

Review Article and Clinical Experience: ASTAXANTHIN – OXIDATIVE STRESS – DIABETES MELLITUS From Basics to Clinics and from General to Specific

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ABSTRACT

Astaxanthin, whose the Haematococcus pluvalis as its richest source, is the strongest and the safest carotenoid without any pro-oxidant nature like β -carotene, lycopene, zeaxanthin, and lutein. Astaxanthin has 550 times powerful than vitamin E and 40 times than β -carotene as a singlet oxygen quencher, and 1000 times powerful than vitamin E against lipid peroxidation. It has superior position in cell membrane and shows 3 important triple effects: anti-oxidant, anti-inflammation, and immuno-modulator properties. Astaxanthin inhibits inflammatory gene expression by suppressing NF- κ B activation, protects cells from oxidative stress, and then improvement of β -cell function, insulin sensitivity and vascular complications (CVDs, etc), suppresses LDL-oxidation and may inhibit lipid peroxidation, and modulates endothelial NO system. In eye health, Astaxanthin reduces ciliary muscle strain during eye fatigue, and this may improve visual activity. Due to its strong antioxidant effects, astaxanthin may be of great important to reduce the risk for the development of ARMD. Due to its effect to quench free radicals and to lower lactic acid, astaxanthin enhances muscle endurance and physical fitness. Treatment of Helicobacter pylori infected mice with astaxanthin reduces gastric inflammation, bacterial load, and modulates cytokines release by splenocytes: a switch from a Th1-response to a mixed Th1/Th2 response during infection. Such an event is: a shift of the T-lymphocyte response from a predominant. Th1-response dominated by IFN- γ is shifted to Th1/ Th2-response with IFN- γ and IL-4. Treatment of H. pylori infected mice with astaxanthin decrease gastric inflammation, and bacterial load, and modulates cytokine release by splenocytes. Astaxanthin supplementation of men decreases lipid peroxidation (decreased 15 hydroxy fatty acid). Astaxanthin and α -tocopherol improve plaque stability by decreasing macrophage infiltration and apoptosis. Astaxanthin and vitamin C prevent gastric ulcerations in stressed rats. The role of antioxidant (Astaxanthin?) in carcinogenesis and chemoprevention in gastric cancer associated with H. pylori has been speculated. The intestinal absorption of astaxanthin delivered as capsules (4 mg bid) for 3 months is adequate and well tolerated, and decreases lipid peroxidation in healthy men

Keywords: astaxanthin, carotenoid, anti-oxidant, anti-inflammation, and immuno-modulator

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Abbreviations: AIR = Acute Insulin Response; ARMD = Age Related Macular Degeneration; Astaxanthin = Astaxanthin; CVD = Cardiovascular Disease; DAG = Diacylglycerol; DVC = Diabetic Vascular Complication; ESADDI = Estimated Safe and Adequate Daily Dietary Intake; GAPDH = Glyceraldehyde 3 Phosphate Dehydrogenase; NADPH = Nicotinamide Adenine Dinucleotide Phosphate; NO = nitric oxide; PAHA = PKC, AGEs, Hexosamine pathway, Aldose reductase; PARP = Poly ADP Ribose Polymerase; PKC = Protein Kinase-C; ROS = Reactive Oxygen Species

INTRODUCTION

The oxidative stress in uncontrolled diabetes mellitus (DM) is caused by four sequential processes as seen in Figure 1 such as : 1. activated Aldose reductase enzyme, 2. activated Hexosamine pathway, 3. increased DAG

