FLORIDA DEPARTMENT OF CITRUS

EXECUTIVE SUMMARY

WHITE PAPER ON INFLAMMATION AND ASSOCIATED DISEASES

BENEFICIAL ROLE OF CITRUS

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I-Introduction

(Fig1)

Recent medical studies have shown that a broad array of diseases, ranging from cardiovascular diseases to some forms of cancer, have in common a series of physiological steps, referred to as "Inflammation"and it is closely related with the immune response. It is now viewed as a major health threat and is even appearing on magazine covers (Fig1). The concept of inflammation can be defined as an extension of the processes taking place after any injury even minor, when the body reacts to any damage and repairs itself. While the injury repair process is beneficial, protracted inflammation can have very detrimental consequences, particularly since it may remain unnoticed for years. There are many causes for the inflammatory events, but pollution and the processes associated with the transformation of food into the energy we use for maintaining life are among the main initiators of this pre-disease state. While food or at least its misuse, is a main cause of inflammation, many compounds that can fight inflammation are also found in food. Fruits and vegetables and more specifically citrus fruits and juices are especially rich in anti-inflammatory compounds. In this review, the successive steps of the inflammation process and the diseases they can promote will be examined. How various classes of citrus compounds may interact with the successive steps of inflammation, limiting or eliminating its effects and contribute in preventing the resulting diseases will also be evaluated.

II-The Inflammation Processes

The main causes of inflammation can be grouped into several categories:

1) Mechanical (traumatic injury).
2) Physical (extremely low or high temperature, ionizing irradiation, microwaves).
3) Chemical (carcinogens, air pollutants, poisons, venoms).
4) Biological (viruses, microorganisms, parasites, allergens).
5) Nutritive (oxygen or vitamin deficiency and excessive food intake, mostly fat and sugar).
6) Aging.
7) Psychosomatic (sleep deprivation, physical and psychological stress, depression).

Inflammation is the general name given to a series of reactions occurring after most kinds of injuries, infections or immunologic stimulation. Inflammation can be divided into **an acute** phase (innate immunity) and **a chronic** phase. **Acquired immunity** has close links to inflammation and will also be examined.

