

Novel Therapeutic Strategies for Adult Obese Asthmatics



Angela L. Linderholm, PhD^a, Jennifer M. Bratt, PhD^a,
Gertrud U. Schuster, PhD^{b,c}, Amir A. Zeki, MD, MAS^a,
Nicholas J. Kenyon, MD, MAS^{a,*}

KEYWORDS

• Severe asthma • L-Arginine • Nitric oxide • Metformin • Statins • Obesity

KEY POINTS

- In the future, treatment regimens for obese, adult asthmatics may include several interventions that interfere with pathways common to several metabolic and nutritional disorders.
- The diabetic drug, metformin, could decrease inflammatory mediators of asthma by improving insulin sensitivity and altering adenosine monophosphate-activated protein kinase (AMPK).
- The cholesterol-lowering class of medications, statins, could have beneficial effects on both airway inflammation and structural remodeling in asthma.
- L-Arginine supplementation may benefit a subset of severe asthmatic patients with impaired nitric oxide (NO) synthase function in the lung.

INTRODUCTION

Adult obese patients with worsening asthma despite appropriate controller drug therapy are extraordinarily complicated to manage and treat. For example, consider a 40-year-old woman with a medical history notable for adult-onset nonallergic

Disclosure: The authors appreciate ongoing support from the National Institutes of Health (T32 HL07013 [J.M. Bratt], K08 HL076415 [A. Zeki], and HL105573, AI097354 [N.J. Kenyon]). The authors have no other disclosures.

^a Division of Pulmonary, Critical Care and Sleep Medicine, Department of Internal Medicine, University of California, Davis, 4150 V Street, Suite 3100, Davis, CA, USA; ^b Nutrition Department, University of California, Davis, 430 West Health Sciences Drive, Davis, CA, USA; ^c Immunity and Diseases Prevention Unit, Western Human Nutrition Research Center, United States Department of Agriculture (USDA), Agricultural Research Services (ARS), 430 West Health Sciences Drive, Davis, CA, USA

* Corresponding author. Division of Pulmonary, Critical Care and Sleep Medicine, University of California, Davis, 451 Health Sciences Drive, GBSF, Suite 6510, Davis, CA 95616.

E-mail address: njkenyon@ucdavis.edu

Immunol Allergy Clin N Am 34 (2014) 809–823

<http://dx.doi.org/10.1016/j.iac.2014.07.006>

immunology.theclinics.com

0889-8561/14\$ – see front matter © 2014 Elsevier Inc. All rights reserved.

asthma, obesity, diabetes mellitus, and sleep apnea whose course has been punctuated by several emergency department admissions in the past year. She already requires continuous oral prednisone and 4-drug therapy for her asthma. How should such a patient be evaluated and treated for the foreseeable future? Although asthma is a complex syndrome that affects an estimated 26 million people in the United States, there are gaps in the recognition and management of asthmatic subgroups. Extrapolating results from short-term, randomized clinical trials to a broad, heterogeneous population of asthmatics treated in community settings is fraught with difficulty and can result in repeated trial-and-error therapeutic interventions. The ability to recognize different asthma phenotypes, to adapt and integrate care when comorbidities exist, and adopt new treatments is still lacking. Although published guidelines, including the National Asthma Education and Prevention Program Expert Panel Report 3 and the World Health Organization Global Initiative for Asthma, present stepwise evaluation and therapeutic recommendations for chronic persistent asthma management, they do not outline coherent plans for the care of adult-onset obese asthmatics.

This article proposes alternative approaches that may prove to be future treatments for adult obese asthmatics who do not respond to the standard controller asthma therapies of inhaled corticosteroids, bronchodilators, and antileukotriene (LT) drugs. Parallels are drawn between seemingly disparate therapeutics through their common signaling pathways (Fig. 1). Specifically, how metformin and statins potentially improve airway inflammation through activation of AMPK, a key regulator of cellular metabolism and energy production, and through their effects on NO is described. In addition, nutritional supplements, such as L-arginine, omega-3 (n-3) fatty acids, and other minerals and vitamins that are currently studied and may potentially be used in combination with conventional therapies are described.

METFORMIN, INSULIN RESISTANCE, AND ASTHMA

The metabolic syndrome with insulin resistance may characterize subsets of asthmatics more than is recognized. The relationship between obesity, insulin resistance, and asthma has been clearly established; however, the mechanisms by which they influence the pathogenesis of asthma is unclear.¹ Metformin is a biguanide class oral antidiabetic drug used to treat type 2 diabetes mellitus and insulin resistance. Although metformin reduces glucose production in the liver through inhibition of gluconeogenesis, the precise mechanisms are unknown and it may have differing modalities in different cell types. Metformin may indirectly activate AMPK by increasing AMP:ATP ratios through mild but specific inhibition of the mitochondrial respiratory chain complex I in hepatocytes, skeletal muscle, endothelial cells, pancreatic B cells, and neurons.² Peroxynitrite, generated by inhibition of complex I, activates AMPK through a c-Src and PI3K-dependent pathway in bovine aortic endothelial cells.³ Metformin also directly activates AMPK through the inhibition of AMP deaminase in isolated skeletal muscle.⁴ In the lung, metformin up-regulates AMPK expression and activity and diminishes proinflammatory cytokine secretion in human bronchial epithelial cells, down-regulating I κ B kinase activity and inhibiting nuclear factor (NF)- κ B.⁵

Obese asthmatics are less responsive to typical asthma controller therapy possibly because of contributing factors, such as an increased proinflammatory environment, that blunt the efficacy of treatment,⁶ yet there have been studies that have shown no difference in induced sputum eosinophils, a biomarker of airway inflammation, between obese and lean asthmatics.^{7,8} In a study of obese and lean asthmatics by Desai and colleagues,⁹ however, there were similarities in sputum eosinophil counts between the 2 groups but an increase in interleukin-5 (IL-5), a mediator of eosinophil

