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PHARMACOLOGICAL EFFECTS OF SPICES: EICOSANOID MODULATING ACTIVITIES AND THEIR SIGNIFICANCE IN HUMAN HEALTH

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SUMMARY

• *Prostanoids/eicosanoids are a group of lipid-soluble compounds which are produced by almost every cell in our body. They play a vital role in the proper functioning of organs. A deviation from the constant rate of formation and release ofprostanoids may lead to pathological situations, sometimes life-threatening. The realization that prevention of chronic diseases by dietary modification would be more effective and economically viable in large population than costly treatment with drugs and hospitalization has stimulated a general interest in the preventive effects of various dietary components, including vegetables, herbs and spices. In Ayurveda, various spices and herbs are described to show medicinal effects, such as anti-thrombotic, anti-atherosclerotic, hypoglycaemic, hypolipidaemic, anti-inflammatory, anti-arthritic etc. Furthermore, as spices modulate eicosanoidproduction they may serve to provide clue(s) to drugs directed to arachidonic acid pathway enzymes as pharmacological targets. Effects of aqueous or ethanolic extracts of few common spices (garlic, onion, ginger, clove, turmeric, cumin and omum) and some pure components isolated from them on in vitro platelet aggregation and their effects on prostanoid production are described. In some preliminary studies consumption of ginger and turmeric has been shown to ameliorate arihitric diseases, while ginger consumption is also shown to exert abortive and prophylactic effects in migraine headache. We propose that search for safe drugs from commonly used spices may open an interesting area in drug research.*

INTRODUCTION

• Spices and herbs are dried parts of different kinds of plants grown for their aromatic, pungent or other desirable substances. They include rhizomes, bulbs, barks, gum, flowerbuds, stigmas, fruits, seeds and leaves. They are characterized by loose terms such as spices, spice seeds, and herbs. Spices are fragrant or pungent plant products of tropical and subtropical regions. Spice seeds are the tiny aromatic fruits and oil containing seeds of herbaceous plants. Herbs are the fragrant leaves of such plants as marjoram, mint, rosemary and thyme. It is not known when man first used spices and herbs in food. As they originate from the tropical and subtropical areas, it is very likely that they were used to impart durability to food. Food is mainly spoiled by microbial growth. Rancidity of fat constituents of the food is another important factor contributing to food spoilage. Food prepared with spices (and herbs) stay longer due to their effects against growth of microorganisms and fat-rancidity. Several spices are known to contain antioxidants which might contribute to the latter effect.

Spices, spice seeds and herbs are added to food or used in food preparation with an intent purpose of imparting flavour and aroma to it. In the small quantities they are used in culinary practice, they are considered to provide little or no nutritive value. They, however, stimulate the appetite and zest to food by enhancing the taste. But there

may be additional benefits too. Use of spices in the recent year has spread to many cultures. This has generated some sort of curiosity to know more about them especially in relation to their biological effects and their modes of action in our body. The illustrious example of garlic may serve to underscore this point. It was used in several ancient cultures for centuries both as food and medicine. Inspite of that, it was only recently that the scientific community has taken garlic seriously. During the last two decades or so, evidence has been produced substantiating the claims made in the folklore.

It might surprise many of us about what has been discovered concerning foods during this century. The foods are full of pharmacological agents, they do act as drugs in the body, and depending on the food one eats, effects at the cellular level can be demonstrated. Many of the food folklores are full of wisdom, and are being confirmed by modern scientific methods. Clues from food folklore have inspired well-known scientists to investigate food-health-disease connections which has led to the realization of amazing powers in the food pharmacy. For example, fruits containing vitamin C were considered to prevent the scurvy in the earlier part of this century but now this vitamin is needed to maintain optimal health and the prevention of some chronic disease(s). Spices described below are not expensive and if their inclusion in the diet could be proven to delay/ward off the onset of chronic diseases (arthritis, atherosclerosis and cancer) billions of dollars could be saved in the health care systems.

In Ayurveda (the traditional Indian system of medicine) and the Graeco-Arabic (Tibb) system of medicine, several spices and herbs are described having medicinal effects, such as being anti-thrombotic, anti-atherosclerotic, hipolipidemic, hypoglycemic, anti-inflammatory, anti-arthritic etc. There are parallels existing between the involvement of eicosanoids in many pathological conditions and the claims in Ayurveda that spices and herbs can cure many of the same diseases. As spices modulate eicosanoid production they may serve to provide clue(s) to drugs directed to arachidonic acid pathways as pharmacological targets.

EICOSANOIDS: PHYSIOLOGICAL, PHARMACOLOGI- CAL AHD CLINICAL RELEVANCE

• Prostanoids are unsaturated lipids derived from fatty acid precursors such as arachidonic acid (AA) which is most abundant in the mammalian tissues compared to other precursors (dihomo-gamma-linolenic acid and eicosapentaenoic acid). The term prostanoids includes a group of chemically related compounds comparising prostaglandins and thromboxanes. In mammalian tissues are synthesized over 20 different prostanoids each with varying biological activity. Even in a single organ, such as the lung or kidney a host of different prostanoids with similar or opposing and at times with no action in that particular organ may be produced. To this list have been added a new group of biologically active substances called leukotrienes which are also synthesized from the same precursors as for prostaglandins. The term eicosanoids was introduced by Corey et al. (1) to encompass prostaglandins, thromboxanes, prostacyclins, hydroperoxy- and hydroxyeicosatetraenoic acids (HPETEs, HETEs), the leukotrienes and lipoxins.

Almost all the mammalian tissues examined so far are known to produce one or the other kind of eicosanoids exibiting specific biological actions. As for example, prostanoids and leukotrienes have important actions in the cardiovacular system regulating the functions of the heart and coronary blood vessels, and blood pressure. Prostanoids are involved not only in the regulation of cardiovascular functions but are implicated in the pathophysiological conditions of this system as well. Similarly, prostaglandins have been shown to influence virtually every aspect of female reproductive functions including the release of gonadotrophic hormones from the pituitary gland, luteolysis (destruction of corpus luteum), menstruation, fertilization of the egg and implantation, parturition, lactation etc. In the gut, prostanoids are known to perform three major physiological functions: control of gut motility, control of gastrointestinal secretions, and providing cytoprotection (protection against ulcers). Prostanoids show a direct action on the nephron to stimulate water and electrolyte reabsorption. They also influence renal function by altering the blood flow within the kidney and by affecting the activity of renal sympathetic nevers. Furthermore, they interact with hormones such as angiotensin II, bradykinin and vasopressin (anti-diuretic hormone, ADH) which influence water and ion transport in this organ. Thus, it would not be surprising that an imbalance in the production of prostanoids should result in diseases of this organ (e.g., Barrier's syndrome due to elevated renal prostanoid - PGE2, PGD2, PG^- production; hepatorenal syndrome due to elevated TxA2 production). Considerable evidence has accumulated implicating prostanoids as mediators of inflammation and in diseases of inflammation (rheumatoid arthritis). Moreover, leukotrienes are considered to be involed in several pathophysiological processes such as, generalized or local immune reactions, i.e., psoriasis, inflammatory diseases, and in asthma, shock and trauma. Even neurological diseases like migraine, epilepsy and schizophrenia are conditions where prostaglandins are associated. Thus, the list of

pathophysiological conditions where eicosanoids play role is long. It is very likely that more information on this count would be added in the future.