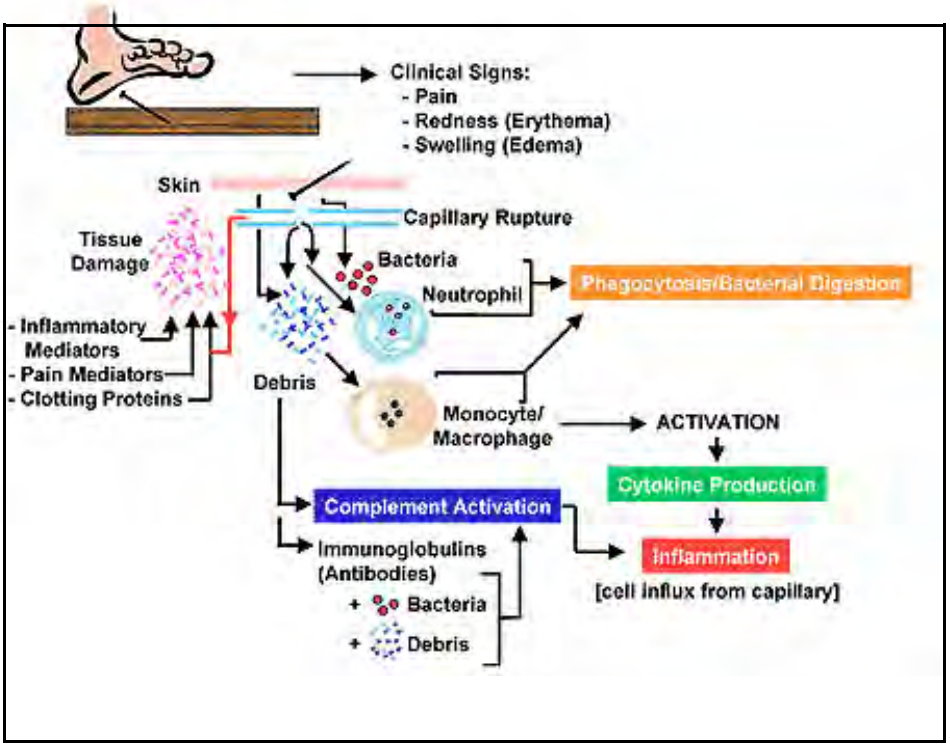


Fig. 2: Acute Inflammation

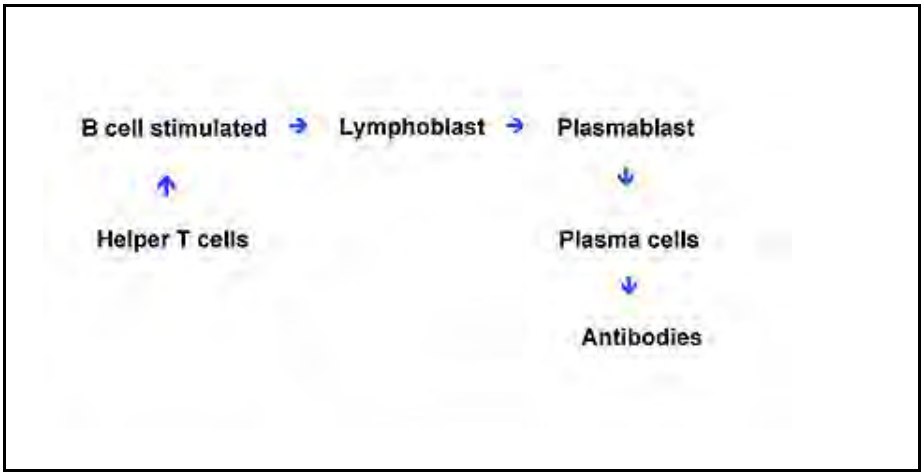


Fig.3 Acquired Immunity

II-I Acute Inflammation (Innate Immunity)

Acute inflammation is the mechanism by which the body repairs itself. For this reason it has also been called ”innate immunity”. It lasts only a short time and is generally beneficial. Following an injury such as a cut (Fig2), a complex series of events is initiated, particularly in the area close to the wound. The classical signs of inflammation are redness, heat and pain. The damaged tissue around the wound starts producing messenger chemicals that attract mainly white blood cells. In turn, the white blood cells and the surrounding tissue generate the production of a cascade of new chemicals and promote the formation of new types of cells such as macrophages (cells that dispose of debris). These cells clean the wound and destroy invading organisms and damaged tissue by producing oxidants such as hydrogen peroxide. The tissue is then repaired and inflammation recedes

II-II Acquired (Adaptive Immunity)

In comparison to innate immunity, acquired (adaptive) immunity develops when the body is exposed to various antigens (foreign invading substances or microorganisms)and builds a defense that is specific to that antigen. Acquired immunity acts much more slowly than the acute phase and is much more specific than innate immunity. Lymphocytes, a special type of white blood cells, contain subgroups, B and T lymphocytes, that are key players in acquired immune responses. B lymphocytes (also called B cells) produce antibodies (Fig3). Antibodies attach to a specific antigen and make it easier for the phagocytes (cell specialized in removing antigens) to destroy the antigen. T lymphocytes (T cells) attack antigens directly, and provide control of the immune response. B cells and T cells are specific for one antigen type. When you are exposed to a different antigen, different B cells and T cells are formed.

Immune system disorders occur when the immune response is inappropriate. Allergies involve an immune response to a substance that, in the majority of people, the body perceives as harmless. Autoimmune disorders (such as systemic lupus and rheumatoid arthritis) occur when the immune system acts to destroy its own body tissues. It should also be pointed out that innate and acquired immunity are not independent processes but interact closely with each other.

II-III Chronic Inflammation and inflammation markers.

If acute inflammation does not progress properly, particularly if the cause of the inflammation is not eliminated, chronic inflammation starts. Chronic inflammation can also be initiated directly by immune system disorders, particularly auto-immune diseases. Inflammation molecules keep being produced and some new ones may appear. These inflammation products are extremely numerous, their activity can be redundant, or opposite and they may be produced until inflammation is resolved. This constant inflammation eventually results in damage to the affected organs. A list of organs that can be affected by inflammation is shown in Fig 4. For example lung inflammation in

smokers never stops and may induce diseases such as emphysema and cancer. Chronic inflammation has been called the silent inflammation since it does not generate the pain associated with acute inflammation and therefore can go untreated for years. Silent inflammation is the underlying cause of heart disease, cancer, and Alzheimer's disease. Virtually every type of chronic disease has a significant inflammatory component as its underlying cause.