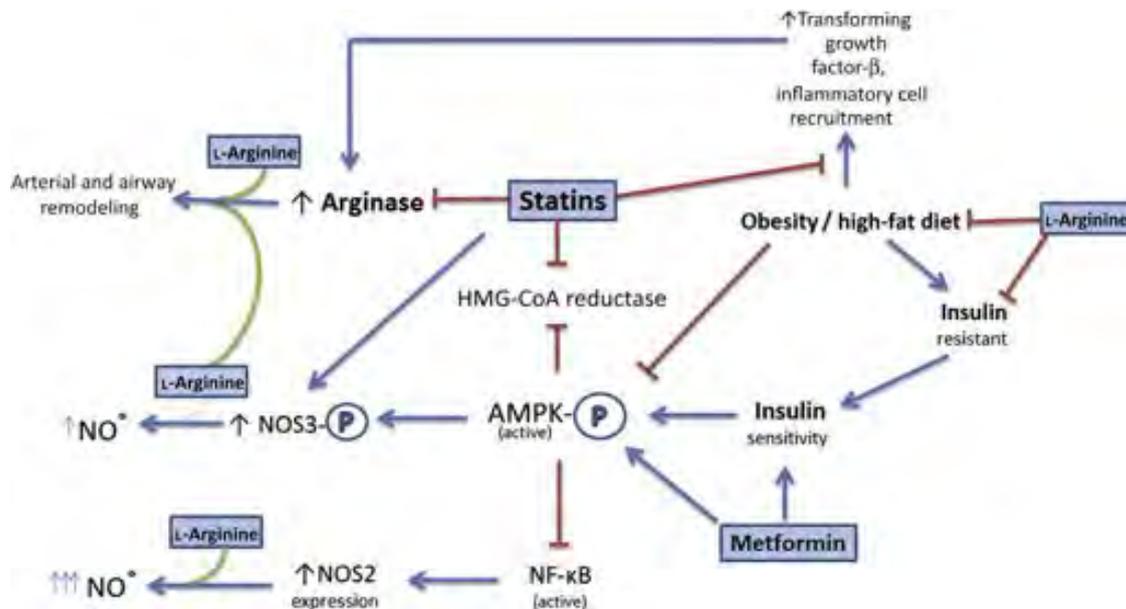


Fig. 1. Potential therapeutics for obese, adult asthmatics described in this review modulate pathways common to several metabolic and nutritional disorders, allowing for the treatment of several comorbidities by targeting their common dysfunction as opposed to individual symptoms. Direct targets include AMPK, which regulates numerous cellular metabolic pathways involved in energy storage, and NOS3, which modulates vascular and bronchial smooth muscle tone. HMG-CoA, hydroxymethylglutaryl-coenzyme A.

activation, in the sputum and increased eosinophil accumulation in the submucosal layer of the obese asthmatic group.

The results from a study using a high-fat diet–induced obese mouse model (male C57BL6/J) of allergic airway inflammation are in agreement with patient observations. Although eosinophil numbers in the bronchoalveolar lavage (BAL) from allergen-challenged obese mice were decreased compared with their lean counterparts, the levels of infiltrated eosinophils in the lung tissue were higher in the obese mice. Treatment of these allergen-challenged obese mice with metformin reduced tissue eosinophil infiltration and increased the number of cells in the BAL fluid suggest differing modes of regulation for eosinophil migration and function between obese and lean asthmatics, possibly through decreased NF- κ B activation.¹⁰ Another mouse study using lean BALB/C female mice demonstrated that metformin decreases eosinophilic inflammation, peribronchial fibrosis, and mucin secretion coupled with increased ratios of activated phospho-AMPK to total AMPK and decreased oxidative stress as measured by the ratio of reduced to oxidized glutathione.¹¹

Metformin can also increase NO synthase 3 (NOS3) dependent production of NO and improve endothelial function through AMPK-dependent positive regulation of NOS3 activity and inhibition of NOS3 negative regulators. Treatment of endothelial cell lines and mice with metformin increases AMPK-dependent NOS3 phosphorylation at the regulatory site Ser1177/1179 and NO production.^{12–14} NOS3 activity is negatively regulated through phosphorylation of Thr495 by protein kinase C- β (PKC β), which is up-regulated in human asthmatics and patients with insulin resistance.¹⁵ Pharmacologic inhibition of PKC β in endothelial cells freshly isolated from diabetics decreases basal levels of Thr495 phosphorylated NOS3 and improved insulin-mediated signaling of NOS3. Overall NOS expression and activity are also reduced in murine models of allergic inflammation and human asthmatics.^{16–18} Overexpression of NOS3 in a mouse model of allergic asthma attenuates airway inflammation and airway hyperresponsiveness, possibly acting through increased levels of S-nitrosothiols in the lung or decreased interferon- γ , IL-5, or IL-10.¹⁹ Further studies are necessary to uncover whether NOS3 could be regulated by metformin in models of asthma or asthmatics.

These findings suggest that introducing metformin in conjunction with standard asthma controller therapy could prove beneficial for outcomes in obese asthmatics by modulation of NOS3 activity or other AMPK-dependent metabolic signaling pathways.

ASTHMA, THE MEVALONATE PATHWAY, AND THE STATIN DRUGS

The statin class of drugs inhibits the enzyme, hydroxymethylglutaryl–coenzyme A reductase (HMGCR), which is the rate-limiting step in cholesterol and isoprenoid biosynthesis in the mevalonate (MVA) pathway.²⁰ Isoprenoids include pyrophosphate lipid molecules (eg, farnesylpyrophosphate [FPP] and geranylgeranylpyrophosphate [GGPP]) that are necessary for post-translational modification of numerous intracellular proteins, including small G-proteins, or GTPases (eg, Rho, Ras, and Rac). GTPases require prenyl tethering to the membrane to maintain proximity to the appropriate transmembrane receptors and signaling transduction machinery. Limiting the availability of the isoprenoid substrate reduces the total amount of GTPase available for activation, potentially dampening cytokine and chemokine signal transduction and the responses that lead to cellular hypertrophy and inflammation.²¹

Statins' effect on isoprenylated GTPases is a mechanism that may be of particular interest in targeting the obese asthmatic population because translational studies in

obese (ob/ob, leptin $-/-$) mice exposed to allergen have indicated increased activity of the GTPase, RhoA, in airway epithelial cells and airway smooth muscle. Increased RhoA activity has been implicated in airway smooth muscle hyperreactivity and hypertrophy. GGPP synthase (GGPPS) expression, the enzyme upstream of FPP and GGPP synthesis, is also increased in obese (ob/ob) mice. Because RhoA requires prenylation by GGPP for activation, the use of statins to manipulate the MVA pathway may have further implications for airway hyperreactivity and remodeling, 2 key features in asthma.^{22,23}

Statins may also regulate L-arginine metabolism and NO production. In addition to being induced by Th2 cytokines IL-4 and IL-13, arginase 1 or 2 expression can be induced via a RhoA/Rho associated protein kinase (ROCK)-mediated pathway. Endothelial cell RhoA/ROCK can be indirectly activated by reactive oxygen and nitrogen species, such as H₂O₂ and ONOO⁻, via protein kinase C. Insulin resistance and diabetes increases the production of these oxidative species through several sources, including uncoupled mitochondrial complex 1, mitochondrial NOS (mtNOS), and NOS3.

