Lipids in Health and Disease

Hypothesis

Can endogenous lipid molecules serve as predictors and prognostic markers of coronary heart disease?

Undurti N Das

Address: UND Life Sciences, 13800 Fairhill Road, #321, Shaker Heights, OH 44120, USA

Email: Undurti N Das - undurti@hotmail.com

Abstract

Dyslipidemia, and inflammatory markers: high-sensitivity C-reactive protein (hs-CRP), myeloperoxidase (MPO), lipoprotein associated phospholipase A_2(Lp-PLA$_2$), and lipid peroxides (LP) are insufficient to predict the onset, extent, and prognosis of CHD. Lipoxins (LXs), resolvins, and protectins are derived from ω-3 fatty acids: eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and ω-6 arachidonic acid in the presence of aspirin; whereas nitrolipids are formed due to the interaction between polyunsaturated fatty acids and nitric oxide (NO). LXs, resolvins, protectins, and nitrolipids are endogenous anti-inflammatory lipid molecules that inhibit production of interleukin-6 (IL-6) and tumor necrosis factor- α (TNF-α), suppress free radical generation, enhance NO generation; and accelerate tissue repair. Thus, beneficial actions of EPA/DHA and aspirin in CHD could be attributed to the formation of LXs, resolvins, protectins, and nitrolipids and suggest that their plasma levels aid in the prediction and prognosis of CHD.

Introduction

An increase in the plasma concentrations of high-sensitive C-reactive protein (hs-CRP), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) occurs in coronary heart disease (CHD) suggesting that it is a low-grade systemic inflammatory condition [1]. Myeloperoxidase (MPO), an abundant leukocyte enzyme, and lipoprotein-associated phospholipase A_2 (Lp-PLA$_2$), produced by macrophages, independently predict the early risk of adverse cardiac events [2,3]. Despite these advances, it is not clear which of these indices are better suited to predict CHD, its severity, and consequent complications.

Although, dyslipidemia, diabetes mellitus, hypertension, and obesity are important risk factors for CHD, there are a significant number of subjects who develop CHD without these risk factors. We and others observed that in CHD, hypertension, diabetes mellitus, hyperlipidemias,

and obesity, EFA (essential fatty acid) metabolism is abnormal such that plasma phospholipid concentrations of arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) are low [4-11]. Despite the fact that deficiency of polyunsaturated fatty acids (PUFAs) is present in CHD, hypertension, diabetes, hyperlipidemias, and obesity and increased intake of PUFAs are of benefit in these diseases [4,7,9-11], exact mechanism(s) of their protective action remained unexplained.

Metabolism of essential fatty acids and its relevance to CHD

Essential fatty acids (EFAs) are important constituents of all cell membranes including endothelial and myocardial cells and determine and influence the behaviour of membrane-bound enzymes and receptors by altering membrane fluidity. EFAs are essential, not synthesized in the

body; and hence, have to be obtained in diet [4]. The two EFAs are ω-6 *cis*-linoleic acid (LA, 18:2) and the ω-3 α-linolenic acid (ALA, 18:3). AA formed from LA is the precursor of 2 series of PGs, thromboxanes (TXs) and 4 series of leukotrienes (LTs); whereas ALA is converted to EPA that gives rise to 3 series of PGs, TXs and 5 series LTs (see Figure 1). LA, γ-linolenic acid (GLA), dihomo-GLA (DGLA), AA, ALA, EPA and docosahexaenoic acid (DHA, 22:6, ω-3) are all PUFAs, but only LA and ALA are EFAs. AA and EPA and their respective PGs, TXs, and LTs play a

significant role in atherosclerosis, CHD, bronchial asthma, inflammatory bowel disease, and other inflammatory conditions [4].