It is therefore clinically important for physicians to be able to monitor inflammation as it relates to various diseases. For a long time, elevated levels of white blood cells have been used as an inflammation marker. More recently, health professionals have started to monitor some of the chemicals generated during inflammation. They are called **inflammation markers**. Cytokines, the serum amyloid A protein (SAA) and fibrinogen are among the most well known (Fig.5). However, the **C reactive protein** which can increase up to a 1000 fold during inflammation has been seen as the best predictor of cardiovascular diseases and is now used clinically (Fig.6). CRP was first described in 1930 by Tillet and Frances, as a protein found in the blood of patients with pneumococcal pneumonia. It was named C-reactive protein because of its ability to react with the C-polysaccharide of the pneumococcus. Homocysteine is another marker but it may be in a class by itself since it appears to be more of genetic or nutritional origin. Homocysteine is a cause of inflammation rather than the result of it. Patients with high homocysteine are likely to have CVDs but homocysteinuria is not an indicator of CVD.

Inflammatory Disease	Infiltrate	Chemokine
Acute respiratory distress syndrome	Neutrophil	Interleukin-8; GRO-α, -β, -γ; ENA-78
Asthma	Eosinophil, T cell, monocyte, basophil	MCP-1, -4; MIP-1α; eotaxin; RANTES
Bacterial pneumonia	Neutrophil	Interleukin-8; ENA-78
Sarcoidosis	T cell, monocyte	IP-10
Glomerulonephritis	Monocyte, T cell, neutrophil	MCP-1, RANTES, IP-10

Inflammatory Disease	Infiltrate	Chemokine
Atherosclerosis	T cell, monocyte	MCP-1, -4; IP-10
Inflammatory bowel disease	Monocyte, neutrophil, T cell, eosinophil	MCP-1, MIP-1α, eotaxin, IP-10, interleukin-8
Psoriasis	T cell, neutrophil	MCP-1, IP-10, MIG, GRO-β, interleukin-8
Bacterial meningitis	Neutrophil, monocyte	Interleukin-8; GRO-α; MCP-1; MIP-1α, -1β
Viral meningitis	T cell, monocyte	MCP-1, IP-10

Fig.4: Main Organs affected by Inflammation. Column one shows the organ affected. Column two shows the type of cells (mainly white blood cells) that invades the inflamed organ and Column 3 shows the inflammation markers produced in the organ.

Detecting Systemic Inflammation

- Markers for the prediction of cardiovascular events
  - IL-6
  - Serum amyloid A (SAA)
  - TNF-α
  - Soluble intracellular adhesion molecule-1 (sICAM-1)
  - Macrophage inhibitory cytokine-1
  - sP-selectin
  - CD 40 ligand
  - hs-CRP

Circulation 109(suppl II):II-11-II-14, 2004. 31

Fig.5

C-Reactive Protein (CRP)

- Pro-inflammatory effects of CRP
  - CRP activates complement system and enhances phagocytosis
  - CRP up-regulates the expression of adhesion molecules in endothelial cells
  - Inhibits endothelial nitric-oxide synthase expression in aortic endothelial cells
  - Stimulates increased release of IL-8
  - Increases LDL uptake by macrophages
  - Increases PAI-1 expression and activity
  - Increases the release of IL-1, IL-6, IL-18 and TNF-α

J Biological Chem. 279:48487-48490, 2004. 13

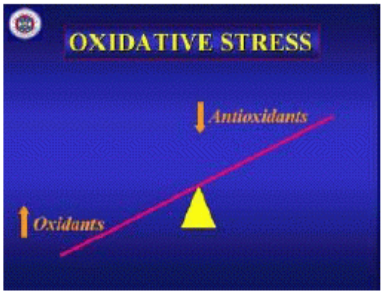
Fig.6

III-Major causes of inflammation

As mentioned previously there are many factors that are responsible for initiating the inflammation process. Some of the major causes will be reviewed.