RhoA-dependent activation of ROCK increases arginase 1 expression in aortas and livers of streptozotocin-induced diabetic rats. Arginase activity depletes intracellular and plasma L-arginine, further reducing endothelium-derived NO production and promoting additional NOS uncoupling. This effect on endothelial NO production is reversible, however, with administration of statins, ROCK inhibitor Y-27632, or L-citrulline.^{24–26} The inhibitory effect of simvastatin on arginase expression and activity has also been observed in the airways of mice, in a model of allergic inflammation, although its mechanism of action has not been determined, because statins also inhibit the expression of IL-13 in airway epithelial cells.^{27,28} Statin-dependent reduction in circulating ADMA further aids production of NO by the NOS enzymes and reduces NOS uncoupling. Thus, simvastatin (and potentially other statins) can function to improve dysfunctional NO metabolism in asthmatic, inflamed lungs.

Statins and AMPK collectively engage in crosstalk that regulates NOS3 activity. Treatment of human endothelial cells with statins results in time- and dose-dependent increases in phosphorylated AMPK, followed by AMPK-dependent phosphorylation of NOS3 at Ser1177.²⁹ These results were confirmed in the aorta and myocardium of mice orally administered atorvastatin.³⁰ Independent of statin administration, AMPK activation also inhibited HMGCR by phosphorylation of the Ser871/Ser872 site in cultured rat hepatocytes and human endothelial cells.^{31,32}

Statins applied to murine models of allergen-induced airway inflammation reduce inflammatory cell influx, Th2 cytokine production, ADMA levels, airway hyperreactivity, and airway remodeling.^{33–36} Specific inhibition of geranylgeranyltransferase by GGTI-2133 reduces airway hyperreactivity, whereas farnesyltransferase inhibition increases inflammation and airway hyperreactivity. This indicates that the effect of statins on airway smooth muscle is dependent on reduced isoprenylation of RhoA by GGPP and the therapeutic effect of statins, at least in allergic airway disease, is through its effects on geranylgeranylation (ie, RhoA), not farnesylation.³⁷

In the authors' severe asthma clinics, female-predominant severe asthmatics with a mean body mass index (BMI) of greater than or equal to 30 were found to benefit from statin treatment. Median statin use for 1 year added to standard inhaler treatment was associated with a higher asthma control test score, indicating improved asthma symptoms.³⁸ Because this is the only study the authors are aware of that evaluates statin use in obese severe asthmatics, clinical application is precluded absent randomized clinical trials to test this observation.

Although data are limited in obese asthmatics, studies by Chiba and Zeki et al.,^{33,34} support the biological plausibility for the role of MVA metabolism and statins in

mitigating asthma in this subgroup. Nonatopic adult-onset asthmatics, especially individuals with the confounding characteristics of obesity and metabolic syndrome, tend to be more severe and refractory to current therapies. These additional comorbidities contribute to systemic inflammation, insulin resistance, and reduced lung function originating from inflammation and mechanical stress derived from increased visceral mass. Statins may address many of these comorbidities; statins reduce proinflammatory signaling by RhoA/ROCK, increase NO production and reduce the production of oxidative species by uncoupled NOS enzyme, and activate AMPK, reversing insulin resistance. Therefore, the authors believe that future experiments in animal models and clinical trials with adult-onset asthmatics with obesity should evaluate the potential therapeutic role of statins and/or statin with L-arginine combination treatment.

L-ARGININE AND ASTHMA

L-Arginine is a substrate in protein synthesis, a molecular component for the conversion of ammonia to urea in the urea cycle, and the substrate in several diverse enzymatic pathways, including the synthesis of NO, creatine, agmatine, ornithine, glutamate, proline, polyamine, and dimethylarginines, including asymmetric dimethylarginine (ADMA).

Dietary supplementation of amino acid formulations is common practice among healthy individuals and those with chronic illnesses. The semi-essential amino acid, L-arginine, has been reported to confer beneficial effects in various small studies of surgery, trauma, and infectious and noninfectious inflammation. Direct infusion of L-arginine to the peripheral or pulmonary arterial beds has been tested in acute and chronic disease states, including coronary heart disease, preeclampsia, and myocardial infarction, because of its bronchodilator and vasodilator actions.^{39–41} L-Arginine can also be used as a primary therapeutic in sickle cell disease patients with acute chest syndrome, where deficiency in NOS3-derived NO contributes to red cell sickling and vasoconstriction,⁴² augmenting NO production and improving outcomes in this disease.

Factors that regulate the output of NO in the airway, endothelium, and adipose tissues of asthmatics include the concentration of L-arginine, the metabolic precursor of NO, and ADMA, an endogenous NOS inhibitor. NO is primarily derived from the oxidative deamination of L-arginine and O₂ into NO and L-citrulline by the NOS family of enzymes. This family includes NOS1 (neuronal NOS), NOS2 (inducible NOS), and NOS3 (endothelial NOS). NOS is also present in mitochondria and referred to as mtNOS but is a variant of NOS1.⁴³ Depletion of L-arginine can cause the NOS isoforms to produce superoxide ($\bullet\text{O}_2^-$) in a process referred to as *uncoupling* that results in the transfer of an electron from NADPH to oxygen instead of L-arginine.^{44,45} NO and superoxide ($\bullet\text{O}_2^-$) can combine to form peroxynitrite (ONOO⁻). Thus, reduced L-arginine availability to NOS enzymes can have the double effect of reducing NO output and producing oxidative and nitrosative species.

Initial studies using DNA microarray analysis in mouse models of allergen-induced inflammation identified the arginase enzymes as potential “asthma signature genes.” Arginase 1 and arginase 2 catalyze the hydrolysis of L-arginine to ornithine and urea. Increased arginase activity in asthmatics and particularly obese asthmatics is important both for the downstream products of arginase activity, including proline and polyamines, which contribute to airway remodeling and cellular proliferation, and its capacity to regulate the availability of L-arginine to the NOS enzymes by substrate depletion. Analysis of lung tissue of asthmatic subjects confirmed that increased

arginase 1 expression correlated with additional effects of increased serum arginase activity and decreased plasma L-arginine compared with controls.^{17,46} Further analysis based on patient subpopulation revealed that serum arginase 1 level in nonatopic asthmatics was further amplified compared with atopic asthmatics.