AA, EPA, and DHA give rise to LXs, resolvins, and protectins. Aspirin converts AA, EPA and DHA to form aspirin-triggered 15 epimer LXs (ATLs) that inhibit local inflammation on the vessel wall by regulating the motility of leukocytes, eosinophils, and monocytes [4,12-14]. COX-2 enzyme is essential for the formation of LXs,
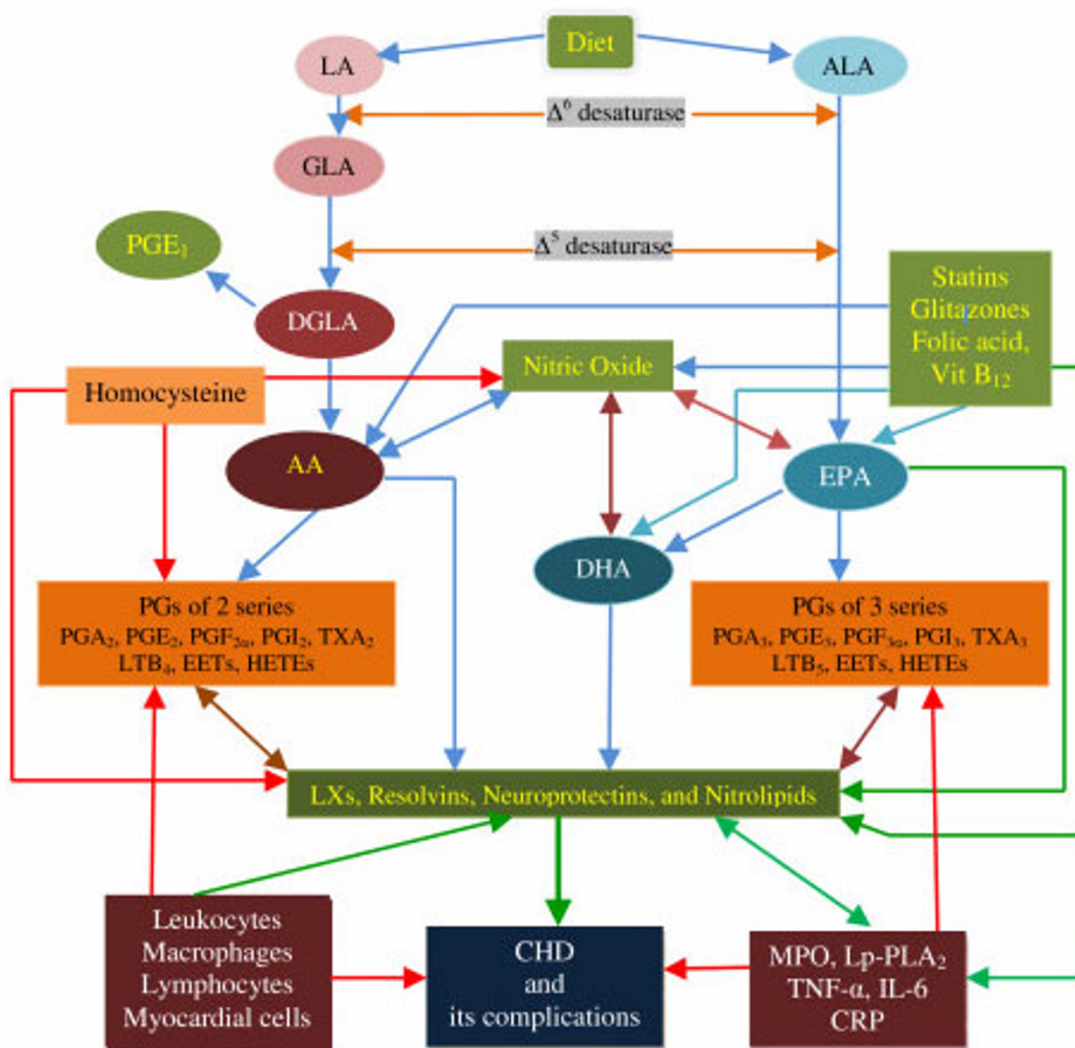

Figure 1
Scheme showing the metabolism of essential fatty acids. Red line indicates initiation and/or progression of disease. Green line indicates protection from disease and better prognosis. Double arrow indicates feedback regulation that could be both positive and negative. Leukocytes, macrophages, lymphocytes, and myocardial cells produce various PGs, LTs, TXs, cytokines, MPO, and other pro-inflammatory molecules that participate in the pathogenesis of CHD. Leukocytes, macrophages, lymphocytes, myocardial cells also produce lipoxins, resolvins, NPD_1, and nitrolipids that have cardioprotective action and enhance healing and prevent or arrest atherosclerosis. Hence, measuring these cardiotoxic and cardioprotective molecules may help in the prediction and prognosis of CHD, and response to therapy.

whose deficiency leads to an interaction between leukocytes (PMNs) and endothelial cells that result in endothelial damage, initiation and progression of atherosclerosis, thrombus formation and CHD.