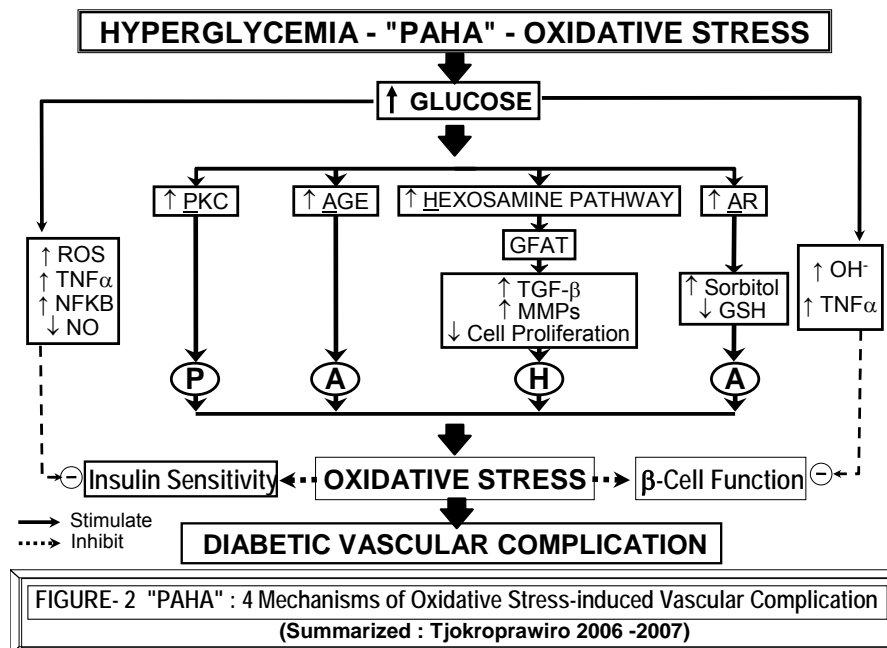
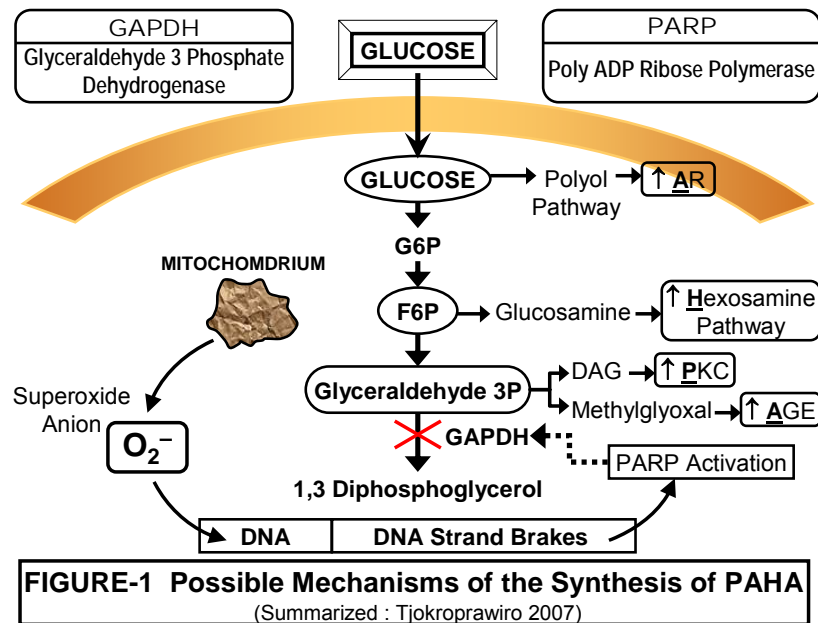
synthesis that activates PKC, and then 4. increased AGE production. For practical point of view, the author abbreviated such 4 components (A, H, P, A) as PAHA. Due to superoxide anion, the DNA standard will be broken down, and overactivated PARP may pursue; consequently, GAPDH activities will be inhibited, and all components of PAHA will be strongly activated (Figure 1).

FOUR PATHWAYS OF PAHA TO OXIDATIVE STRESS IN DM

Type 2 diabetes mellitus (T2DM) is associated with an increased risk of developing cardiovascular events.