Animal models of allergic airway inflammation have shown that inhibition of arginase ameliorated lung inflammation; reduced airway and peribronchial eosinophilia; and decreased production of Th2 proinflammatory cytokines, IL-4 and IL-13, and eotaxins. Arginase inhibition reduced characteristics of airway remodeling, including goblet cell hyperplasia, airway smooth muscle proliferation, and subepithelial collagen accumulation. Airway hyperreactivity and airway maximal contraction were also reduced.^{47–50} In lieu of arginase inhibition, supplementation with oral or aerosolized L-arginine produced comparable results, indicating that the driving force for these improvements was maintaining adequate levels of L-arginine.⁵¹

Increased arginase activity in the obese, nonatopic asthmatic subpopulation may be derived from sources beyond the lung. Obese subjects have heightened expression of arginase 1 (4.5-fold) in circulating mononuclear cells compared with normal-weight subjects.⁵² Exposure to high levels of insulin causes endothelial cells to have increased arginase 2 activity that leads to the uncoupling of NOS3 and increased inflammatory cell adhesion via soluble intercellular cellular adhesion molecule 1. Abrogation of these effects can be achieved through arginase inhibition.⁵³ Hyperglycemic rats have increased arginase 1 and arginase 2 expression in muscle arteriole beds, which inhibit the vasodilation resulting from increased flow. Inhibition of arginase reverses the arteriole flow impedance.⁵⁴ In these models, the beneficial effects of arginase inhibition include mitigation of hypertension, insulin resistance, and systemic inflammation.

In an ongoing phase II study (NCT01841281), the authors are examining a subset of adult severe asthmatics who are predominantly overweight/obese and female. The go-to biomarkers that are indicative of asthma do not necessarily apply for this obese, late-onset population, because this subset is nonatopic (low circulating IgE) and exhibit low exhaled NO, which inversely correlates with BMI and deceptively low sputum eosinophilia.⁵⁵ Shifts in NO metabolism are indicative of the vascular dysfunction relating to obesity, and the changes in exhaled NO may represent corresponding changes in the lung.⁹ The authors hypothesize that this subset of asthmatics will respond favorably to L-arginine supplementation in addition to their standard therapy because in obese asthmatics, the age of asthma onset seems to dictate how they respond to controller therapies, whether their obesity is causative or a comorbid condition.

ADMA AND PHOSPHODIESTERASE INHIBITION

NO production by the NOS enzymes can be inhibited by endogenous competitive inhibitor, ADMA, which can uncouple NOS enzymes, producing superoxide. ADMA is derived from the posttranslational modification of L-arginine by protein-arginine methyltransferase type 1.⁵⁶ The lung is a major source of protein-bound asymmetric dimethylarginine with greater than 3-fold higher levels of bound ADMA compared with heart, kidney, or liver tissue, and an estimated 1.4% of arginine residues present in lung proteins asymmetrically dimethylated.⁵⁷ Proteolytic degradation of these proteins releases methylarginases to the free amino acid pool^{58,59} where they can be enzymatically converted into L-citrulline and dimethylamine by the dimethylarginine dimethylaminohydrolase (DDAH) enzymes or excreted in the urine.⁶⁰

The lung arginine:ADMA ratio is significantly altered in asthmatics compared with nonasthmatics, with sputum ADMA inversely correlating to exhaled NO.⁶¹ Increased

plasma ADMA has been observed in numerous disease states, including pulmonary arteriole hypertension, chronic obstructive pulmonary disease (COPD), cardiovascular disease, and diabetes mellitus.^{61–63} The subgroup consisting of obese, nonatopic asthmatics, independent of other confounding metabolic risk factors, may have significantly increased levels of plasma ADMA and reduced L-arginine:ADMA ratio due to obesity alone.⁶⁴ Other potentiating factors, such as insulin resistance, even in normotensive individuals, also correlate with increased ADMA.^{65,66}

Mouse models of allergic airway inflammation have given further insight into the role of ADMA metabolism in disease. Allergen exposed mice, like human asthmatics, have significantly increased levels of ADMA in their serum and BALF⁶⁷ and further examination revealed increased synthesis of ADMA in alveolar epithelial cells and macrophages and reduced expression of ADMA catabolic enzyme, DDAH2, in airway epithelial cells compared with control mice.⁶⁷ Altering ADMA degradation by overexpressing DDAH1 reduced markers of inflammation, including eosinophilia, Th2 cytokines IL-4 and IL-13, arginase, and IgE.⁶⁸

DDAH2 expression is regulated by intracellular cyclic adenosine monophosphate (cAMP) concentration via protein kinase A (PKA)-dependent phosphorylation of the transcription factor, cAMP response element-binding (CREB) protein. PKA activation is mediated by cAMP binding to PKA-regulatory subunits, resulting in the release of active PKA, which can phosphorylate numerous targets, including CREB, which require phosphorylation to facilitate binding to the CRE domains upstream of the human DDAH2 gene. Inhibiting cAMP to AMP conversion through the use of phosphodiesterase (PDE) 3/4 inhibitors increases catabolism of the endogenous NOS inhibitor, ADMA, and increasing NOS3 activity in endothelium and alveolar macrophages.^{69,70} Activation of PKA by PDE4 inhibitors may also target RhoA pathways. PKA-dependent RhoA phosphorylation inhibits RhoA activation, and phosphorylation of guanine nucleotide dissociation inhibitor (GDI) by PKA enhances GDI binding to RhoA, deactivating it.⁷¹ Because the RhoA/ROCK pathway also regulates expression of arginase, this pathway may further alleviate the depletion of L-arginine and NOS uncoupling.^{26,72}

PDE4 inhibitors tested in murine models of chronic allergic airway inflammation reduce airway eosinophilia, inflammatory cell recruitment, matrix metalloproteinase 9 activity, fibroblast migration and hypercontractility, and subepithelial collagen deposition.^{73,74} For patient use, second-generation PDE4 inhibitors for treatment of COPD have completed multiple randomized clinical trials, noting improvement in prebronchodilator forced expiratory volume in the first second of expiration, with limited side effects, including nausea, diarrhea, headaches, and weight loss. In combination with other asthma control therapies, the use of PDE4 inhibitors to treat severe, obese asthmatics, especially those with measurably heightened plasma ADMA levels, may provide therapeutic benefits.

The potential treatment options reviewed in this section are united by the common principle that decreasing NOS uncoupling has significant clinical benefit in the subgroup of obese, nonatopic asthmatics in which the compounding factors of obesity, endothelial dysfunction, metabolic syndrome, and lung inflammation all contribute to perpetuating NOS dysfunction. These treatments approach the issue of deficient NO production from numerous directions. Supplementation with L-arginine bolsters the L-arginine:ADMA ratio, effectively outcompeting the NOS inhibitor molecule based on enzyme saturation and directly addressing the issue of limited substrate uncoupling NOS enzymes. Arginase inhibition indirectly produces the same effect, increasing both intracellular and plasma L-arginine availability and increasing the L-arginine:ADMA ratio. Finally, inhibition of PDE4 increases ADMA catabolism by decreasing

cAMP turnover and subsequently increasing expression of DDAH2. The uncoupling of NOS by ADMA and/or substrate depletion results in the production of oxidative and nitrosative species in the airways, inflammatory cells, endothelial cells and adipose tissue, contributing to the progression of disease.