EPA can be converted to 18R-HEPE (18R- hydroxy-eicosapentaenoic acid), 18-HEPE, and 15R-HEPE. Activated human PMNs, in turn, convert 18R-HEPE to 5,12,18R-triHEPE and 15R-HEPE to 15-epi-LXA$_5$ by 5-lipoxygenase. Both 18R-HEPE and 5,12,18R-triHEPE inhibited LTB$_4$-stimulated PMN transendothelial migration similar to 15-epiLXA$_4$. 5,12,18R-triHEPE competed with LTB$_4$ for its receptors and inhibited PMN infiltration, and thus, 5,12,18R-triHEPE suppresses LT-mediated responses when present at the sites of inflammation [4,12-15].

Similarly, DHA is transformed enzymatically to 17R series of hydroxy DHAs (HDHAs) that, in turn, is converted by PMNs to di- and tri-hydroxy containing docosanoids [4,16]. Similar small molecular weight compounds (similar to HDHAs) are generated from AA and EPA. Thus, 15R-hydroxy containing compounds are formed from AA, 18R series from EPA, and 17R-hydroxy series from DHA that have potent anti-inflammatory actions and induce resolution of the inflammatory process and are called "resolvins" (see Figure 1). Resolvins inhibited cytokine generation, leukocyte recruitment, leukocyte diapedesis, and exudate formation. AA, EPA, and DHA-derived resolvins inhibited brain ischemia-reperfusion injury. 10,17S-dihydroxydocosatriene derived from DHA termed as neuroprotectin D1 (NPD1) reduced infiltration of PMNs, showed anti-inflammatory and neuroprotective properties [4,17], and inhibited oxidative stress-induced apoptosis of human retinal pigment epithelial cells [18]. Both LXs and NPD1 enhanced wound healing [19], and promoted brain cell survival [20,21]. Thus, lipoxins, resolvins, and protectins derived from AA, EPA, and DHA have cardio-protective, neuroprotective, and other cyto-protective actions. It is noteworthy that leukocytes, macrophages, endothelial cells, and myocardial cells have the complete enzyme system for the conversion of PUFAs to PGs, TXs, LTs, LXs, resolvins, protectins and nitrolipids. Based on these findings, I propose that failure to produce adequate amounts of LXs, resolvins, NPD1 (and other protectins), and nitrolipids or interference with their action could lead to initiation and persistence of inflammation and tissue damage including CHD.

Nitrolipids and CHD
NO reacts with PUFAs to yield their respective nitroalkene derivatives, termed as nitrolipids, produce vascular relaxation, inhibit neutrophil degranulation and superoxide formation, and inhibit platelet activation [22-24]. Nitrolipids have endogenous PPAR-γ ligand activity and release NO [24]. Thus, nitrolipids prevent platelet aggregation,

thrombus formation, atherosclerosis, and suppress inflammation. Thus PUFAs not only form precursors to various eicosanoids, resolvins, LXs, and NPD1 but also react with NO to form nitrolipids that prevent platelet aggregation and thrombus formation and hence, arrest of atherosclerosis. Nitrolipids also have anti-inflammatory actions, and so, their deficiency can cause CHD.

Pro- and anti-inflammatory molecules in CHD
Coronary heart disease (CHD) is a low-grade systemic inflammatory condition since these subjects have enhanced plasma levels of reactive oxygen species (ROS), CRP, IL-6, and TNF-α and low circulating levels of NO and various PUFAs [1-6]. Increased ROS decreases anti-oxidant content of the cells/tissues. This leads to an imbalance between the pro- and anti-oxidant molecules that favor tissue damage in CHD [25]. Hs-CRP, LP-LPA$_2$, ROS, and MPO have cytotoxic actions, whereas LXs, resolvins, protectins, and nitrolipids show cytoprotective and cardioprotective actions, in view of their ability to inhibit production of IL-6 and TNF-α, suppress free radical generation, and enhance tissue repair [4,12-24,26-28]. Hence, an increase in hs-CRP, LP-LPA$_2$, ROS, and MPO and a decrease in LXs, resolvins, protectins, and nitrolipids or both not only predisposes to CHD but also indicates poor prognosis. When EPA, DHA, AA, and aspirin are given together in adequate amounts, LXs, resolvins, protectins, and nitrolipids are formed that prevent myocardial damage. This explains the beneficial actions of EPA/DHA/AA and aspirin in both primary and secondary prevention of CHD [4,26-28].