Hyperglycemia – PAHA – Oxidative Stress is metabolic chain which is strongly associated with diabetic vascular complications. Four major hypothetical mechanisms of hyperglycemia – induced tissue damage through the formation of ROS (oxidative stress) abbreviated by the author as PAHA (PKC, AGE, Hexosamine Pathway, Aldose Reductase) are the causative components of oxidative stress (Figure 2). Oxidative stress may cause β -cell dysfunction

(decreased AIR or first phase of insulin secretion, and increased β -cell apoptosis) as well as insulin resistance (decreased all activities of PI3K, Akt, and GLUT-1 and GLUT-4 translocations). Long-term excellent glycemic control and strong antioxidant (f.e Astaxanthin = Asthin Force®) may offer the preservation of β -cell function and improve insulin resistance, and reduce markers of oxidative stress in T2DM.



Four Pathways of PAHA are shortly described below (Tjokropawiro, 2007). As seen in Figure 2, the short description of each component of PAHA can be followed, PAHA in Indonesian language means “THIGH”

PKC Pathway (P)

Body proteins become irreversibly modified by sugars in a process known as the Maillard reaction, leading to tissue “browning”. Diacylglycerol (DAG) and PKC are critical intracellular signaling molecules that can regulate many vascular functions, including permeability, vasodilator release, endothelial activation, and growth factor signaling. Activation of PKC may also be involved in the induction of growth factor expression (VEGF, TGF- β) and signaling molecules (VEGF, ET-1). In addition, PKC activation can also impact other signaling pathways such as those using MAP kinase or nuclear transcription factor. In the glomeruli of rats with diabetes, the α , β , δ , ϵ , and ζ are isoforms of PKC which all have been shown to be activated. Ruboxistaurin mesylate, a PKC inhibitor, has high affinity for the β_1 and β_2 isoforms, and has been shown to block many vascular abnormalities in endothelial cells and contractile cells from the retina, arteries, and renal glomeruli. In animal with diabetes, ruboxistaurine mesylate has been shown to prevent or reserve many early hemodynamic changes observed in diabetic retinopathy, nephropathy, and even neuropathy. Chronic oral treatment with this PKC- β isoform inhibitor in genetically diabetic mice prevented mesangial expansion and glomerular dysfunction. Ruboxistaurins mesylate is now being in clinical trials for diabetic retinopathy and neuropathy.

AGE Pathway (A)

AGEs can alter cellular function by binding to receptors, such as the receptor for AGEs (RAGEs), a transmembrane receptor. This binding may produce a cascade of cellular signaling events, such as activation of MAP kinase or PKC, which can lead to cellular dysfunction. Other receptors, such as the macrophage scavenger receptor, p60, p90, and galectin-3 have been reported to bind AGEs. Clinical trial using amiguanidine, an inhibitor of AGE formation, have been shown to be inconclusive due to the presence of limiting toxicity. However, the use of soluble RAGE-inhibitor to block binding to RAGE in animal models of diabetes has been reported to prevent several effects of hyperglycemia

Hexosamine Pathway (H)

As seen in Figure 1, via the activated GFAT due to high glucose blood level, TGF- β may increase which in turn induces the accumulation of matrix protein components of the mesangium and inhibits cell proliferation (increased MMPs and decreased cell proliferation). The accumulation of mesangial matrix is a marker to diabetic glomerulosclerosis, and a progressive decline in the surface area available for glomerular filtration and diabetic nephropathy may pursue.

Aldose Reductase or Polyol Pathway (A)

Aldose reductase uses nicotinamide adenine dinucleotide phosphate (NADPH) to reduce glucose to sorbitol which is then oxidized level of sorbitol is believed to contribute to the development of cataract. The decline in cellular NADPH caused by increases in aldose reductase flux may decrease the generator of nitric oxide (NO) in endothelial cell (decreased NO may increase the expression of ICAM and VICAM, induces platelet aggregation, suppresses vasodilator, induces SMC proliferation), and alter the cellular redox balance. Aldose reductase inhibitors have been shown to prevent some of the pathologic changes in rodent models of diabetic retinopathy, nephropathy, and neuropathy.