VITAMINS AND OMEGA-3 POLYUNSATURATED FATTY ACIDS: OTHER POTENTIAL CONSIDERATION IN ASTHMA AND OBESITY

The bioavailability of fat-soluble vitamins A, D, and E in asthmatic patients has not been intensely explored but it can have an impact on obese asthmatic patients because body fat likely acts as a reservoir for storage of fat-soluble vitamins. Although more research must be done to evaluate the adequate status of these vitamins in obese asthmatic patients, a summary of these vitamins and omega-3 polyunsaturated fatty acid (PUFAs) and their impact on asthma is included.

Recent epidemiologic studies point to an inverse relationship between vitamin A levels and severity of asthma.^{75,76} Vitamin A affects a broad array of immune responses through retinoic acid (RA), its major oxidative metabolite. Previous data indicate that vitamin A deficiency can impair immune function, whereas excess RA induces inflammatory disorders. Vitamin A or RA limits the differentiation of naïve T cells to T-helper cell (Th)1 by reducing IL-12, INF- γ , and NF- κ B signaling, resulting in the increase of Th2-mediated processes. RA can oppose Th17 cell commitment by increasing TGF- β signaling and reducing the expression of IL-6 receptor, whereas excess vitamin A results in the induction of different subsets of Foxp3⁺ regulatory T (Treg) cells and their mediated processes.⁷⁷ RA has also been shown to inhibit eosinophilic and basophilic differentiation and regulate IgA and IgG production in response to T-cell-dependent antigens. Results from studies in mice and rodents suggest strongly that adequate vitamin A status is the key for maintaining a balance of well-regulated T-cell differentiation and function.

The clinicaltrials.gov web site cites more than 25 asthma studies that are ongoing with vitamin D. Vitamin D supplementation and asthma have been studied for years and the conflicting findings are likely due to differences in study design, sample size, and method for assessing vitamin D status through measuring levels of 25-hydroxyvitamin D (25(OH)D).^{78,79} Potential beneficial effects of 1,25-dihydroxyvitamin D (calcitriol) synthesized by bronchial epithelial cells include increasing antimicrobial peptide production by facilitating Toll-like receptor signaling, regulation of the inflammatory response, airway remodeling, and respiratory muscle function. Circulating 25(OH)D can suppress IL-17- and IL-4-mediated expression of IL-13 and shift the Th1/Th2 balance toward Th2 dominance. These controversial functions may be due to the direct effect of vitamin D on CD4⁺ T cells by promoting an IL-10 secreting population of Treg cells.⁷⁹ In addition, supplementation of calcitriol has been associated with decreases in body fat mass and improved insulin sensitivity in obese people.⁸⁰ Despite supplementation of vitamin D, large populations remain vitamin D deficient. This could apply to the adult obese asthmatic population, because the bioavailability seems to be decreased with increased body fat mass.

Although vitamin A and D exert most of their effects through the binding of nuclear receptors and regulating of gene transcription, vitamin C and E act as potent antioxidants. Deficiencies in vitamin C and other plasma antioxidants are associated with lung disease and case-control and cross-sectional studies suggest that vitamin C supplementation may decrease asthma severity and exacerbation frequency through antioxidant mechanisms.⁸¹ Vitamin E supplementation in asthmatics has been shown to help stabilize lung epithelia membranes and protect against ozone-induced

membrane injury by interrupting lipid peroxidation.⁸² Increased levels of vitamin E are associated with less allergic skin sensitization, lower IgE secretion, and suppressed neutrophil recruitment. Vitamin E supplementation in animal studies improves vasodilation and decreases oxidation of LDL through increased antioxidants levels in the vasculature. Randomized controlled clinical trials of supplemental vitamin E in asthmatics have not, however, consistently demonstrated that higher intake of vitamin E reduces asthma events.⁸³

Several clinical studies have been designed to test the hypothesis that diets supplemented with the omega-3 PUFAs, eicosapentanoic acid (EPA) and docosahexaenoic acid (DHA), the major component of fish oil, ameliorate the development of asthma or improve asthma outcomes.⁸⁴ In the context of asthma, in the obese patient subgroup, intervention trials with n-3 PUFAs show beneficial effects in patients with type 2 diabetes mellitus and cardiovascular disease.^{85,86} EPA has an impact on inflammatory signaling by competitively inhibiting enzymatic pathways that convert arachidonic acid (AA) to potent proinflammatory 4-series LTs and 2-series prostaglandins (PGs), mediated by 5-lipoxygenase and cyclooxygenase (COX). EPA may also inhibit IgE production through COX.⁸⁷ In addition, EPA is metabolized to less inflammatory 5-series LTs and 3-series PGs and potent anti-inflammatory mediators, resolvins of the E-series (RvE1 and Rv3). The effects of DHA are distinct from EPA; DHA can decrease transcription of AA metabolizing enzymes, such as COX-2, by inhibiting NF- κ B activation. DHA can also be converted into anti-inflammatory mediators, including the resolvins of the D-series (RvD1, RvD2, RvD3, and RvD4), docosatrienes, and protectins.⁸⁸ Results of the studies testing effects of n-3 PUFAs on asthma pathogenesis over the past 20 years have been conflicting and at least 1 meta-analysis determined that n-3 PUFAs does not affect asthma outcomes.⁸⁴ There is a consensus, however, that the total number of subjects in these trials is insufficient to make firm conclusions about the effects of the supplements.

FUTURE CONSIDERATIONS/SUMMARY

Controller drug therapies for asthma may be partly or wholly ineffective in adult obese, nonatopic asthma patients, leaving them poorly controlled and exposed to adverse drug side effects, such as relative adrenal insufficiency, osteoporosis, and more frequent respiratory viral infections. One approach to therapy does not fit all and physicians must demonstrate a willingness to try several treatment alternatives. The authors recommend that asthmatic patients not appropriately responding to controller drug therapy be re-evaluated. Adult obese, nonallergic asthmatics may have concomitant conditions that potentiate systemic inflammation; in turn, they may respond to treatment targeted at these associated conditions. Patients with the metabolic syndrome, hypercholesterolemia, and diabetes treated with either metformin or the statin drugs may find benefit for their asthma as well as their metabolic derangements. Although firm recommendations cannot be made until further studies are performed, these medications, L-arginine, omega-3 fatty acids, and other nutritional supplements are exciting considerations for future treatment of asthma. A move toward more targeted therapies for asthma subgroups is needed, and, biologically, these therapies account for and treat the comorbidities associated with obese, nonatopic asthmatic patients.