A general outline on the bioconversion of arachidonic acid into prostaglandins and some other AA metabolites is shown in Fig. 1.

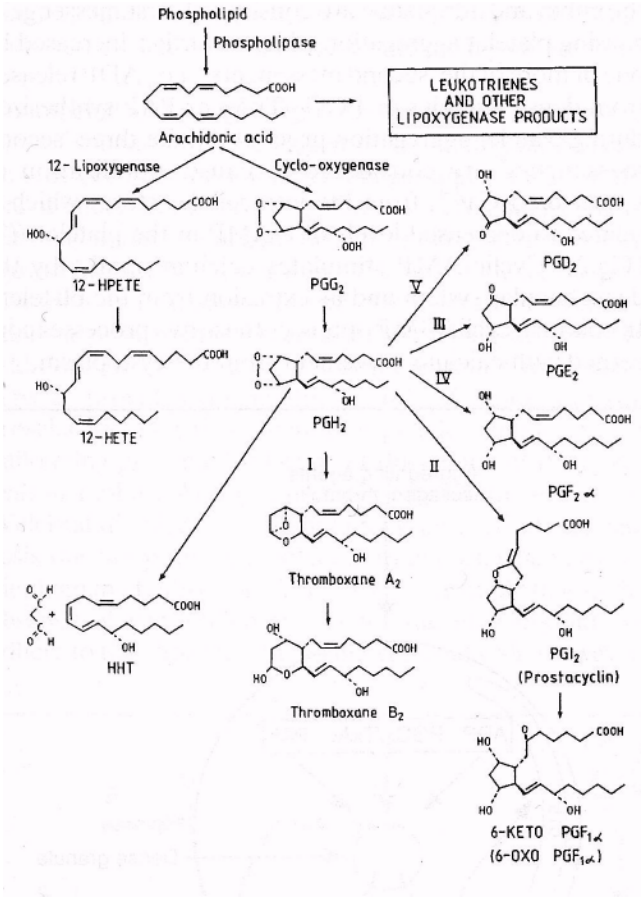

Figure 1. An outline of the cydooxygenase catalyzed conversion of arachidonic acid into the pivotal intermediates - prostaglandin endoperoxides (PGG2 and PGH2> - together with other AA metabolites: thromboxane, prostaglandins and prostacyclin produced therefrom, and 12-lipoxygenase-derived products. I Thromboxane synthase; II Prostacyclin synthase; III PG-endoperoxide E isomerase; IV Prostaglandin F reductase; V PG-endoperoxide D isomerase (From: Mustafa T and Srivaslava KC. Prostaglandins (eicosanoids) and their role in ectothermic organisms, p. 157 In: Advances in Comparative and Environmental Physiology, Vol. 5, Springer-Verlag, Berlin Heidelberg 1989)

PROSTANOIDS AND PLATELETS

• Platelets are small particles which circulate in the blood of mammals. Naturally, they appear as disc-shaped, roughly about 2-3 pm in diameter and 0.7 Mm in thickness. Human blood contains about 150000 - 400000 platelets per microliter. They play an important role in cardiovascular haemostasis. The major function of the blood platelets is to maintain the haemostatic integrity of the blood vessel and to stop bleeding after injury. To achieve this, platelets interact with the subendothelial components of the vessel wall, such as collagen, that activate the platelets in which it brings about a series of changes ranging from change of shape (from discoidal to spherical form), production of pseudopodia and adherence to collagen fibrils. The platelets in this acivated state release substances from their storage granules that cause other platelets to form aggregates at the site of the injury. This haemostatic plug is reversible and thus unstable. Stability is confirmed through activation of a coagulation system generating thrombin which produces insoluble fibrin from fibrinogen. It is the generation of fibrin that stabilizes the plug. Several steps of the coagulation cascade are dependent on the stimulated platelets for optimal activity.

On stimulation platelets exhibit six responses: shape change, aggregation, arachidonic acid liberation, a-granule release, dense granule release, and acid hidrolase release. The platelet responses are distinguishable on the basis of requirement for different concentrations of the aggregating agent. The concentration requirement increases for various responses in the above mentioned order. On the basis of strength, aggregation producing agents are divided into groups. ADP, adrenaline, nor-epinephrine, vasopressin and serotonin are weak activators capable of triggering only shape change and aggregation. Thromboxane *A.2* (formed from liberated AA) is of an intermediate strength, and produces not only shape change and aggregation, but also the release of contens from the alpha- and dense granules. Thrombin, collagen (applies to platelets that actually adhere to it) and divalent ionophore A23187 (a non-physiological stimulus) are characterized as strong inducers that can produce all the six responses.

When platelets are challenged with an aggregating agent (ADP, adrenaline, thrombin, collagen or calcium ionophore A23187) irreversible aggregation takes place accompanied by release of the contents of platelet storage granules. However, when platelets are challenged by strong inducers such as collagen, calcium ionophore A23187 or thrombin, a rapid deacylation of platelet phospholipids takes place. This results in the generation of free AA which is quickly metabolized to eicosanoids. In the case of stim-

uli, such as collagen and thrombin which activate platelets by a receptor-mediated mechanism, the story is not so simple in regard to the release of AA. Phospholipase C is activated following the activation of a receptor via G protein. It causes hydrolysis of the minor membrane lipid phosphatidyl inositol-4,5-biphosphate (PIP2). The two products of this hydrolysis are inositol 1,4,5-triphosphate (IP3) and 1,2-diacylglycerol (DAG). *IP3* acts to mobilize intracellular calcium, and DAG stimulates the same protein kinase C that can be activated by tumor promoting phorbol esters (2). IPg and DAG are considered second messengers in this transduction mechanism. Thus, involvement of inositol which is uniquely rich in stearic acid at the - position and AA at the -position, a coupled system of phospholipase C and diglyceride lipase has assumed a greater significance in eicosanoid biosynthesis. It is likely that specific stimuli induce eicosanoid production by the activation of phospholipase C, whereas in pathophysiological situations unspecific stimuli induce the formation of these compounds in which phospholise A2 is involed. However, platelet agonists like ADP and adrenaline, though capable of producing irreversible aggregation, do not generate large amounts of eicosanoids. Thus, in the case of ADP undetectable amounts of eicosanoids are generated, and with adrenaline as agonist very small amounts of these are produced (3).