ASTAXANTHIN IN NATURE AND CLINICAL APPLICATIONS

Astaxanthin (Astaxanthin) is the main carotenoid pigment found in aquatic animals, however *Haematococcus pluvialis* is the richest source of natural Astaxanthin and is now cultivated at industrial scale. There are 732 known carotenoids, and humans cannot synthesise it, hence, need to obtain carotenoids from food such as salmon, trout, red seabream, shrimp, lobster, fish eggs, and also found in birds like flamingoes, quails, and other species. There are 3 stereoisomers of Astaxanthin such as 3S,3'S; 3R,3'S; 3R,3'R. The 3S,3'S stereomer is the main form found in *H. pluvialis*, while synthetic Astaxanthin contains primarily the 3R,3'S stereomer. Carotenoids can be categorized into Polar Carotenoids (astaxanthin, canthaxanthin, tunaxanthin, zeaxanthin, lutein) and Non-Polar Carotenoids (β -carotene, α -carotene, lycopene). The algae *Haematococcus pluvialis* is rich in Astaxanthin with 3 important properties (Mortensen et al 1997; Krinsky et al 1989): anti-oxidative properties, anti-inflammatory properties, immuno-modulatory properties in vitro (Jyonouchi et al 1995, 1996; Okai et al 1996), which is comprising the enhancement of T-cell dependent antibody production by mouse T-lymphocyte

clones and unprimed T-lymphocytes; and the enhancement of TNF α and IL-1 α release of mouse peritoneal adherent cells and down regulation of IFN-release by mouse Th1 clones and primed spleen cells (splenocytes).

Astaxanthin (Asthin Force®) at the time being in clinical use is the only strongest and safest antioxidant without any pro-oxidant effect with minimally 10 properties: Photo-oxidative Damage Protectant, Mitochondrial and Cellular Membrane Protector, Immune Response Safer, Anti-inflammation, Anti-Cancer Properties, Eye Health- ARMD, Skin Health Effects, Anti Neuro-degenerative Properties, Cardiovascular Properties, Diabetes Mellitus, Hypertension, and CVDs (Prevention of β -cell Function, and Increased Insulin Sensitivity). Only selected topics will be briefly described after four pathways of PAHA to reach oxidative stress are presented.

Antioxidant Effects of Astaxanthin

As seen in Figure 1, oxidative stress may cause β -cell dysfunction and decreased insulin sensitivity (insulin resistance). On the basis of its strong antioxidant, astaxanthin (Asthin Force®) may preserve β -cell function and improves insulin sensitivity. Within cells, free radicals (e.g., hydroxyl and peroxy radicals) as well as highly reactive form of oxygen (e.g., singlet oxygen) can damage DNA, protein, and lipid membrane (lipid peroxidation). This oxidative damage has been linked to β -cell apoptosis, aging (Harman 1981, Ames, 1993), and atherosclerosis (Steinberg 1989, Francis 2000), ischemia-reperfusion injury (Simpson 1987; Takayama 1992) age related macular degeneration = ARMD (Gerster 1991), and carcinogenesis (Breimer 1990; Marnett 1987; Moody 1982). Dietary antioxidants such as vitamin C or E and carotenoids (astaxanthin, β -caroten, etc) have been shown to help fight this oxidative damage in in vitro and in vivo studies (Anderson 1999; Kurashige 1990; Tinkler 1994). Potent antioxidant carotenoids (e.g., astaxanthin) may quench singlet oxygen and other reactive species, by absorbing the excited energy of singlet oxygen onto the carotenoid chain, leading to the degradation of the carotenoid molecule, but preventing other molecules or tissues from being damage (Beutner 2001; Mortensen 1997; Tinkler 1994). They also can prevent the chain reaction production of free radicals initiated by the degradation of lipid membrane (Mortensen 1997; Tinkler 1994).