III-I Oxidative stress

(Fig 7)



One of the family of compounds that has been shown to cause significant damage to the body is the oxidants. They are divided into free radicals and reactive oxidative species (ROS). Free radicals are very unstable and extremely reactive compounds. They can be characterized as molecules lacking an electron. They cannot survive as such for any significant amount of time and must get their missing electrons from the first cellular molecule they encounter. The ROS are a little more stable but usually give rise to free radicals. One of the best-known example is ozone (O_3) mostly generated by car exhaust. Ozone is unstable and breaks down into oxygen (O_2) and a very powerful oxidant "native oxygen" (O). Native oxygen oxidizes (burns) any organ molecules it meets within a fraction of a second. Body fats (lipids), proteins and DNA are the main targets of free radicals and ROS.

Free radicals and ROS are generated by pollutants, UV rays, and radiation. Another source of ROS is inflammation itself. White blood cells and macrophages destroy invading organisms and damaged tissue by producing oxidants, which can also damage healthy tissue. This can become significant during chronic inflammation.

However, the main cause of oxidant generation is linked to energy generation by the body. This involves: (1) The intake of food and its break down in the digestive tract into its elementary components: sugars, fatty acids and amino acids. (2) The transport by the cardiovascular system of elementary food (amino acid, lipids and glucose)and oxygen from the lungs to the various organs and cells (3) The production of energy by the cellular mitochondria (the cell's power generators). The body behaves like any power generating engine such as the car engine. We ingest food (fuel) that is burned (oxidized) by oxygen into water, carbon dioxide and energy.

ROS are produced during food oxidation and energy generation. Oxidants are generated immediately after a meal (postprandial oxidation), when large quantities of food are processed into energy or stored. The production of oxidants can also peak during exercise when stored food is converted rapidly to energy. It has been estimated that up to 2% of the energy production process is misdirected toward the production of free radicals and oxidants
The body has the ability to neutralize oxidants, using some of its own enzymes or antioxidants provided by food and more specifically fruits and vegetables. **The condition called "oxidative stress" occurs when the amount of oxidant generated exceeds the body ability to neutralize them** (Fig 7)

III-II Metabolic syndrome

Besides oxidants, food itself is a major cause of inflammation. Excessive food intake, particularly sugars and fats, leads to a condition called **metabolic syndrome**. It is not a disease *per se* , but a series of symptoms which leads to serious health disorders.

It has been assumed that if someone has three or more of following symptoms, the person is affected by the metabolic syndrome

1) Central obesity (excessive fat tissue in and around the abdomen- waist measurement of more than 40 inches for men and more than 35 inches for women.
2) Blood fat disorders — mainly high levels of triglycerides (150 mg/dL or higher, low levels of HDL, or "good," cholesterol (below 40 mg/dL for men and below 50 mg/dL for women).
3) Insulin resistance or glucose intolerance.
4) Pro thrombotic state (high fibrinogen in the blood).
5) High blood pressure (130/85 mmHg or higher).

III-II-1 Sugar intake

Sugars are among the foods responsible for initiating the metabolic syndrome. All the sugars present in food are eventually incorporated in the glucose energy production pathways. During transport storage and utilization, glucose is under the control of two pancreatic hormones insulin and glucagon. The insulin, glucagon interactions are shown in figure 8.

Insulin's role is to control the distribution and storage of glucose after a mea,l when blood glucose level is high, and therefore maintains blood glucose at a proper level. Excess glucose is stored as a glucose polymer similar to starch (glycogen). Glycogen is the readily available stored energy and is rapidly depleted during exercise since only about 500g of glycogen can be stored in the liver and in the muscle. The remaining excess energy is transformed into fat, a very concentrated form of energy, that can be stored indefinitely in very large amounts, as we are all aware. Insulin acts by interacting with receptors located on the organs using glucose. If the amount of glucose in the blood is constantly too high the receptors become less and less sensitive and need higher and higher levels of insulin to respond and initiate glucose storage (Fig.9). This condition is called pre-diabetes or insulin resistance. It leads to type 2 diabetes when insulin can no longer manage glucose properly and it remains constantly high in the blood. Diabetes can promote many other health problems and particularly all the cardiovascular diseases (CVD) as well as blindness and gangrene.

Glucagon activity is the opposite of that of insulin, it releases glucose from storage when needed between meals, stops liver glycogen synthesis, increases liver glycogen breakdown and stimulates gluconeogenesis (synthesis of new glucose by the body) in the liver to further increase the circulating blood glucose concentration.