REFERENCES

1. Weiss ST. Obesity: insight into the origins of asthma. *Nat Immunol* 2005;6(6):537–9.

2. Viollet B, Guigas B, Sanz Garcia N, et al. Cellular and molecular mechanisms of metformin: an overview. *Clin Sci (Lond)* 2012;122(6):253–70.
3. Zou MH, Kirkpatrick SS, Davis BJ, et al. Activation of the AMP-activated protein kinase by the anti-diabetic drug metformin in vivo. Role of mitochondrial reactive nitrogen species. *J Biol Chem* 2004;279(42):43940–51.
4. Ouyang J, Parakhia RA, Ochs RS. Metformin activates AMP kinase through inhibition of AMP deaminase. *J Biol Chem* 2011;286(1):1–11.
5. Myerburg MM, King JD Jr, Oyster NM, et al. AMPK agonists ameliorate sodium and fluid transport and inflammation in cystic fibrosis airway epithelial cells. *Am J Respir Cell Mol Biol* 2010;42(6):676–84.
6. Clark AR. MAP kinase phosphatase 1: a novel mediator of biological effects of glucocorticoids? *J Endocrinol* 2003;178(1):5–12.
7. Todd DC, Armstrong S, D'Silva L, et al. Effect of obesity on airway inflammation: a cross-sectional analysis of body mass index and sputum cell counts. *Clin Exp Allergy* 2007;37(7):1049–54.
8. McLachlan CR, Poulton R, Car G, et al. Adiposity, asthma, and airway inflammation. *J Allergy Clin Immunol* 2007;119(3):634–9.
9. Desai D, Newby C, Symon FA, et al. Elevated sputum interleukin-5 and submucosal eosinophilia in obese individuals with severe asthma. *Am J Respir Crit Care Med* 2013;188(6):657–63.
10. Calixto MC, Lintomen L, Andre DM, et al. Metformin attenuates the exacerbation of the allergic eosinophilic inflammation in high fat-diet-induced obesity in mice. *PLoS One* 2013;8(10):e76786.
11. Park CS, Bang BR, Kwon HS, et al. Metformin reduces airway inflammation and remodeling via activation of AMP-activated protein kinase. *Biochem Pharmacol* 2012;84(12):1660–70.
12. Morrow VA, Fougelle F, Connell JM, et al. Direct activation of AMP-activated protein kinase stimulates nitric-oxide synthesis in human aortic endothelial cells. *J Biol Chem* 2003;278(34):31629–39.
13. Davis B, Rahman A, Arner A. AMP-activated kinase relaxes agonist induced contractions in the mouse aorta via effects on PKC signaling and inhibits NO-induced relaxation. *Eur J Pharmacol* 2012;695(1–3):88–95.
14. Cheang WS, Tian XY, Wong WT, et al. Metformin protects endothelial function in diet-induced obese mice by inhibition of endoplasmic reticulum stress through 5' adenosine monophosphate-activated protein kinase-peroxisome proliferator-activated receptor delta pathway. *Arterioscler Thromb Vasc Biol* 2014;34:830–6.
15. Tabit CE, Shenouda SM, Holbrook M, et al. Protein kinase C-beta contributes to impaired endothelial insulin signaling in humans with diabetes mellitus. *Circulation* 2013;127(1):86–95.
16. Ahmad T, Mabalirajan U, Joseph DA, et al. Exhaled nitric oxide estimation by a simple and efficient noninvasive technique and its utility as a marker of airway inflammation in mice. *J Appl Physiol* (1985) 2009;107(1):295–301.
17. Morris CR, Poljakovic M, Lavrisha L, et al. Decreased arginine bioavailability and increased serum arginase activity in asthma. *Am J Respir Crit Care Med* 2004;170(2):148–53.
18. Bratt JM, Williams K, Rabowsky MF, et al. Nitric oxide synthase enzymes in the airways of mice exposed to ovalbumin: NOS2 expression is NOS3 dependent. *Mediators Inflamm* 2010;2010. pii:321061.
19. Ten Broeke R, De Crom R, Van Haperen R, et al. Overexpression of endothelial nitric oxide synthase suppresses features of allergic asthma in mice. *Respir Res* 2006;7:58.