This implies that deficiency of PUFAs seen in CHD and other conditions leads to reduced formation of NO, LXs, resolvins, protectins, and nitrolipids that would initiate atherosclerosis and CHD and/or worsen an existing disease. It is predicted that plasma levels of hs-CRP, ROS, MPO, Lp-PLA$_2$ and LP will be increased whereas those of LXs, resolvins, NPD$_1$ and nitrolipids will be decreased in patients with CHD. Furthermore, a balance between these pro- and anti-inflammatory molecules may aid in predicting prognosis of CHD. For instance, subjects with low levels of LXs, resolvins, NPD$_1$, and nitrolipids may have poor outcome and have higher incidence of cardiac failure, arrhythmias, and recurrence of myocardial infarction. Statins and thiazolidinediones enhance the formation of myocardial 15-epi-LXA$_4$ and EFAs mediate some of their actions that could explain the ability of statins and glitazones to prevent CHD [29-31]. This implies that those who have low levels of EFAs and their metabolites could be resistant to the beneficial action of statins and glitazones; and such subjects will be benefited from supplementation of AA/EPA/DHA.

Hypothesis: EFAs/PUFAs and their metabolites are low in CHD

One of the risk factors for CHD is elevated levels of homocysteine. Southeast Asians especially, Indians (people from Indian subcontinent) are at high risk of CHD [32]. The increased incidence of CHD in Indians has been attributed to their proatherogenic diet, lack of exercise, smoking, high levels of homocysteine and LP(a) lipoprotein, endothelial dysfunction and enhanced plaque and low-grade systemic inflammation [32]. They also have increased prevalence of metabolic syndrome X including insulin resistance, hypertension, abdominal obesity, and dyslipidemia, and type 2 diabetes mellitus, factors that could explain the high incidence of CHD. These factors coupled with the observation that only limited number of patients with CHD receive thrombolytic therapy (36.8%), antiplatelet drugs (~98%), β-blockers (59.3%), lipid-lowering drugs (52%), ACE inhibitors/angiotensin-II receptor blockers (56.8%), anticoagulants (81.3%), percutaneous coronary intervention (7.5%), coronary artery bypass graft (2.9%) and that the incidence of death and complications are more common in Indians [32,33] suggests that as a race Indians are the most vulnerable population for cardiovascular diseases. It is estimated that by 2010, 60% of the world's heart disease is expected to occur in India [34] and are likely to suffer from CHD at an earlier age than do people in developed countries [35]. Unfortunately, all the studies performed in Indians, residing both in India and the developed countries; concentrated on the incidence of the disease and the prevalence of risk factors but none have looked at the pathophysiology of the disease. In a previous study, I showed that even healthy Indians have lower concentrations of various PUFAs compared to Canadians and Americans (USA) [36]. This suggests that as a race Indians have low activity of Δ^6 and Δ^5 desaturases that is responsible for decreased concentrations of various PUFAs in their plasma and tissues as a result of which the formation of PGE_1, PGI_2, PGI_3, lipoxins, resolvins and protectins will be low that may render them more susceptible to develop low-grade systemic inflammatory conditions including CHD [36-41]. This is further supported by the observation that Indians and Europeans with CHD have low levels of PUFAs in their plasma phospholipid fraction [6,37,38]. Furthermore, low activity of Δ^6 and Δ^5 desaturases could lead to the initiation and progression of insulin resistance and atherosclerosis in Indians as previously proposed [42,43]. These evidences suggest that Indians are at high risk of developing CHD and its associated death and complications [32,33] partly due to a defect in the activity of Δ^6 and Δ^5 desaturases that result in low plasma and tissue concentrations of PUFAs and their products: PGE_1, PGI_2, PGI_3, lipoxins, resolvins, protectins, and nitrolipids [36-41]. PUFAs and their products prevent platelet aggregation, lower blood pressure, reduce LDL-C, ameliorate the adverse actions of homocysteine, inhibit ACE (angiotensin converting enzyme) and HMG-CoA enzyme activities and thus, may function as endogenous "polypill" [44]. These evidences suggest that PUFAs of both ω-3 and ω-6 series can be given to high risk subjects to prevent CHD and other cardiovascular diseases [44]. It is likely that subjects with low levels of various PUFAs and their products: PGE_1, PGI_2, PGI_3, lipoxins, resolvins, protectins, and nitrolipids are not only vulnerable to develop CHD but also have poor outcome and higher incidence of cardiac failure, arrhythmias, and recurrence of myocardial infarction (Figure 1) since these endogenous lipid molecules have anti-arrhythmic action, enhance wound healing and suppress inflammation [45-49], though some studies disputed the anti-arrhythmic action of PUFAs [50,51]. In this context, it is interesting to note that folic acid not only reduces plasma homocysteine levels but also augments the metabolism of essential fatty acids such that plasma and tissue levels of various PUFAs are enhanced (reviewed in [44,52-55]). It is possible that some subjects may have normal concentrations of EFAs/PUFAs in their plasma and tissues but the formation of lipoxins, resolvins, protectins, and nitrolipids may be defective. Recently it was noted that the gene that encodes the 5-lipoxygenase activating protein (FLAP) and its risk variant results in an almost 2-fold increased risk of CHD by leading to the production of leukotriene B_4 (LTB_4), a potent chemokine mediators of arterial inflammation [56-58]. This is supported by the observation that patients with CHD produce more LTB_4 than controls, suggesting that LTB pathway is upregulated in CHD [56]. It is interesting to note that lipoxins are potent inhibitors of LTB_4 and leukotrienes [59].