The liberated AA is converted by the enzyme cyclooxygenase first into pivotal compounds of the AA cascade, prostaglandin-endoperoxides (PGG2, PGH_2) which involves incorporation of two moles oxygen per mole of AA. This reaction is known as the "oxygen-burst" when oxygen consumption of stimulated platelets is monitored (4). Prostaglandin-endoperoxides are short-lived AA-metabolites with a biological half-life of 5 min. Finally they end up in small amounts of stable metabolites called primary prostaglandins (PGE_2, PGF_{2a}, PGD_2). In the platelets, PG-endoperoxides are converted mainly to another class of eicosanoids - thromboxanes (Tx) produced by a specific enzyme thromboxane synthase. Thromboxane A_2 (TxA_2) is a very potent aggregating agent, and contracts aorta and other blood vessel preparations. TxA_2 is very unstable with a biological half-life of about 30 seconds (5). Both TxA_2 and PG-endoperoxides inhibit elevation of the platelet c-AMP levels by natural and physiological agents PGE^ and PGI_2 without affecting the basal levels of c-AMP. Increased intracellular c-AMP inhibits aggregation by preventing mobilization of intracellular Ca^+ from the platelet dense tubular system or by phosphorylating specific proteins catalyzed by c-AMP-dependent protein kinases. Prostacyclin, a very potent antiplateled and blood vessel relaxing substance of physiological origin, is also a product of AA metabolism. It is produced from PG-endoperoxides by a specific enzyme called prostacyclin synthase localized mainly in the blood vessels (6). Antiplatelet activity of prostacyclin is affected through increased c-AMP levels.

Physiologically important platelet aggregating agents in vivo include collagen, thrombin, ADP and possibly adrenaline and platelet activating factor (PAF). Collagen, thrombin and adrenaline are considered 'first messengers' causing platelet aggregation which is further increased by one or more of the 'second messengers', i.e., ADP released from dense granules or $PGG2/TxA_2$ or PAF synthesized during platelet aggregation process. All the three 'second messengers' are considered to cause mobilization of sequestered Ca^+ from the intracellular stores which is related to decreased levels of c-AMP in the platelets (7) (Fig.2). Cyclic AMP stimulates calcium uptake by the dense tubular system and its extrusion from the platelets. In contrast, cyclic GMP opposses these two processes concerned with calcium movement from the cystoplasm.

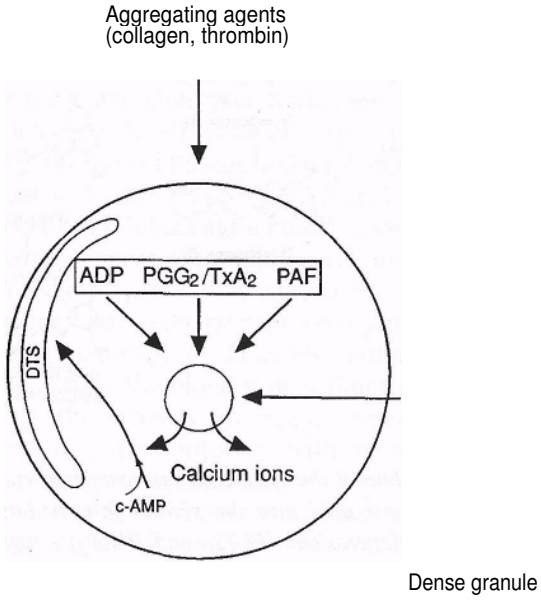

***Figure** 2. Three-tier mechanism of platelet aggregation.*

ROLE OF PLATELETS IN THROMBOSIS AMD ATHEROSCLEROSIS

• Atherosclerosis and thrombosis are the major cause of death in the affluent societies. Of the several factors that are considered to contribute to its development, diet is the most important one. In such societies, a major part of the dietary energy is provided by fat which comes chiefly from the animal products. A high fat intake may cause serum cholesterol and plasma fibrinogen levels to

increase, simultaneously decreasing the fibrinolytic activity and blood coagulation time. It has become more and more established in recent years that platelets play a key role in the development of atherosclerosis. The concept of the "loss of endothelial integrity" is very tenable (8). There may be several causes producing endothelial damage. Mechanical forces at the sites of flow effect, cholesterol deposition and foam cell infiltration into the subendothelium, vasculitis due to toxins or bacterial or viral infections, and causes related to immunology are the important ones.

Endothelial injury (loss of endothelium) leads to the exposure of subendothelial collagen to circulating blood cells, and the accumulation of macrophages and platelets at the site of injury. In this process, platelets secrete many chemicals, including vasoactive substances and platelet-derived growth factor (PGDF) of which the latter induces the proliferation and migration of smooth muscle cells with the result a proliferative atherosclerotic lesion appears. In addition, two important observations made in animals serve to provide evidence for the role of platelets in atherosclerosis. Drastic reduction of platelet number before ballooning prevented subsequent thickening of the aortic wall in rabbits. And in a strain of pigs with severe von Willebrand's disease experimentally induced atherosclerosis was not produced, unlike normal pigs, when given a diet high in choresterol. From this it may imply that in the absence of von Willebrand factor the platelets did not adhere to the cholesterol-damaged arterial wall. However, there may be other stimuli contributing to this process. Lymphokines from different sources and growth factors derived from the endothelium and other cells of the vessel wall may play an equally improtant role. The evidence that platelets directly enhanced cholesterol esterification and macrophage cholesterol ester accumulation further underscores the important role platelets play in the development of atherosclerosis (9).

The fundamental lesion in more than 95% of all cases of coronary heart disease (CHD) is coronary atherosclerosis which is a universal process occurring in most humans and is characterized by a local manifestation of a generalized disease. It may begin at quite an early age (10 yr) as fatty streaks and develop into progressive atheroma from the time of adolescence. Coronary atheroma may behave in either of the two ways: in some individuals it may remain clinically silent throughout life but in others it may develop into CHD which results from the narrowing and occlusion of the coronary artery. Therefore, the problem concerning the prevention of clinical coronary artery disease has two aspects: (i) prevention of occurrence of atheroma and its progression, and (ii) protection against complications resulting from thrombosis.