20. Yeganeh B, Wiechec E, Ande SR, et al. Targeting the mevalonate cascade as a new therapeutic approach in heart disease, cancer and pulmonary disease. *Pharmacol Ther* 2014;143:87–110.
21. Rikitake Y, Liao JK. Rho GTPases, statins, and nitric oxide. *Circ Res* 2005; 97(12):1232–5.
22. Takeda N, Kondo M, Ito S, et al. Role of RhoA inactivation in reduced cell proliferation of human airway smooth muscle by simvastatin. *Am J Respir Cell Mol Biol* 2006;35(6):722–9.
23. Viganò T, Hernandez A, Corsini A, et al. Mevalonate pathway and isoprenoids regulate human bronchial myocyte proliferation. *Eur J Pharmacol* 1995;291(2): 201–3.
24. Romero MJ, Platt DH, Tawfik HE, et al. Diabetes-induced coronary vascular dysfunction involves increased arginase activity. *Circ Res* 2008;102(1):95–102.
25. Romero MJ, Iddings JA, Platt DH, et al. Diabetes-induced vascular dysfunction involves arginase I. *Am J Physiol Heart Circ Physiol* 2012;302(1):H159–166.
26. Holowatz LA, Santhanam L, Webb A, et al. Oral atorvastatin therapy restores cutaneous microvascular function by decreasing arginase activity in hypercholesterolaemic humans. *J Physiol* 2011;589(Pt 8):2093–103.
27. Zeki AA, Thai P, Kenyon NJ, et al. Differential effects of simvastatin on IL-13-induced cytokine gene expression in primary mouse tracheal epithelial cells. *Respir Res* 2012;13:38.
28. Zeki AA, Bratt JM, Rabowsky M, et al. Simvastatin inhibits goblet cell hyperplasia and lung arginase in a mouse model of allergic asthma: a novel treatment for airway remodeling? *Transl Res* 2010;156(6):335–49.
29. Rossoni LV, Wareing M, Wenceslau CF, et al. Acute simvastatin increases endothelial nitric oxide synthase phosphorylation via AMP-activated protein kinase and reduces contractility of isolated rat mesenteric resistance arteries. *Clin Sci (Lond)* 2011;121(10):449–58.
30. Sun W, Lee TS, Zhu M, et al. Statins activate AMP-activated protein kinase in vitro and in vivo. *Circulation* 2006;114(24):2655–62.
31. Gillespie JG, Hardie DG. Phosphorylation and inactivation of HMG-CoA reductase at the AMP-activated protein kinase site in response to fructose treatment of isolated rat hepatocytes. *FEBS Lett* 1992;306(1):59–62.
32. Fisslthaler B, Fleming I, Keseru B, et al. Fluid shear stress and NO decrease the activity of the hydroxy-methylglutaryl coenzyme A reductase in endothelial cells via the AMP-activated protein kinase and FoxO1. *Circ Res* 2007;100(2):e12–21.
33. Chiba Y, Sato S, Misawa M. Lovastatin inhibits antigen-induced airway eosinophilia without affecting the production of inflammatory mediators in mice. *Inflamm Res* 2009;58(7):363–9.
34. Zeki AA, Franzi L, Last J, et al. Simvastatin inhibits airway hyperreactivity: implications for the mevalonate pathway and beyond. *Am J Respir Crit Care Med* 2009;180(8):731–40.
35. Ahmad T, Mabalirajan U, Sharma A, et al. Simvastatin improves epithelial dysfunction and airway hyperresponsiveness: from asymmetric dimethyl-arginine to asthma. *Am J Respir Cell Mol Biol* 2011;44(4):531–9.
36. Xu L, Dong XW, Shen LL, et al. Simvastatin delivery via inhalation attenuates airway inflammation in a murine model of asthma. *Int Immunopharmacol* 2012;12(4):556–64.
37. Chiba Y, Sato S, Hanazaki M, et al. Inhibition of geranylgeranyltransferase inhibits bronchial smooth muscle hyperresponsiveness in mice. *Am J Physiol Lung Cell Mol Physiol* 2009;297(5):L984–991.

38. Zeki AA, Oldham J, Wilson M, et al. Statin use and asthma control in patients with severe asthma. *BMJ Open* 2013;3(8). pii:e003314.
39. Boger RH, Mugge A, Bode-Boger SM, et al. Differential systemic and pulmonary hemodynamic effects of L-arginine in patients with coronary artery disease or primary pulmonary hypertension. *Int J Clin Pharmacol Ther* 1996;34(8): 323–8.
40. Dorniak-Wall T, Grivell RM, Dekker GA, et al. The role of L-arginine in the prevention and treatment of pre-eclampsia: a systematic review of randomised trials. *J Hum Hypertens* 2014;28(4):230–5.
41. Bednarz B, Jaxa-Chamiec T, Maciejewski P, et al. Efficacy and safety of oral L-arginine in acute myocardial infarction. Results of the multicenter, randomized, double-blind, placebo-controlled ARAMI pilot trial. *Kardiol Pol* 2005;62(5): 421–7.
42. Sullivan KJ, Kissoon N, Sandler E, et al. Effect of oral arginine supplementation on exhaled nitric oxide concentration in sickle cell anemia and acute chest syndrome. *J Pediatr Hematol Oncol* 2010;32(7):e249–258.
43. Zaobornyj T, Ghafourifar P. Strategic localization of heart mitochondrial NOS: a review of the evidence. *Am J Physiol Heart Circ Physiol* 2012;303(11): H1283–1293.
44. Xia Y, Dawson VL, Dawson TM, et al. Nitric oxide synthase generates superoxide and nitric oxide in arginine-depleted cells leading to peroxynitrite-mediated cellular injury. *Proc Natl Acad Sci U S A* 1996;93(13):6770–4.
45. Chen CA, Wang TY, Varadharaj S, et al. S-glutathionylation uncouples eNOS and regulates its cellular and vascular function. *Nature* 2010;468(7327):1115–8.
46. North ML, Khanna N, Marsden PA, et al. Functionally important role for arginase 1 in the airway hyperresponsiveness of asthma. *Am J Physiol Lung Cell Mol Physiol* 2009;296(6):L911–920.
47. Maarsingh H, Dekkers BG, Zuidhof AB, et al. Increased arginase activity contributes to airway remodelling in chronic allergic asthma. *Eur Respir J* 2011; 38(2):318–28.
48. Meurs H, McKay S, Maarsingh H, et al. Increased arginase activity underlies allergen-induced deficiency of cNOS-derived nitric oxide and airway hyperresponsiveness. *Br J Pharmacol* 2002;136(3):391–8.
49. Takahashi N, Ogino K, Takemoto K, et al. Direct inhibition of arginase attenuated airway allergic reactions and inflammation in a *Dermatophagoides farinae*-induced NC/Nga mouse model. *Am J Physiol Lung Cell Mol Physiol* 2010; 299(1):L17–24.
50. Bratt JM, Franzi LM, Linderholm AL, et al. Arginase inhibition in airways from normal and nitric oxide synthase 2-knockout mice exposed to ovalbumin. *Toxicol Appl Pharmacol* 2010;242(1):1–8.
51. Mabalirajan U, Ahmad T, Leishangthem GD, et al. Beneficial effects of high dose of L-arginine on airway hyperresponsiveness and airway inflammation in a murine model of asthma. *J Allergy Clin Immunol* 2010;125(3):626–35.
52. Kim OY, Lee SM, Chung JH, et al. Arginase I and the very low-density lipoprotein receptor are associated with phenotypic biomarkers for obesity. *Nutrition* 2012; 28(6):635–9.
53. Giri H, Muthuramu I, Dhar M, et al. Protein tyrosine phosphatase SHP2 mediates chronic insulin-induced endothelial inflammation. *Arterioscler Thromb Vasc Biol* 2012;32(8):1943–50.
54. Johnson FK, Johnson RA, Peyton KJ, et al. Arginase promotes skeletal muscle arteriolar endothelial dysfunction in diabetic rats. *Front Immunol* 2013;4:119.