Thus, it is proposed that subjects at high risk for CHD have increased hs-CRP, MPO, Lp-PLA$_2$, LP, IL-6, TNF-α, LTB_4 and low levels of various PUFAs, LXs, resolvins, protectins, and nitrolipids, and the balance between these pro-inflammatory and anti-inflammatory molecules may also aid in the prediction and prognosis of CHD.

If this proposal is true, it implies that PUFAs are not only useful in the prevention of CHD but development of stable and synthetic analogues of LXs, resolvins, NPD_1, and nitrolipids may form a new approach in the management of cardiovascular diseases.

References
1. Das UN: **Clinical laboratory tools to diagnose inflammation.** *Adv Clin Chem* 2006, **41:**189-229.
2. Brennan ML, Penn MS, Lente FV, *et al.*: **Prognostic value of myeloperoxidase in patients with chest pain.** *N Engl J Med* 2003, **349:**1595-1604.
3. Oei HHS, Meer IM van der, Hofman A, *et al.*: **Lipoprotein-associated phospholipase A$_2$ activity is associated with risk of coronary heart disease and ischemic stroke. The Rotterdam study.** *Circulation* 2005, **111:**570-575.

4.   Das UN: **Essential fatty acids: Biochemistry, physiology and pathology.** *Biotech J* 2006, **1(4):**420-439.
5.   Das UN: **Essential fatty acid metabolism in patients with essential hypertension, diabetes mellitus and coronary heart disease.** *Prostaglandins Leukot Essent Fatty Acids* 1995, **52(6):**387-391.
6.   Kumar KV, Das UN: **Lipid peroxides and essential fatty acids in patients with coronary heart disease.** *J Nutritional Med* 1994, **4:**33-37.
7.   Das UN: **Nutritional factors in the pathobiology of human essential hypertension.** *Nutrition* 2001, **17:**337-346.
8.   Das UN: **A defect in the activity of $\Delta 6$ and $\Delta 5$ desaturases may be a factor predisposing to the development of insulin resistance syndrome.** *Prostaglandins Leukot Essen Fatty Acids* 2005, **72:**343-350.
9.   Wang L, Folsom AR, Eckfeldt JH: **Plasma fatty acid composition and incidence of coronary heart disease in middle aged adults: the Atherosclerosis Risk in Communities (ARIC) Study.** *Nutr Metab Cardiovasc Dis* 2003, **13:**256-66.
10.   Suresh Y, Das UN: **Differential effect of saturated, monounsaturated, and polyunsaturated fatty acids on alloxan-induced diabetes mellitus.** *Prostaglandins Leukot Essent Fatty Acids* 2006, **74(3):**199-213.
11.   Mozaffarian D, Ascherio A, Hu FB, Stampfer MJ, Willett WC, Siscovick DS, Rimm EB: **Interplay between different polyunsaturated fatty acids and risk of coronary heart disease in men.** *Circulation* 2005, **111:**157-164.