Palozza et al (1992) and Naguib et al (2000) demonstrated astaxanthin with its strong antioxidant property at protecting membranous phospholipids and other lipids against peroxidation. Most studies showed that astaxanthin is stronger antioxidant (against singlet

oxygen) than vitamin E (500 fold stronger) and other carotenoids like β -caroten (38 fold stronger) or lutein (Kurashige 1990; Shimidzu 1996); in addition, astaxanthin showed 1.000 fold stonger against lipid peroxidation than vitamin E. In connection with its effects as a strong antioxidant, astaxanthin is belived to play a key role in a number properties such as protection or promotion against: UV-light photooxidation, inflammation, cancer, ulcer's *Helicobacter pylori*, aging, aged-related disease (ARMD, etc), immune response, diabetes, dyslipidemia, hypertension, CVDs, liver function, joint health, prostate health, and eye health.

Miki et al (1991) was the first to demonstrate that astaxanthin had a stronger protective effects against photooxidation than lutein and β -carotene. O'Connor et al (1998) further demonstrated that astaxanthin could be more effective than β -carotene and lutein at preventing UV-light photooxidation of lipids by a factor of up to 200 and 1.000 fold, respectively. Oxidative damage to the eye and skin by UV light has been widely documented (McVean 1999; Trevithick 1999). Hence, astaxanthin may be very important for eye and skin health. Normally, LDL in plasma is not oxidized, and oxidation of LDL is believed to contribute to development of atherosclerosis or cardiovascular diseases (CVDs). Hence, antioxidant supplementation will be of benefit to reduce the risk of CVDs.

Astaxanthin like cholesterol, is carried by VLDL, LDL, and HDL in human blood. Both in an in vivo test and in study with human subjects ingesting daily dosages as low as 3.6 mg astaxanthin per day for two consecutive weeks (Miki et al 1998) demonstrated that astaxanthin protects LDL-cholesterol against induced in vitro oxidation. This suggests that astaxanthin may help prevention of atherosclerosis and the risk of CVD by preventing oxidation of LDL-cholesterol in the blood. In addition, Murillo et al (1992) observed that astaxanthin supplementation led to an increase in blood level of HDL. By reducing inflammation (that is associated with the development of CHD), astaxanthin may be beneficial to heart health.

Hussein et al (2005) reported antihypertensive potential of astaxanthin in hypertensive rats through its positive effects on vascular reactivity and hemorheology. Salonen et al (2001) demonstrated in healthy men that astaxanthin (after 3 months) supplementation may decrease lipid peroxidation (decreased 15-hydroxy fatty acid as indicator of decreased lipid peroxidation). Li et al (2004) demonstrated in hyperlipidemic rabbits that astaxanthin and α -tocopherol may improve plaque stability by decreasing macrophage infiltration and