55. Holguin F, Comhair SA, Hazen SL, et al. An association between L-arginine/asymmetric dimethyl arginine balance, obesity, and the age of asthma onset phenotype. *Am J Respir Crit Care Med* 2013;187(2):153–9.
56. Bedford MT, Richard S. Arginine methylation an emerging regulator of protein function. *Mol Cell* 2005;18(3):263–72.
57. Bulau P, Zakrzewicz D, Kitowska K, et al. Analysis of methylarginine metabolism in the cardiovascular system identifies the lung as a major source of ADMA. *Am J Physiol Lung Cell Mol Physiol* 2007;292(1):L18–24.
58. Rawal N, Rajpurohit R, Lischwe MA, et al. Structural specificity of substrate for S-adenosylmethionine:protein arginine N-methyltransferases. *Biochim Biophys Acta* 1995;1248(1):11–8.
59. Cantoni GL. Biological methylation: selected aspects. *Annu Rev Biochem* 1975;44:435–51.
60. MacAllister RJ, Fickling SA, Whitley GS, et al. Metabolism of methylarginines by human vasculature; implications for the regulation of nitric oxide synthesis. *Br J Pharmacol* 1994;112(1):43–8.
61. Scott JA, North ML, Rafii M, et al. Asymmetric dimethylarginine is increased in asthma. *Am J Respir Crit Care Med* 2011;184(7):779–85.
62. Kato GJ, Wang Z, Machado RF, et al. Endogenous nitric oxide synthase inhibitors in sickle cell disease: abnormal levels and correlations with pulmonary hypertension, desaturation, haemolysis, organ dysfunction and death. *Br J Haematol* 2009;145(4):506–13.
63. Abbasi F, Asagmi T, Cooke JP, et al. Plasma concentrations of asymmetric dimethylarginine are increased in patients with type 2 diabetes mellitus. *Am J Cardiol* 2001;88(10):1201–3.
64. Eid HM, Arnesen H, Hjerkin EM, et al. Relationship between obesity, smoking, and the endogenous nitric oxide synthase inhibitor, asymmetric dimethylarginine. *Metabolism* 2004;53(12):1574–9.
65. Stuhlinger MC, Abbasi F, Chu JW, et al. Relationship between insulin resistance and an endogenous nitric oxide synthase inhibitor. *JAMA* 2002;287(11):1420–6.
66. Sydow K, Mondon CE, Cooke JP. Insulin resistance: potential role of the endogenous nitric oxide synthase inhibitor ADMA. *Vasc Med* 2005;10(Suppl 1):S35–43.
67. Ahmad T, Mabalirajan U, Ghosh B, et al. Altered asymmetric dimethyl arginine metabolism in allergically inflamed mouse lungs. *Am J Respir Cell Mol Biol* 2010;42(1):3–8.
68. Kinker KG, Gibson AM, Bass SA, et al. Overexpression of dimethylarginine dimethylaminohydrolase 1 attenuates airway inflammation in a mouse model of asthma. *PLoS One* 2014;9(1):e85148.
69. Pullamsetti SS, Savai R, Schaefer MB, et al. cAMP phosphodiesterase inhibitors increases nitric oxide production by modulating dimethylarginine dimethylaminohydrolases. *Circulation* 2011;123(11):1194–204.
70. Hwang TL, Tang MC, Kuo LM, et al. YC-1 potentiates cAMP-induced CREB activation and nitric oxide production in alveolar macrophages. *Toxicol Appl Pharmacol* 2012;260(2):193–200.
71. Qiao J, Huang F, Lum H. PKA inhibits RhoA activation: a protection mechanism against endothelial barrier dysfunction. *Am J Physiol Lung Cell Mol Physiol* 2003;284(6):L972–980.
72. Ming XF, Barandier C, Viswambharan H, et al. Thrombin stimulates human endothelial arginase enzymatic activity via RhoA/ROCK pathway: implications for atherosclerotic endothelial dysfunction. *Circulation* 2004;110(24):3708–14.

73. Belleguic C, Corbel M, Germain N, et al. Reduction of matrix metalloproteinase-9 activity by the selective phosphodiesterase 4 inhibitor, RP 73-401 in sensitized mice. *Eur J Pharmacol* 2000;404(3):369–73.
74. Kumar RK, Herbert C, Thomas PS, et al. Inhibition of inflammation and remodeling by roflumilast and dexamethasone in murine chronic asthma. *J Pharmacol Exp Ther* 2003;307(1):349–55.
75. Allen S, Britton JR, Leonardi-Bee JA. Association between antioxidant vitamins and asthma outcome measures: systematic review and meta-analysis. *Thorax* 2009;64(7):610–9.
76. Ahmad SM, Haskell MJ, Raqib R, et al. Vitamin A status is associated with T-cell responses in Bangladeshi men. *Br J Nutr* 2009;102(6):797–802.
77. Ross AC. Vitamin A and retinoic acid in T cell-related immunity. *Am J Clin Nutr* 2012;96(5):1166S–72S.
78. Finklea JD, Grossmann RE, Tangpricha V. Vitamin D and chronic lung disease: a review of molecular mechanisms and clinical studies. *Adv Nutr* 2011;2(3):244–53.
79. Paul G, Brehm JM, Alcorn JF, et al. Vitamin D and asthma. *Am J Respir Crit Care Med* 2012;185(2):124–32.
80. Pathak K, Soares MJ, Calton EK, et al. Vitamin D supplementation and body weight status: a systematic review and meta-analysis of randomized controlled trials. *Obes Rev* 2014;15:528–37.
81. Milan SJ, Hart A, Wilkinson M. Vitamin C for asthma and exercise-induced bronchoconstriction. *Cochrane Database Syst Rev* 2013;(10):CD010391.
82. Trenga CA, Koenig JQ, Williams PV. Dietary antioxidants and ozone-induced bronchial hyperresponsiveness in adults with asthma. *Arch Environ Health* 2001;56(3):242–9.
83. Keaney JF Jr, Gaziano JM, Xu A, et al. Dietary antioxidants preserve endothelium-dependent vessel relaxation in cholesterol-fed rabbits. *Proc Natl Acad Sci U S A* 1993;90(24):11880–4.
84. Schuster GU, Kenyon NJ, Stephensen CB. Asthma. In: Caballero B, editor. *Encyclopedia of human nutrition*, vol. 1, 3rd edition. Elsevier, Ltd; 2013. p. 122–8.
85. Nakamura T, Azuma A, Kuribayashi T, et al. Serum fatty acid levels, dietary style and coronary heart disease in three neighbouring areas in Japan: the Kumihama study. *Br J Nutr* 2003;89(2):267–72.
86. Bjerregaard P, Pedersen HS, Mulvad G. The associations of a marine diet with plasma lipids, blood glucose, blood pressure and obesity among the inuit in Greenland. *Eur J Clin Nutr* 2000;54(9):732–7.
87. Schmitz G, Ecker J. The opposing effects of n–3 and n–6 fatty acids. *Prog Lipid Res* 2008;47(2):147–55.
88. Serhan CN, Chiang N, Van Dyke TE. Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. *Nat Rev Immunol* 2008;8(5):349–61.