PLATELET AGGREGATION

- Since in the present review, one of the essential features is the effects of spices on platelet aggregation and

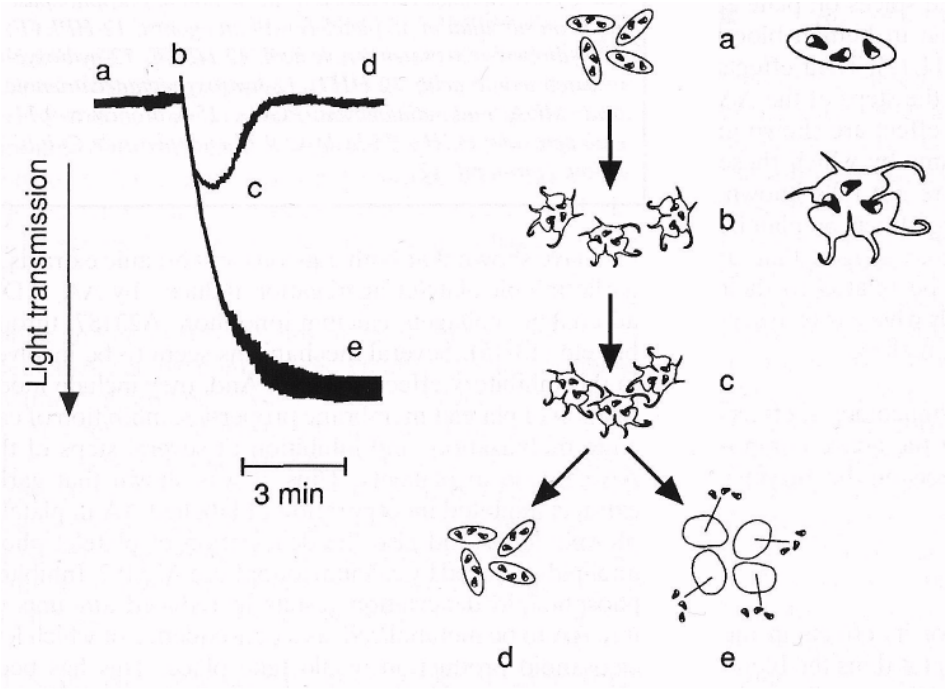

Figure 3. *A typical platelet aggregation pattern induced by ADP showing reversible and irreversible phases of aggregation (left) with associated changes in platelets (right). A platelet aggregometeris used to study aggregation based on a simple photometric principle. As platelets aggregate increase in light transmission takes place, (a) Normal, unstimulated disc-shaped platelets with a variable number of organelles randomly distributed in the cytoplasm enclosed by a plasma membrane; (b) platelet shape change with associated pseudopodia; (c) primary aggregation in which platelets adhere to each other through fibrinogen-Ca^ + linkages which are considered loose, hence unstable; (d) platelets move apart (reversible aggregation); (e) platelets undergo secondary (irreversible) aggregation following the release of proaggregatoiy agents from platelet granules.*

the mechanisms by which this is affected, a brief description of the various pathways involved in platelet aggregation would be appropriate. The platelet function is dependent on several inter-dependent pathways which can be selectively inhibited without blocking platelet function. Thus, even though treatment of platelets with aspirin results in irreversible inhibition of the enzyme cyclooxygenase (reduced formation of TxA2), the platelets can be induced to aggregate and release their contents when challenged with higher concentrations of collagen and thrombin. Thromboxane *A.^* produced in the platelets promotes release of ADP which concomitantly acts on the platelets in concert with TxA2- Removal of ADP enzymatically followed by treatment of platelets with aspirin can not block the platelet function, because such platelets still respond to thrombin. The mediator which produces aggregation in these platelets is termed platelet activating factor (PAF, a biologically active phospholipid, 1-O-alkyl, 2-acetyl, glyceryl-3-phosphocholine). PAF is generated by the action of thrombin, collagen or A23187 on human platelets. In contrast, AA and ADP do not trigger its formation (10). Thus, three different pathways of platelet aggregation have been characterized: (i) thromboxane A2-dependent, (ii) ADP-dependent, and (iii) PAF-mediated. A typical aggregation pattern induced by ADP showing reversible and irreversible aggregation with associated changes in platelets is shown in Fig. 3.

PHARMACOLOGICAL EFFECTS OF SPICES

• Effects of some common food spices on platelet aggregation and eicosanoid formation in human blood platelets have been reviewed earlier (11,12). Their effects on the generation of eicosanoids and the steps of the AA cascade in platelets where they show effect are shown in Fig. 4. Although the exact mechanisms by which these spices ellicit their medicinal effects are not fully known, their in vitro and in some cases ex vitro effects on platelet aggregation and on AA metabolism may suggest that, at least, one of the mechanisms could be related to their effects on the formation of eicosanoids whose role in several pathophysiological states is being realized.

Below we give in some detail the pharmacological effects of some selected spices together with the active components contained therein and their effects on the enzymes of the AA cascade.

Garlic (Allium sativum)

Garlic has been extensively studied for its effects in the cardiovascular system. The folklore that it thins the blood has been confirmed in several studies. We, as well as others, have shown that both aqueous and organic extracts of garlic inhibit platelet aggregation-induced by AA, ADP, adrenaline, collagen, calcium ionophore A23187, thrombin etc (13-15). Several mechanisms seem to be involved in this inhibitory effect of garlic. And, they include modification of platelet membrane properties, inhibition of calcium mobilization, and inhibition of several steps of the AA-cascade in platelets. Thus, it was shown that garlic extracts inhibited incorporation of labelled AA in platelet phospholipids and also the deacylation of platelet phospholipids induced by calcium ionophore A23187. Inhibited phospholipid deacylation results in reduced amounts of free AA to be metabolized, as a consequence of which less eicosanoid production would take place. This has been observed by us (13,14). A direct inhibitory effect of garlic

