreased in 2 studies (71,79), HDLs increased in 3 studies (79,133,134), and apoA-1, involved with HDL metabolism, was increased in 1 other study (131). Improvements in TGs were reported in 1 study (79).

A major drawback of the tomato-based clinical trials is that few compared effects against a control diet, and therefore relied on examining changes from baseline. Comparator group differences have tended to be stronger. Among the investigations reporting no effect of tomato products on lipid endpoints, 5 investigations had a comparison group (59,65,74,135,136). These data emphasize the importance of having proper controls to better understand the efficacy of specific interventions.

One research area that deserves more attention is the role of tomato and lycopene intake on HDL metabolism. Three tomato-based studies (79,133,134) and 1 lycopene supplement study (80) revealed improvements in HDL cholesterol. HDLs must be functional to be protective (137); apoA-1 is a critical component of HDLs and when its production is lacking or displaced from HDLs, HDLs are rendered relatively dysfunctional. In states of chronic low-grade inflammation, such as obesity, apoA-1 can be displaced by the inflammatory protein SAA; the adipose tissue is a major source of SAA. HDLs rendered dysfunctional by this exchange promote a proatherogenic phenotype and increase cholesterol ester (CE) deposition in the arterial wall by enhancing CE uptake by macrophages and reducing reverse CE transport. The modified HDLs act much like modified/oxidized LDLs to promote an overall unstable vessel environment. Modified HDLs are also less effective in protecting LDLs from oxidation via HDL–paraoxonase-1 (PON-1) activity and presence (137), adding to the inflamed vessel environment. Therefore, dietary strategies that can increase HDL cholesterol and or at minimum preserve the functionality of HDLs would have important therapeutic and disease risk–lowering implications.

In a recent investigation by McEneny et al. (111), 54 moderately overweight, middle-aged individuals consumed <10 mg/wk of lycopene (control), 224–350 mg/wk dietary lycopene from tomato-based products, or 70 mg/wk lycopene supplement for 12 wk. The associations with SAA and HDL fractions were evaluated along with other functional indicators such as PON-1 activity. Serum and HDL fractions were enriched with lycopene compared with control after the food-based and supplement intervention. SAA was reduced in the HDL<sub>3</sub> fraction and PON-1 activity increased after lycopene supplementation. The dietary/tomato lycopene intervention produced intermediate results. Overall, supportive evidence continues to accumulate for a role of lycopene in HDL metabolism and functionality.

**Summary and Conclusions**

The purpose of this review was to examine the available human clinical trials assessing the efficacy of lycopene supplements versus tomato products on CVD risk factors. Epidemiologic investigations describing an inverse relation between blood and tissue lycopene concentrations and risk of CVD along with preclinical data revealing biologic activity...
of lycopene support the hypothesis that lycopene and/or tomatoes may be associated with reductions in CVD risk. A major question, however, is whether lycopene as a dietary supplement can deliver cardiovascular benefits equivalent to tomatoes. History indicates that single-nutrient approaches often fail and on occasion are harmful (138). Nonetheless, there continues to be interest by consumers and a booming dietary supplement industry built on sales of naturally and synthetically derived individual nutrients and dietary components, of which lycopene and tomatoes are 1 example.

We have reviewed the results of lycopene supplementation and tomato-based food interventions on traditional and emerging CVD risk factors. Our goal was to determine whether there was compelling evidence in favor of lycopene supplementation or tomato intake to reduce CVD risk. We focused on oxidative stress and damage, inflammation, endothelial function, BP, and lipid metabolism for this review. Lycopene is a well-established phytochemical with numerous clinical trials published to date.

Overall, there was more support for consuming tomato products versus taking a lycopene supplement daily for improvements in lipoproteins (e.g., LDL oxidation), lipids, and protein and DNA from oxidative damage. Results were overwhelming for both tomato products and lycopene supplements on selected inflammatory markers such as CRP. However, the available evidence remains limited and represents an opportunity for future research on the anti-inflammatory activity of tomatoes and lycopene. Specifically, the study of tomato and lycopene activity on acute disturbances, such as metabolic inflammation during postprandial metabolism, may provide insight for designing efficacy trials assessing long-term benefits.

Lycopene supplementation was shown in 3 of 5 studies to reduce BP, whereas tomato intake reduced BP in 1 of 3 clinical trials. The evidence favors lycopene supplementation as a therapeutic strategy in lowering BP among hypertensive individuals (60,129). However, Kim et al. (84) reported reduced systolic BP at a similar dose (15 mg/d lycopene) in healthy participants, suggesting that lycopene may also have a role in reducing or maintaining BP within the normal range.

Finally, improvements in lipid metabolism are modest but supportive for tomato intake versus taking a lycopene supplement. The emerging focus on HDL metabolism and function for tomato intake is a promising area of research. Likewise, although only 1 study examined LDL particle size to date (84), this represents another potential research opportunity for lycopene and tomatoes.

Tomatoes provide a number of key nutrients and phytochemicals, including lycopene, that support cardiovascular health. The study of bioactive components in foods and/or supplements is growing, in part due to consumer interest and new market opportunities. Tomatoes and lycopene are good examples of intensified attention by consumers, researchers, and industry. Considering the diverse interests, some of which may be overlapping (intellectual, health, economical), research is of paramount importance to support appropriate messaging, claims, and recommendations about tomatoes and lycopene intake. The present review highlights the need for more targeted research; however, at present, the available trials support consuming tomato-based foods as a first-line approach compared with lycopene supplementation, with the exception of BP management. Future research that is well designed, clinically focused, mechanistically revealing, and relevant to human intake will undoubtedly add to the growing body of knowledge unveiling the promise of tomatoes and/or lycopene supplementation as an integral component of a heart-healthy diet.

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