Fruits and particularly citrus contain three main sugars; glucose, fructose and sucrose (a disaccharide made of glucose and fructose). During digestion sucrose is split into fructose and glucose and fructose is mainly incorporated into the glucose metabolism. The introduction of high fructose corn syrup has drastically increased the amount of fructose ingested by the US population. It is believed that exposure of the liver to large quantities of fructose leads to rapid stimulation of lipid production and accumulation, which in turn contributes to reduced insulin sensitivity and hepatic insulin resistance and nutritive inflammation. The consumption of high amounts of fructose is view as a main contributor to insulin resistance. However it has also been shown that in smaller quantities fructose, less than 10% of the total sugar intake, can have a beneficial effect in controlling blood glucose level. This may be of importance for citrus juices which contain glucose and fructose in similar proportions. **Therefore, during a meal rich in starch which produces large quantities of glucose, citrus could be absorbed and provide the small amount of fructose necessary to improve sugar management.**

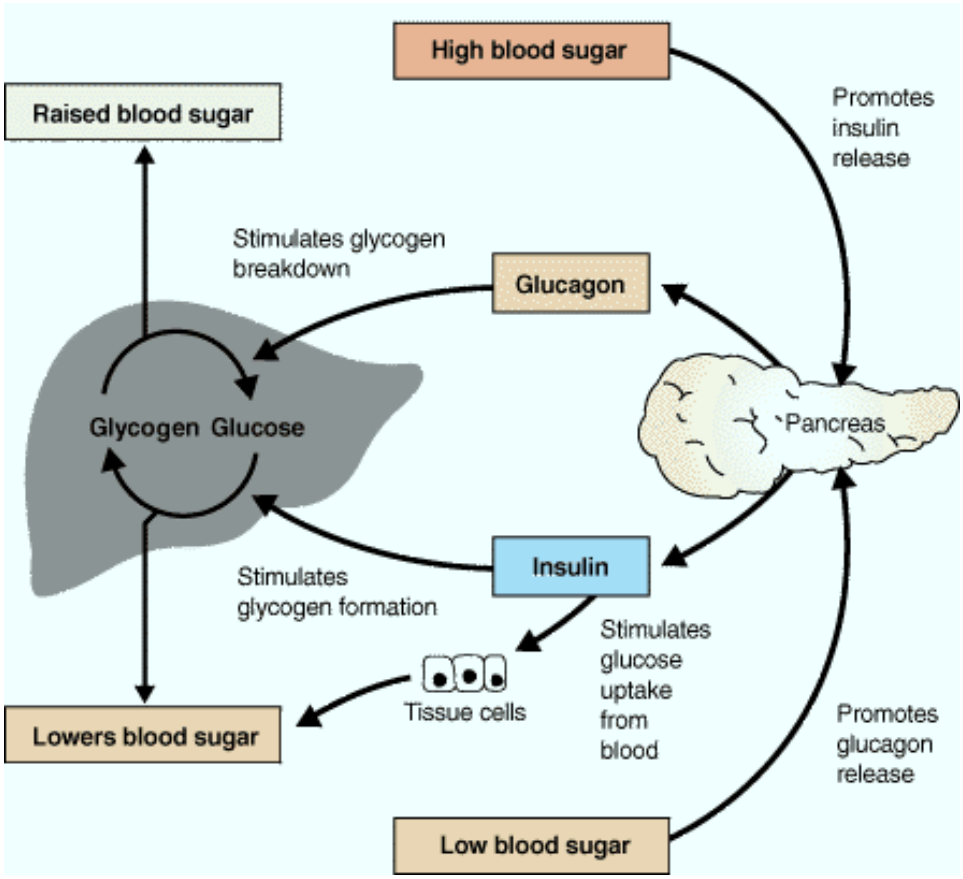


Fig. 8**:** Interaction between insulin and glucagon

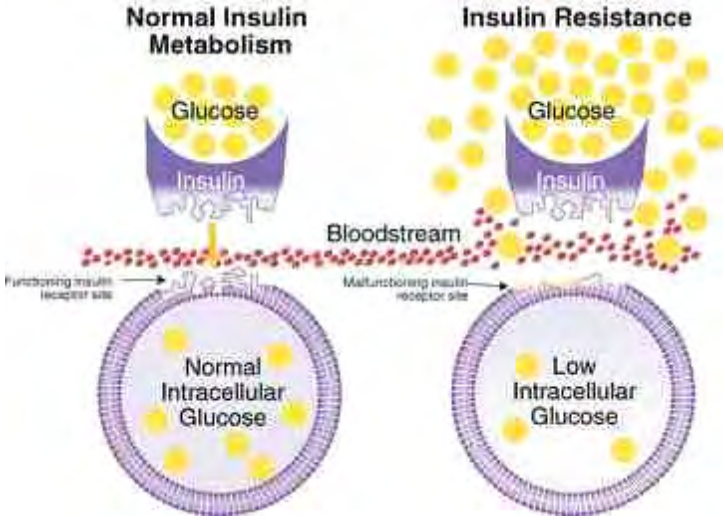


Fig. 9**:** Insulin resistance occurs when the insulin receptors become insensitive and do not allow glucose to move from the bloodstream into the cells

III-II-2 Fat intake

Besides sugar, an excessive fat intake is also of concern. Fatty meals are one of the main cause of cardiovascular diseases (CVD), particularly hardening of the arteries (atherosclerosis). After being broken down into elementary lipids (fatty acids), fat is transported from the intestine into the blood and in the blood as lipoprotein (LP) (Fig.10), little balls of fat, with embedded proteins. Fat is less dense than protein, therefore lipoproteins with more proteins than lipids are called high density LP (HDL). They are classified as the "good" LP since they can be excreted by the body. The LP with more lipid than proteins are the low density lipoproteins (LDL) and are the bad LP. They are oxidized by the oxidants generated in the body and are deposited in the arteries as plaque (atherosclerosis). Plaque decreases the arteries' diameter, limiting or stopping blood flow which can lead to heart attacks. Plaques can also make the blood vessels more rigid increasing blood pressure. Complications occur if the plaque surface becomes damaged and induce the formation of blood clots (similar to the clots that stop bleeding after a cut). Fragments of plaque and clots can break away. The dislodged material travels to other parts of the body (e.g., brain, heart, kidneys, and legs), where they block blood flow through smaller arteries resulting in serious injury to tissues and organs many of them lethal (Fig.11).