12.   Serhan CN, Clish CB, Brannon J, Colgan SP, Chiang N, Gronert K: **Novel functional sets of lipid-derived mediators with antiinflammatory actions generated from omega-3 fatty acids via cyclooxygenase 2-nonsteroidal antiinflammatory drugs and transcellular processing.** *J Exp Med* 2000, **192:**1197-1204.
13.   Serhan CN, Hong S, Gronert K, Colgan SP, Devchand PR, Mirick G, Moussignac RL: **Resolvins: A family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals.** *J Exp Med* 2002, **196:**1025-1037.
14.   Das UN: **COX-2 inhibitors and metabolism of essential fatty acids.** *Med Sci Monit* 2005, **11:**RA233-RA237.
15.   Levy BD, Clish CB, Schmidt B, Gronert K, Serhan CN: **Lipid mediator class switching during acute inflammation signals in resolution.** *Nat Immunol* 2001, **2:**612-619.
16.   Hong S, Gronert K, Devchand PR, Moussignac RL, Serhan CN: **Novel docosatrienes and 17S-resolvins generated from docosahexaenoic acid in murine brain, human blood, and glial cells. Autacoids in anti-inflammation.** *J Biol Chem* 2003, **278:**14677-14687.
17.   Marcheselli VL, Hong S, Lukiw WJ, Tian XH, Gronert K, Musto A, Hardy M, Gimenez JM, Chiang N, Serhan CN, Bazan NG: **Novel docosanoids inhibit brain ischemia-reperfusion-mediated leukocyte infiltration and pro-inflammatory gene expression.** *J Biol Chem* 2003, **278:**43807-43817.
18.   Mukherjee PK, Marcheselli VL, Serhan CN, Bazan NG: **Neuroprotectin D1: a docosahexaenoic acid-derived docosatriene protects human retinal pigment epithelial cells from oxidative stress.** *Proc Natl Acad Sci USA* 2004, **101:**8491-8496.
19.   Gronert K, Maheshwari N, Khan N, Hassan IR, Dunn M, Laniado Schwartzman M: **A role for the mouse 12/15-lipoxygenase pathway in promoting epithelial wound healing and host defense.** *J Biol Chem* 2005, **280:**15267-15278.
20.   Lukiw WJ, Cui J-G, Marcheselli VL, Bodker M, Botkjaer A, Gotlinger K, Serhan CN, Bazan NG: **A role for docosahexaenoic acid-derived neuroprotectin D1 in neural cell survival and Alzheimer disease.** *J Clin Invest* 2005, **115:**2774-2783.
21.   Calon F, Lim GP, Morihara T, Yang F, Ubeda O, Salem N Jr, Frautschy SA, Cole GM: **Dietary n-3 polyunsaturated fatty acid depletion activates caspases and decreases NMDA receptors in the brain of a transgenic mouse model of Alzheimer's.** *Eur J Neurosci* 2005, **22:**617-626.
22.   Baker PRS, Lin Y, Schopfer FJ, Woodcock SR, Groeger AL, Batthyany C, Sweeney S, Long MH, Iles KE, Baker LM, Branchaud BP, Chen YE, Freeman BA: **Fatty acid transduction of nitric oxide signaling: Multiple nitrated unsaturated fatty acid derivatives exist in human blood and urine and serve as endogenous peroxisome proliferator-activated receptor ligands.** *J Biol Chem* 2005, **280:**42464-42475.