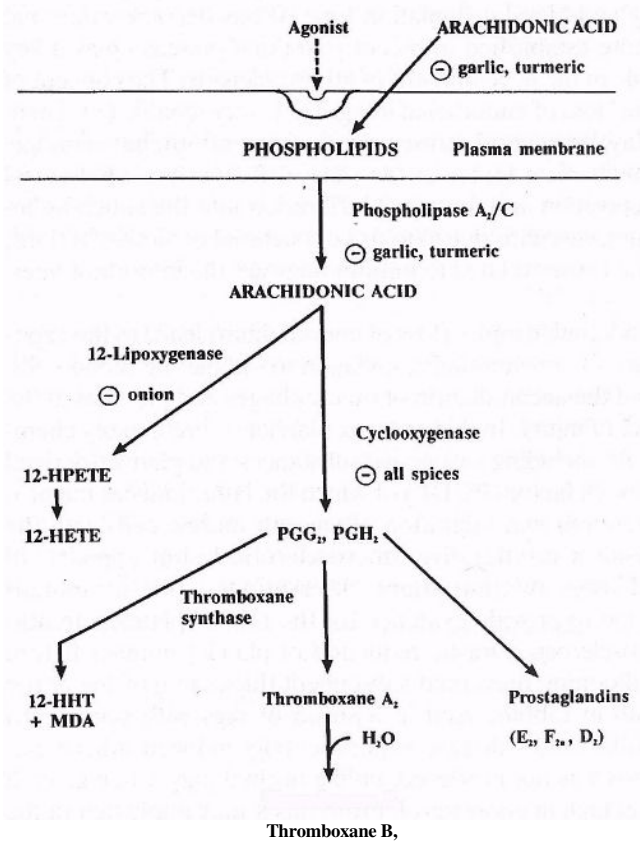

Figure 4. *Transformations of arachidonic acid in human platelets and the steps where spices show their inhibitory efects. Arachidonc acid esterified in the ^-position of the platelet membrane phospholipids is released by the action of phospholipase A^C on stimulation of platelets with an agonist. J2-HPETE, 12-hydroperoxyeicosatetraenoic acid; J2-HETE, 12-hydroxyeicosatetraeonic add; 12-HHT, 12-hydroxyheptadecatrienoic acid; MDA, malondialdehyde; PGG^ 15-hydroperoxy-9,Jl-endoperoxide; PGH^ 15-hydroxy-9,ll-endoperoxide;Q inhibition. (From ref. 12)*

extracts and its components on the enzymes of the AA cascade has also been reported (13,14,16,17).

On examining the effects of methanolic extract of garlic and some pure fractions obtained therefrom, it was found that though the extract and its components inhibited platelet aggregation, they failed to modify the metabolism of AA in platelets (15). However, the methanolic extract and a pure fraction isolated from it did inhibit by more than of TxE$2 formation in collagen-stimulated platelets. The anti-platelet behavior of garlic and especially of one of its components (E,Z)-ajoene is, according to the authors, apparently due to its ability to create membrane perturbation achieved by deep insertion of this substance in between the two monolayers of plasma membranes of the intact platelets (Apitz-Castro, personal communication). Ajoene did not alter AA metabolism in intact platelets.

While it is probable that garlic modifies platelet membrane properties as inferred from the restricted entry of labelled AA into platelets (13,15), there are several studies reported by various groups which indicate that garlic extracts and some of its components, including (E,Z)-ajoene, modify AA metabolism in platelets and other biological systems containing the enzymes (including 5-lipoxygenase) of the AA cascade (13,16,17). Moreover, aqueous extract of garlic when administered to rabbits was found to inhibit TxA2 formation in clotting blood and also in blood vessel preparations of this animal. Interestingly, this treatment did not affect the generation of prostacyclin in whole blood and aortic tissue (18). In another study, pretreatment of rabbits with an aqueous extract of garlic provided protection against thrombocytopenia and hypotension on administering collagen or arachidonic acid intravenously. The amount of thromboxane synthesized in these animals was reduced to levels not sufficient to induce thrombocytopenia and hypotension. All animals that were pretreated with garlic survived against the lethal dose of collagen or AA (19).

Intact and undisturbed bulbs of garlic contain only a few medicinally active compounds. Treatments like chopping, steaming of food processing brings about a radical change in the chemistry of garlic; the nature of the compounds produced depends largely on the mode garlic is handled. Crushing triggers the formation of a cascade of compounds that are quite reactive and take part in a complex system of chemical reactions. In such a process at least 100 sulphur-containing compounds are produced. Garlic sulphur compounds are linked to its medicinal uses.

Thus, the biological activity of an extract of garlic would depend on the mode of its preparation. The extract prepared at room temperature consists mainly of allicin, an antibacterial component. Allicin is unstable at room temperature with a half-life of ca. 2.4 hours. It is generated instantly when garlic is chopped or crushed due to the action of an enzyme called alliinase on the parent compound alliin, which is otherwise odourfree. Besides allicin, are also present small amounts of several other dialkyl thiosulphinates [RS (O)SR'; R and R' are allyl, methyl and propyl] and complex sulphinyl components including the antithrombotic ajoenes. Garlic when processed by steam distillation, yields an oily mass consisting of diallyl, methyl allyl, dimethyl, and allyl 1-propenyl oligosulphides - all originating from the thiosulphinates (20). The steam distilled essential oil, though lacking most of the antibacterial and antithrombotic activity (with the exception of components such as diallyl trisulphide, allyl methyl trisulphide and other paraffinic polysulphides which show antiplatelet activity in vitro), has been found to possess other equally interesting biological properties, such as antitumor and antioxidant effects (21,22). When garlic is subjected to cold aging process, besides oil-soluble organosulphur compounds (diallyl sulphide, diallyl disulphide, methyl allyl trisulphide and other polysulphides, ajoene, dithiins etc) major water-soluble organosulphur compounds, such as S-allyl cysteine, S-allyl mercaptocysteine, and several sulphur-containing amino acids are produced. S-allyl cysteine and S-allyl mercaptocysteine have been shown to be anticancer in animals (23), and to provide protection against liver damage (24).

Role of adenosine vis-a-vis other components

One of the ingredients of garlic is adenosine. About half of the antiplatelet activity of garlic has been attributed to adenosine (16). As adenosine is water soluble, a part of the antiaggregatory effects observed with the aqueous extracts of garlic and onion (and possibly ginger) in vitro (11) could be due to adenosine present in the extracts. To clarify the mode of action of adenosine on platelet aggregation and AA metabolism in platelets, we used commercially available adenosine for this purpose. Adenosine abolished AA- and collagen-induced aggregation at 1-2 MM and 10 MM respectively. However, adenosine (i) did not inhibit the formation of thromboxane and 12-lipoxygenase products from labelled AA in platelets even when used up to 990 MM; (ii) did not inhibit the release of labelled AA from platelet phospholipids [which were prelabelled with (14C)AA] on stimulation with calcium ionophore A23187 at 50, 200 and 560 MM; (iii) did not inhibit ionophore A23187-induced aggregation even up to 1 MM concentration (14). As the reactions in steps (i) to (iii) were found to be influenced by garlic extracts (aqueous and

organic), it was concluded that garlic contains also components, other than adenosine, which contribute to antiplatelet activity (13,14). Our observations with adenosine are similar to those of other who have reported that adenosine and allicin present in garlic inhibited platelet aggregation without altering AA metabolism in platelets (16). Polysulphides, such as diallyl trisulphide (25), dimethyl trisulphide (16) and methyl allyl trisulphide (26) were shown to be antiaggregatory and altered AA metabolism (16,25,26).