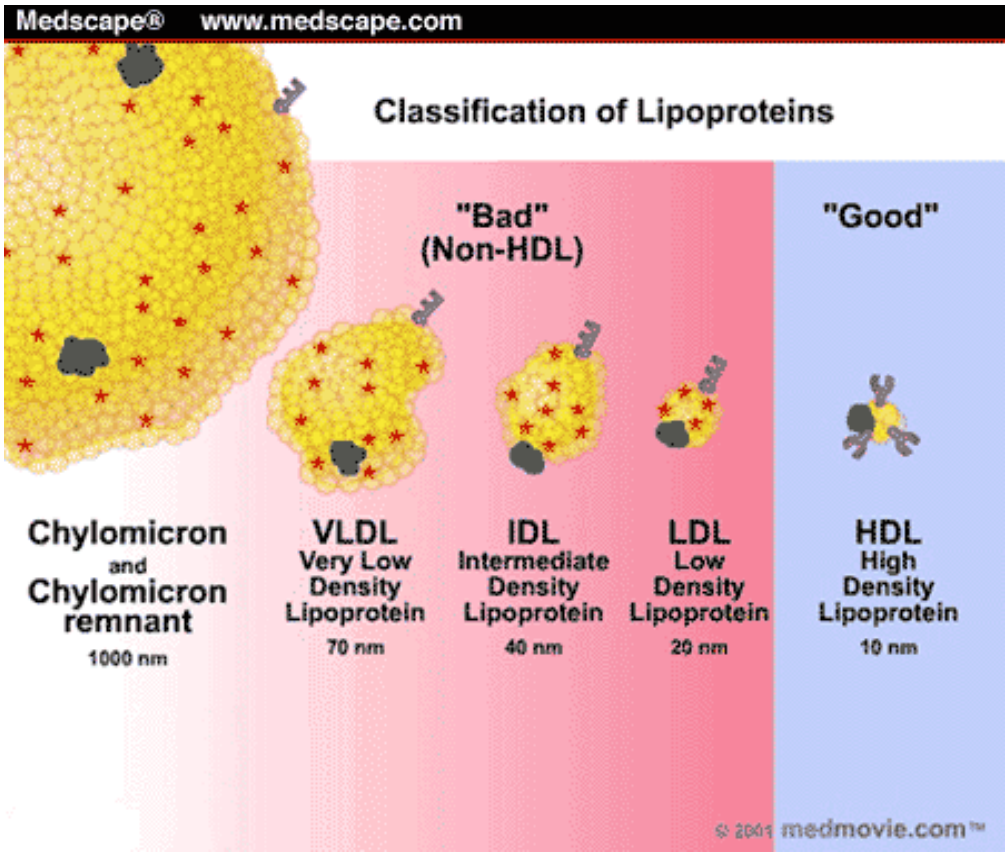


Fig.10: Various types of lipoproteins

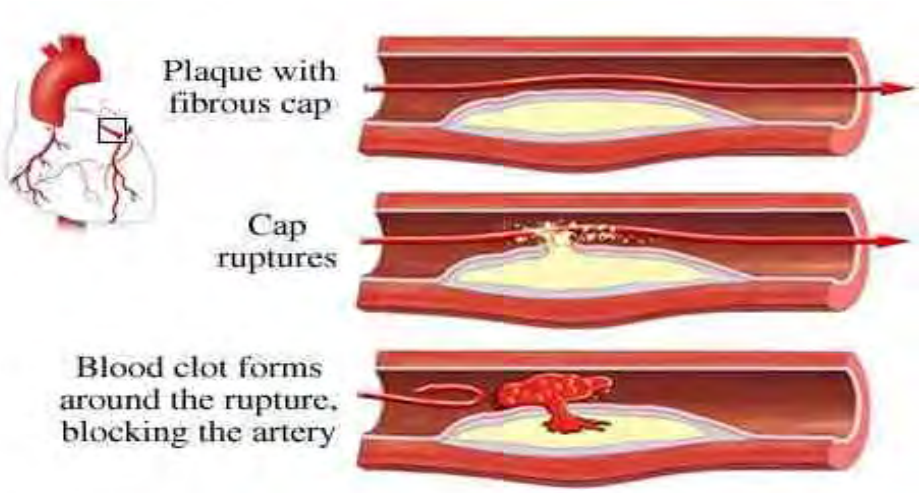


Fig. 11: Plaque

III-II-3 High Blood Pressure and Ion (mineral) intake

Not directly related to the metabolic syndrome but closely involved with CVD are the minerals (ions) provided by food. Sodium(Na) is the main body ion and its concentration determine the osmotic pressure of our fluids and ultimately blood pressure. Cellular ions are controlled by pumps that regulate their intracellular concentrations. Pumps very often cannot allow an ion in if they don't excrete another called the counter ion (Fig.12). Potassium (K) is the sodium counter ion. The Na/K pump excretes three sodium ions for two potassium ions it takes in. It has been proposed that the proper ratio Na/K is more important than the absolute amount of sodium ingested. Citrus juices with a high K level should favor the excretion of sodium.

About 90% of all high blood pressure cases are called essential hypertension. The causes of essential hypertension are unknown but are certainly based on complex processes in all major organs and systems, including the heart, blood vessels, nerves, hormones, and the kidneys. When blood pressure falls (for systolic, to 100 mm Hg or lower), the kidneys release the enzyme renin into the bloodstream. Renin splits angiotensinogen, a large protein that circulates in the bloodstream, into pieces. One piece called Angiotensin I is further cut by angiotensin-converting enzyme (ACE) into angiotensin II. Angiotensin II (Ang II), causes the muscular walls of small arteries (arterioles) to constrict, increasing blood pressure. Angiotensin II also triggers the release of the hormone aldosterone from the adrenal glands. Aldosterone causes the kidneys to retain salt (sodium) and excrete potassium (Fig.13). The sodium causes water to be retained, thus increasing blood volume and blood pressure. Besides its activity in regulating blood pressure, Ang II starts an inflammatory process with the production of Reactive Oxygen Species, adhesion molecules, chemokines, and as well as other mediators. Ang II and interleukin 6 (IL-6) induce other pro-inflammatory compounds and superoxide anions. Thus, the induction of IL-6 by Ang II may represent one important mechanism by which Ang II could be involved in the development of atherosclerosis. **Citrus with a low sodium and high potassium levels as well as high antioxidant content may alleviate some of the damages caused by high blood pressure.**