23.   Coles B, Bloodsworth A, Clark SR, Lewis MJ, Cross AR, Freeman BA, O'Donnell VB: **Nitrolinoleate inhibits superoxide generation, degranulation, and integrin expression by human neutrophils.** *Circ Res* 2002, **91:**375-381.
24.   Lima ES, Bonini MG, Augusto O, Barbeiro HV, Souza HP, Abdalla DS: **Nitrated lipids decompose to nitric oxide and lipid radicals and cause vasorelaxation.** *Free Radic Biol Med* 2005, **39:**532-539.
25.   Das UN: **Free radicals, cytokines and nitric oxide in cardiac failure and myocardial infarction.** *Mol Cell Biochem* 2000, **215:**145-152.
26.   Claria J, Serhan CN: **Aspirin triggers previously undescribed bioactive eicosanoids by human endothelial cell-leukocyte interactions.** *Proc Natl Acad Sci USA* 1995, **92:**9475-9479.
27.   Chiang N, Gronert K, Clish CB, O'Brien JA, Freeman M, Serhan CN: **Leukotriene B$_4$ receptor transgenic mice reveal novel protective roles for lipoxins and aspirin-triggered lipoxins in reperfusion.** *J Clin Invest* 1999, **104:**309-316.
28.   Arita M, Bianchini F, Aliberti J, Sher A, Chiang N, Hong S, Yang R, Petasis NA, Serhan CN: **Stereochemical assignment, antiinflammatory properties, and receptor for the omega-3 lipid mediator resolvin E1.** *J Exp Med* 2005, **201:**713-722.
29.   Birnbaum Y, Ye Y, Lin Y, Freeberg SY, Nishi SP, Martinez JD, Huang MH, Uretsky BF, Perez-Polo JR: **Augmentation of myocardial production of 15-epi-lipoxin A$_4$ by pioglitazone and atorvastatin in the rat.** *Circulation* 2006, **114:**929-935.
30.   Levy BD: **Myocardial 15-epi-lipoxin A4 generation provides a new mechanism for the immunomodulatory effects of statins and thiazolidinediones.** *Circulation* 2006, **114:**873-875.
31.   Das UN: **Essential fatty acids as possible mediators of the actions of statins.** *Prostaglandins Leukot Essent Fatty Acids* 2001, **65:**37-40.
32.   Kuppuswamy VC, Gupta S: **Excess coronary heart disease in South Asians in the United Kingdom.** *BMJ* 2005, **330:**1223-1224.
33.   Xavier D, Pais P, Devereaux PJ, Xie C, Prabhakaran D, Reddy KS, Gupta R, Joshi P, Kerkar P, Thanikachalam S, Haridas KK, Jaison T, Naik S, Maity AK, Yusuf S, on behalf of the CREATE registry investigators: **Treatment and outcomes of acute coronary syndromes in India (CREATE): a prospective analysis of registry data.** *Lancet* 2008, **371:**1435-1442.
34.   Ghaffar A, Reddy KS, Singhi M: **Burden of non-communicable diseases in South Asia.** *BMJ* 2004, **328:**807-310.
35.   Joshi P, Islam S, Pais P, Reddy S, Dorairaj P, Kazmi K, Pandey MR, Haque S, Mendis S, Rangarajan S, Yusuf S: **Risk factors for early myocardial infarction in South Asians compared with individuals in other countries.** *JAMA* 2007, **297:**286-294.
36.   Das UN, Kumar KV, Ramesh G: **Essential fatty acid metabolism in South Indians.** *Prostaglandins Leukot Essent Fatty Acids* 1994, **50(5):**253-255.
37.   Das UN: **Essential fatty acids- a review.** *Current Pharmaceutical Biotechnology* 2006, **7:**467-482.
38.   Harris WS, Assad B, Poston CP: **Tissue omega-6/omega-3 fatty acid ratio and risk for coronary artery disease.** *Am J Cardiol* 2006, **98(4A):**19i-26i.
39.   Das UN: **Beneficial effect(s) of n-3 fatty acids in cardiovascular diseases: But, why and how?** *Prostaglandins Leukot Essent Fatty Acids* 2000, **63(6):**351-362.
40.   Das UN: **Metabolic syndrome X is common in South Asians: But, why and how?** *Nutrition* 2002, **18:**774-776.
41.   Das UN: **Beneficial actions of polyunsaturated fatty acids in cardiovascular diseases: But, how and why?** *Current Nutrition Food Sci* 2008, **4:**2-31.
42.   Das UN: **A defect in the activity of Δ^6 and Δ^5 desaturases may be a factor predisposing to the development of insulin resistance syndrome.** *Prostaglandins Leukotrienes Essen Fatty Acids* 2005, **72:**343-350.
43.   Das UN: **A defect in the activity of Δ^6 and Δ^5 desaturases may be a factor in the initiation and progression of atherosclerosis.** *Prostaglandins Leukot Essen Fatty Acids* 2007, **76:**251-268.
44.   Das UN: **Do polyunsaturated fatty acids behave as endogenous "polypill"?** *Med Hypotheses* 2008, **70:**430-434.
45.   Das UN: **Prostaglandins and cardiac arrhythmias.** *Med J Australia* 1981, **2:**157.
46.   Das UN, Lee AM, Barritt GJ: **Prostanoids can modify response to electrical stimulus and $^{45}Ca^{++}$ exchange in isolated myocardial cells.** *Prostaglandins Leukot Med* 1983, **12:**305-312.