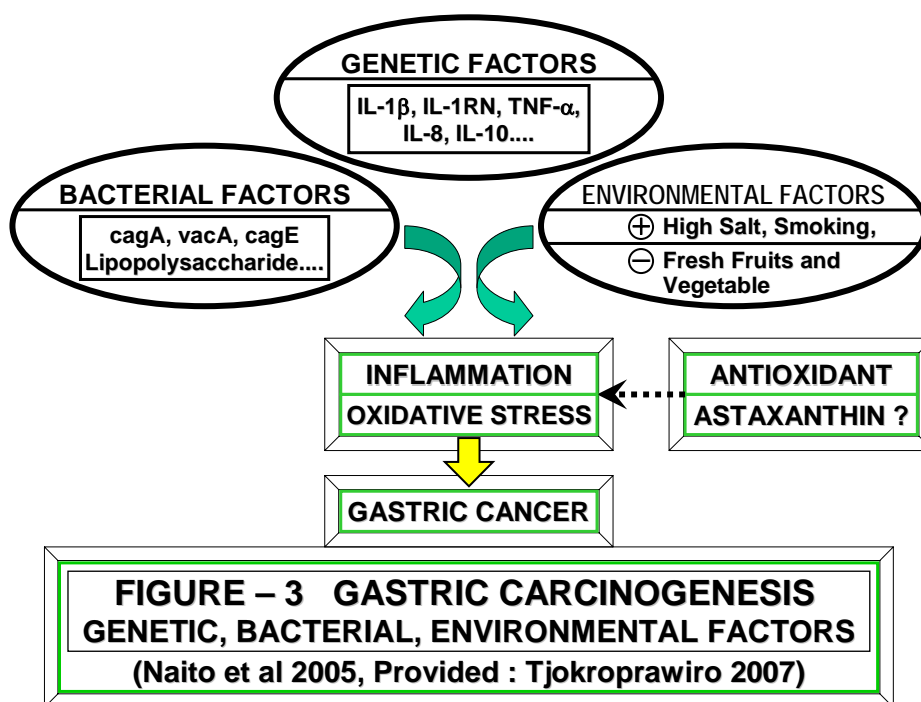
apoptosis (due to antioxidant properties) in this experimental animals.

Recently, Karppi et al (2005) investigated in healthy men that supplementation of astaxanthin 4 mg bid for 3 months reduced 15-hydroxy fatty acid statistically significant. This finding suggest that astaxanthin supplementation as a strong antioxidant may play an important role in the prevention of CVD.

In the centre of, where visual acuity is highest, a yellow spot called the macula lutea is visible. The yellow color is caused by the presence of nutritional carotenoid, lutein, and zeaxanthin, which accumulate there to a greater extent than in any other tissue. In the center of macula lutea, zeaxanthin is more predominant than lutein, whereas in the peripheral region lutein (as in plasma) predominates. The presence of lutein and zeaxanthin can contribute to quench the photo chemically induced ROS, attenuate chromatic aberration, and to inhibit apoptosis. Thus, these two carotenoids may contribute to reduce the risk for age related macular degeneration (ARMD). Furthermore, intake of lutein and zeaxanthin can specifically

increases their levels in the macula. Astaxanthin has not been isolated in human eye, yet it is found in the eye or eye parts of a number of animals (Egeland, 1993). However, an animal study has demonstrated that astaxanthin is capable of crossing the blood brain barrier, and like lutein will deposit in the retina of animals if included in the diet (Furr et al 1997). The composition of astaxanthin is very close to that of lutein and zeaxanthin, yet it has demonstrated in in vitro studies, a stronger antioxidant activity and UV-light protection effect than these two other carotenoids (O'Connor et al 1998). Hence, deposition of astaxanthin in the eye may provide superior protection against UV-light and oxidation of retinal tissue or protection against ARMD. Importantly, Tso et al (1996) reported that retinal photoreceptors of rats fed astaxanthin were less damaged by a UV-light injury and recovered faster than animals fed no astaxanthin, supporting the potential of astaxanthin for eye health.

As seen in Figure 3, the role of antioxidant in carcinogenesis of *Helicobacter pylori* was reported by Naito et al (2005).



It has been demonstrated that oxidative and nitrosative stress associated with inflammatory plays an important role in gastric carcinogenesis as mediator of carcinogenic compound formation, DNA damage, and cell proliferation. *Helicobacter pylori* – associated

inflammation not only activates various oxidant-producing enzymes such as NADPH oxidase and inducible nitric oxide synthase (iNOS), but also lowers the antioxidant ascorbic acid in the stomach (Benerjee et al 1994; Sobala et al 1993). Intake fresh vegetable

containing antioxidants such as vitamin-C and β -carotene reduces the risk for gastric cancer (Nishikawa et al 2005) as seen in Figure 3. It can be speculated that astaxanthin, a strong antioxidant and anti inflammation, may minimized the risk for development of *Helicobacter pylori* to gastric cancer.

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