What all these would mean in an in vivo situation (that is, after consumption of garlic). In aggregation studies employing whole blood, adenosine was ineffective probably because it is taken up by erythrocytes which have a highly active nucleoside transport system and adenosine deaminase activity (ADA) of about 0.2 pmolar units per ml of cells (27). This would correspond to whole body erythrocyte ADA activity capable of degrading 135 mg adenosine per min. Other tissues such as liver and the intestinal mucosa have a high ADA activity. This means that adenosine would be poorly absorbed from the intestinal tract, and whatsoever reaches the blood would not survive more than a few minutes after absorption. Similarly, allicin was not traceable in blood after administration of garlic (28). Because of its chemical nature as a strong oxidizing agent and reacting rapidly with sulphydryl group of cysteine, it is likely that it renders itself ineffective by reacting with the peptides containing cysteine in the intestine and producing S-allyl mercaptocysteine. Nevertheless, as garlic and garlic products have been found to show effects in animals and humans, it seems reasonable that other products derived from allicin and other thiosulphinates (alkyl polysulphides, dithiins, ajoene) could reach circulation and body tissues since they are non-reactive (non-oxidative) and are lipid soluble. May be, it is these compounds which produce some of the medicinal effects. However, it can not be excluded that some hitherto unknown metabolite(s) derived from allicin with biological activity might be discovered in future. The active principles identified in garlic and onion are shown in Table 1.

Table 1. Active principles of garlic and onion.

Antiplatelet
Garlic:
alliin
allicin
allyl 1,5-hexadienyl trisulphide
allyl methyl trisulphide
S-allyl 2-propene thiosulphinate
ajoene
diallyl disulphide
diallyl trisulphide
1,5-hexadienyl trisulphide
methyl allyl trisulphide
2-vynil 1,3-dithiene
3-vinyl 1,2-dithiene

Onion:
adenoside
alliin
l-(methyl sulphynil)-propyl methyl disulphide
9,10,13-trihydroxy-ll-octadecenoic acid
9,12,13-trihydroxy-10-octadecenoic acid
trans-5-ethyl-4,6,7-trithia-2-decene-4-oxide
trans, trans (and trans, cis) 5-ethyl 4,6,7-trithia 2,8-decadiene-4-oxide

Antibiotic
Garlic:
allicin
ajoene
diallyl disulphide
diallyl trisulphide / :

Fibrinolysis
Garlic:
methane-thiol-3,4-dimethylthiophene
methyl cysteine sulphoxide
propyl allyl disulphide
propyl cysteine sulphoxide

Onion:
cycloalliin

Blood sugar, insulin
Garlic:
allicin
diallyl disulphide .

Onion:
allyl propyl disulphide

Blood lipids, cholesterol
Garlic:
alliin
allicin
allyl propyl disulphide
diallyl disulphide
S-methyl-L-cysteine sulphoxide

(From: Jos Kleijnen et al., Br J Clin Pharmacol 28: 535-544, 1989)

• Onion (Allium cepa)

An oily extract of onion inhibits both the cyclooxygenase and 12-lipoxygenase in platelets, and inhibits also platelet aggregation induced by ADP, adrenaline and AA (11,12). The compounds inhibiting the two enzymes have been identified (29).

Crude ethanolic onion extracts have been shown to prevent allergen-induced bronchoconstriction in laboratory animals and humans. This interesting observation has promoted search for the chemicals which bring about this effect. More than 150 chemicals have been isolated from onion. An a,p-unsaturated thiosulphinate and six unsaturated a-sulphinyldisulphides with biological activity have been characterized. These compounds have been collectively termed "cepaenes". They strongly inhibit cyclooxygenase in the microsomes from sheep seminal vesicular gland and also 5-lipoxygenase (5-LO) of porcine leukocytes (29). Alk(en)ylsulphinithioic acid esters are reported to inhibit histamine release, leukotriene and thromboxane biosynthesis in vitro and prevent PAF- and allergen-induced bronchial obstruction in vivo (30). Recently, a compound structurally related to a-sulphinyldisulphides and identified as l-(methylsulphinyl)-propyl methyl disulphide has been isolated from onion. This compound inhibits collagen-induced platelet aggregation. As it does not occur as such in onion, it is rather formed by an interaction of thiopropanal-S-oxide and methyl sulphinic acid produced when onion is crushed (31). Several possible therapeutic benefits of onion include: retards blood clotting, thins the blood, boosts beneficial HDL cholesterol, lowers total blood cholesterol (all suggesting onion's potential as a multifaceted heart-blood medicine), regulates blood sugar etc.

• Ginger (Zingiber officinale)

Ginger is used in folkmedicin, and is a popular food spice. It has many therapeutic uses. It is mentioned in British Pharmacopoea as a carminative. It occupies an important place in Ayurvedic and Graeco-Arabic (Tibb) systems of medicine where, among other things, it is recommended against rheumatism (32).

Chemically ginger, as one may expect, contains several classes of compounds. The chemical composition of dried ginger is as follows: starch 40-60%, proteins 10%, fats 10%, firbes 5%, inorganic material 6%, residual moisture 10%, and essential oil (oleoresin) 1-4%. In all more than 200 different volatile substances have been characterized in the essential oil fraction wherein the pharmacologically active compounds are to be found.