47. Das UN: **Prostacyclin as an endogenous anti-arrhythmic agent.** *Basic Res Cardiol* 1983, **78:**716-719.
48. Den Ruijter HM, Berecki G, Verkerk AO, Bakker D, Baartscheer A, Schumacher CA, Belterman CN, de Jonge N, Fiolet JW, Brouwer IA, Coronel R: **Acute administration of fish oil inhibits triggered activity in isolated myocytes from rabbits and patients with heart failure.** *Circulation* 2008, **117:**536-544.
49. Kang JX, Leaf A: **Prevention of fatal cardiac arrhythmias by polyunsaturated fatty acids.** *Am J Clin Nutr* 2000, **71(1 Suppl):**202S-207S.
50. Brouwer IA, Zock PL, Camm AJ, Bocker D, Hauer RN, Wever EF, Dullemeijer C, Ronden JE, Katan MB, Lubinski A, Buschler H, Schouten EG, SOFA Study Group: **Effect of fish oil on ventricular tachyarrhythmia and death in patients with implantable cardioverter defibrillators: the Study on Omega-3 Fatty Acids and Ventricular Arrhythmia (SOFA) randomized trial.** *JAMA* 2006, **295:**2613-2619.
51. Raitt MH, Connor WE, Morris C, Kron J, Halperin B, Chugh SS, McClelland J, Cook J, MacMurdy K, Swenson R, Connor SL, Gerhard G, Kraemer DF, Oseran D, Marchant C, Calhoun D, Shnider R, McAnulty J: **Fish oil supplementation and risk of ventricular tachycardia and ventricular fibrillation in patients with implantable defibrillators: a randomized controlled trial.** *JAMA* 2005, **293:**2884-2891.
52. Das UN: **Folic acid says NO to vascular diseases.** *Nutrition* 2003, **19:**686-692.
53. Das UN: **Can essential fatty acids reduce the burden of disease(s)?** *Lipids Health Dis* 2008, **7:**9.
54. Pita ML, Delgado MJ: **Folic acid administration increases N-3 polyunsaturated fatty acids in rat plasma and tissue lipids.** *Thromb Haemost* 2000, **84:**420-423.
55. Durand P, Prost M, Blache D: **Pro-thrombotic effects of a folic acid deficient diet in rat platelets and macrophages related to elevated homocysteine and decreased n-3 polyunsaturated fatty acids.** *Atherosclerosis* 1996, **121:**231-243.
56. Helgadottir A, Manolescu A, Thorleitsson G, Gretarsdottir S, Jonsdottir H, Thorsteinsdottir U, Samani NJ, Gudmundsson G, Grant SF, Thorgeirsson G, Sveinbjornsdottir S, Valdimarsson EM, Matthiasson SE, Johannsson H, Gudmundsdottir O, Gurney ME, Sainz J, Thorhallsdottir M, Andresdottir M, Frigge ML, Topol EJ, Kong A, Gudnason V, Hakonason H, Gulcher JR, Stefansson K: **The gene encoding 5-lipoxygenase activating protein confers risk of myocardial infarction and stroke.** *Nat Genet* 2004, **36:**233-239.
57. Ford-Hutchinson AW: **Leukotriene B$_4$ in inflammation.** *Crit Rev Immunol* 1990, **10:**1-12.
58. Samuelsson B: **Leukotrienes: mediators of immediate hypersensitivity reactions and inflammation.** *Science* 1983, **220:**568-575.
59. Conti P, Reale M, Barbacana RC, Panara MR, Bongrazio M: **Inhibition of leukotriene B4 in neutrophils by lipoxins A4 and B4.** *Agents Actions* 1991, **32:**85-87.