Ginger, its extracts and fractions thereof have been shown to inhibit platelet aggregation both in vitro (33) and ex vivo (34). The last one is related to an incidental observation by Dr. Dorso (the donor of blood) that only one-time consumption the previous evening of a large quantity of an excellent marmalade (Ginger with Grapefruit, Crabtree and Evelyn, London) whose major ingredient (15%) was ginger produced inhibition of AA-induced platelet aggregation which returned to normal after about a week time. We and others have shown that ginger strongly inhibits prostaglandin synthesis (11,33,35). Even some of the components identified in ginger were more potent than indomethacin in inhibiting prostaglandin synthesis (35). Ginger contains also several 5-lipoxygenase (5-LO) inhibitors (36). Ginger consumption resulted in reduced formation of thromboxane in human blood (37). Ginger has been shown to ameliorate symptoms of pain and swelling in musculo-skeletal diseases, which is brought about, at least, by its inhibition of cyclooxygenase and 5-LO pathways (that is, a dual inhibitor of AA metabolism) (38-40). In addition, ginger was found to exert abortive and prophylactic effects in migraine headache (41).

Ginger is known to possess antihistamic and antioxidant properties (32). The role of free radicals as endogenous histamine liberators has attracted attention. Significant peroxidation of membrane lipids could have damaging effects on membrane fluidity and cell compartmentalization with a consequent leakage of endocellular compounds, including histamine. In this context, AA may be considered to be a source of free radicals. Release of histamine from mast cells dependent on the generation of PG-endoperoxides from AA has been demonstrated (42). The release of histamine from mast cells by metabolitically activated AA is not associated with a significant leakage of LDH; this may suggest it to be an exocytotic secretory process. As this process was blocked by reduced glutathione, D-mannitol, and by lipoxygenase and cyclooxygenase pathway inhibitors, and was not reproduced by AA and by the end-products of the AA cascade, it may be suggested that free radical intermediates generated in the metabolic activation of AA are involved in mast cell histamine release (42,43).

• Clove (Syzygium aromaticum)

Clove, a pungent aromatic dried flower bud of myrtaceous tree, is frequently used in spice mixtures in the Indian subcontinent. Clove is also used as a crude drug for home medicine. It is reputed to be an aromatic stomachic in traditional medicine. There have been reported only a few pharmacological studies with clove; its active principles possess cholagogue effects (44). We have observed that an

ethereal extract of clove (oil of cloves) inhibits strongly platelet aggregation, an effect which is largely mediated by reduced prostanoid synthesis (45). Further, we have isolated and characterized eugenol and acetyl eugenol, two potent antiplatelet compounds present in the oil of cloves. They inhibit AA-, adrenaline- and collagen-induced platelet aggregation; they are more potent in inhibiting aggregation induced by the first two agonists. Their inhibitory effect was reversible. These components behave antiaggregatory, at least, by a combination of two effects: (i) inhibition of platelet thromboxane formation, and (ii) increased formation of 12-lipoxygenase (12-LO) product (12-HPETE) which has been shown to be antiaggregatory, and inhibits platelet thromboxane formation. Furthermore, plasma proteins (especially albumin) may act as a 'condiut' diverting free AA from the cyclooxygenase to the lipoxygenase pathway, i.e., it ensures increased production of 12-lipoxygenase products (46,47). This might explain why relatively lower amounts of these compounds inhibit AA-induced aggregation in contrast to the amounts needed for inhibition of thromboxane synthesis in washed platelets. This observation with platelets in plasma (aggregation experiments) and washed platelets (AA metabolism studies) becomes more interesting if one considers that in aggregation experiments plasma proteins are very likely to bind these compounds, thus lowering their effective concentrations considerably. The two compounds were more potent than aspirin in inhibiting aggregation induced by AA, adrenaline and collagen. In AA-induced aggregation eugenol was on par with indomethacin (48). Eugenol is a phenolic compound, and several phenolic cyclooxygenase inhibitors have been shown to be anti-inflammatory in animal models. The best examples are served by eugenol and curcumin (49,50). In fact, naturally occurring phenolic compounds are reputed in the folklore as "counter irritants". For centuries they have been used in plasters, liniments, poultices and rubs for their anti-inflammatory effects. Properties like cyclooxygenase inhibition, antioxidation and free radical scavanging might contribute to their anti-inflammatory effects. A structure-activity relationship for phenolic compounds in relation to cyclooxygenase inhibition has been reported (51).

- Turmeric (Curcuma longa L.)

Curcuma longa is a perennial herb widely cultivated in tropical regions of Asia. Its dried rhizome (turmeric powder) is used for imparting colour and flavour to food. Turmeric is used for medicinal purposes. There major components, 1,7-bis(4-hydroxy-3-methoxyphenil)-1,6-heptadiene-3,5-dione (curcumin), p,p'-dihydroxydicinnamoylmethane, and p-hydroxycinnamoyl (feruloyl)methane have been identified in turmeric; of these curcumin is the most important fraction. This substance is effective in reducing inflammation in animals and provides relief in arthritis in humans (52). In a short double-blind cross-over clinical trial involving 18 patients with confirmed rheumatoid arthritis, curcumin was found to produce significant improvement of symptoms such as, morning stiffness, walking time, and joint swelling following two weeks of therapy with an oral dose of 120 mg per day in all the patients (53). It was demonstrated to show hypercholesterolemic effects in rats (54). It has been shown to possess antihepatotoxic properties (55). It lowered blood sugar in a patient with diabetes (56). Curcuma powder has been reported to increase mucin content of gastric juice in rabbits (57). Thus, it may prove useful in providing protection to gastric mucosa against irritants. Curcumin has been shown to have strong antioxidant properties (58). It has been demonstrated to be an effective antimutagen in vitro against several environmental and standard mutagens and carcinogens that require metabolic activation for their activity. Curcumin provides protection against lipid peroxide-induced DNA damage (59). Turmeric fed at 0.5% of the diet and above inhibited benzo(oc)pyrene- and 3-methylcholanthrene-mediated mutagenicity. Water soluble compounds from turmeric possess antioxidant/anticlastogen and anti-promoter properties, and provide protection against mutagenicity of direct-acting carcinogens as well as in benzo(oc) pyrene-induced genotoxicity and carcinogenicity (60). Turmeric and its active component, curcumin, have been reported to provide symptomatic relief in patients with external cancerous lesions (61). Topical application of curcumin strongly inhibited 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced ornithine decarboxylase activity, DNA synthesis and tumor promotion in mouse skin (62). The inhibitory effect of curcumin in TPA-induced tumor promotion in mouse epidermis was found parallel to its inhibitory effect on TPA-induced epidermal inflammation and epidermal lipoxygenase and cyclooxygenase activities (62). The anticarcinogenicity of curcumin, which has been shown to be a potent inhibitor of the genotoxicity of various environmental mutagens/carcinogens, is probably mediated by its inhibitory effect on DNA-carcinogen adduct formation (63). It appears that the anticarcinogenicity is mediated by a series of mechanisms. Thus populations, like those in India, which include in their daily diet considerable amounts of turmeric [ca. 2-2.5 g per person per day for an average individual of 60 kg body weight (WHO/FAO Report 1974); the dry rhizome contains about 3-5% curcumin] (64), may be protected from the damaging effects of several mutagens/carcinogens present in the diet (65), besides those originating from occupational sources and life-style (tobacco habits) (66). When turmeric is used as a medication, it may be taken in doses up to 5 g daily.

Further, curcumin is reported to be non-toxic even at 100 times the usual intake (67).

The three active components of turmeric, including curcumin, were found to possess anticoagulation activity. Curcumin at doses between *25* and 100 mg/kg (i.p.) inhibited collagen- and adrenaline-induced aggregation of Tosta-
cyclin synthesis in rat thoracic aorta. As collagen-induced aggregation is associated with increased production of TxA2> it may by assumed that curcumin inhibits aggregation by reducing thromboxane synthesis.

In our studies, ethereal extract from turmeric was found to exert influence at several steps of the AA cascade in platelets. Thus, this spice, besides inhibiting cyclooxygenase, inhibited the incorporation of AA into platelet phospholipids, and also inhibited the release of (^C)AA from labelled platelets on stimulation with A23187 (68)(ref 13 and 14 for comparsion with garlic). It would, therefore, be interesting to examine if these effects on the AA cascade were common to other cell types or were specific for blood plateles. If incorporation and release of AA were also inhibited by turmeric in other cells (e.g., PMNs) it might explain its anti-inflammatory effects. For a concise information on the pharmacological properties of Curcuma longa (turmeric) the reader may refer to a recent review (69).

SOME LESS KNOWN SPICES

• Spices, such as omum *(Trachyspermum ammi),* asafoetida *(Ferula foetida)* and fenugreek *(Trigonella foenumgraecum)* are used less frequently, and in relatively small quantities in culinary practice in the Indian subcontinent. Cumin (Cuminum cyminum) is used though in relatively higher amounts compared to the three mentioned above, but not to the extent of other spices. In Ayurveda, omum is described to possess antispasmodic properties, and is prescribed in stomach trouble. In India it is a common practice to give women post parturition (after childbirth) a decoction of omum seeds. Cumin is known to provide ameliorative effects in stomach disorders. Organic extracts of both the spices inhibites AA-induced aggregation and production of TxB2 from exogenous AA and in AA-labelled platelets (68,70). Cumin extract inhibited also collagen- and adrenaline-induced aggregation (68).

Asafoetida is an odorous resin which is used only in very small amounts (milligram quantities) in food making because of its strong flavour and bitter taste. The essential oil isolated from the resin has been shown to have firbrinolytic and hypocholesterolemic properties. Fenugreek seeds and also its leaves are used as spice. Especially the seeds are known to provide relief in diarrhoea and gastrointestinal spasms. In rats, feeding with fenugreek-supplemented diet for five weeks improved clinical parameters, such as hyperglycemia, free fatty acids, cholesterol and triglycerides to normal values in experimental diabets (71).

COMCLUSIOMS AND PERSPECTIVES

• The food species that we have studied, and also several others form a part of the diet of large populations of Asia and other regions of the world. Their regulatory effects on the eicosanoid production may suggest that they might provide protection against diseases in which elevated levels of eicosanoids have been reported. Many spices (e.g., ginger, garlic, onions, clove, hot peppers) contain antioxidants which might potentiate the effects of physiologically significant dietary antioxidants, such as selenium, and vitamins A, C and E. These micronutrients, as is well recognized, provide synergistic multilevel defense system againts free radical injury and lipid peroxidation. Several reports have appeared showing an inverse relationship between the levels of anti-oxidants in the blood and ischaemic heart disease. Besides, a number of antioxidants are known to possess anti-carcinogenic properties. Thus, spices (e.g. turmeric) and their components may supplement, facilitate and/or enhance the activity of natural antioxidant systems protecting against the oxidative stress presumbly involved in the multistep process of carcinogenesis. In addition, scientific confirmation of the medicinal properties of food constituents as are found in the folklore may contribute to the development of dietary supplements (fish oil supplement, various garlic preparations on the market) and may also provide clues to the development of useful drugs. Futhermore, guided by the observation with the Eskimos who ingest large amounts of omega-3 fatty acids, which do not seem to cause them any harm, rather protect them from cardiovascular disorders and other chronic diseases, it would be of interest to pursue epidemiological survey of populations which consume spices regularly with regard to the prevalence of diseases typical of the the industrialized societies. ;

The enthusiasm about spices and their possible protective role in coronary heart disease (CHD) may be lessened when one takes into account the reports of epidemiological studies made on populations consuming spices with regard to prevalence of CHD in such populations. High rates of CHD in populations of South Asian origin were reported from Singapore, South Africa and Trinidad in 1950s. Similar finding were reported from U.K. for popu-

lations of South Asian origin in 1971. The high rates of CHD in Asian immigrants could not be explained on the basis of well-known risk factors such as plasma total cholesterol and smoking habbits. Non-insulin dependent diabetes is present in about 20% of South Asian men and women aged over 40 years in the United Kindom, comparted with about 5% of Europeans. Similar prevalence rates have been reported for other overseas South Asian populations and for an urban population in South India. A strong etiological predictor is a combination of genetic components, non-insulin dependent diabetes and obesity. Insulin resistance is associated with a particular type of obesity in which a high portion of body fat is deposited intra-abdominally. This would lead to physiological disturbances including hyperinsulinaemia, hypertriglyceridaemia, low concentrations of plasma high density lipoprotein cholesterol and hypertension (72). In these populations, spiced food consumption is a common practice. Should it be assumed then that spices do not have any protective effect? In answer, a point of caution we would like to make here. The way spices are used in food preparation in Northern India and Pakistan (and this may be so for the entire Indian sub-continent) may not be a healthy practice at all. During cooking spices are almost always exposed to high temperatures (frying) for periods long enough which would destroy the active/useful components some of which are anti-oxidants and modulate the arachidonic acid pathway. Such a cooking practice is likely to obscure results of any future epidemiological study of populations consuming spices if they use spices in cooking the way it is practized in the Indian sub-continent, and occurrence of diseases mediated by eicosanoids. In contrast, in Greece CHD is minimal though people eat fat-laden meat and smoke relatively much. But they love to eat peppers, onion, garlic and spices, besides fresh fruits providing physiologically important antioxidants. Furthermore, consumption of olive oil may have additional benefits. The same is true for Italy and Spain.

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