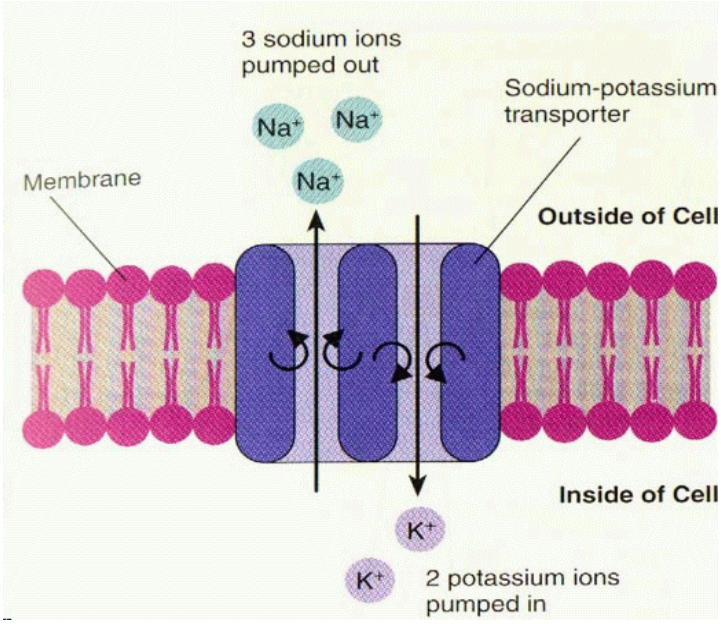


Fig.12: Sodium/Potassium Pump

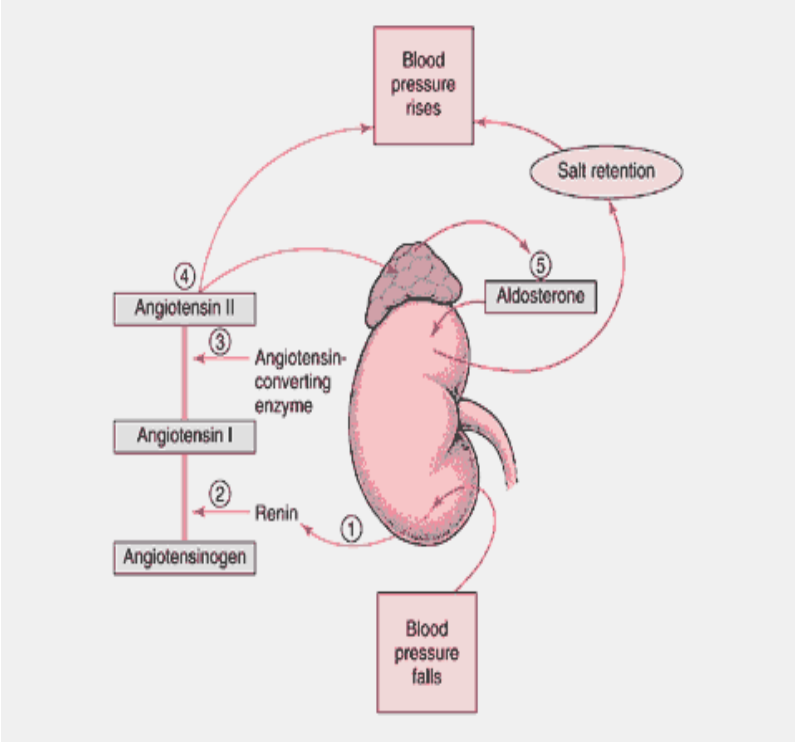


Fig.13: Angiotensin cycle and blood pressure control

III-III Aging and stress

Unfortunately for all of us, aging can be viewed as a result of chronic inflammation. It is thought that human genes are programed for a life span of about 120 years. However as we grow older, the ability to absorb and use antioxidants decreases while at the same time the production of oxidants increases. Although all organs are affected by aging, the brain has been one of the main targets of the senescence process. It has also been reported that physiological problems such as stress and depression can induce inflammation in the body. Reports have shown that people suffering from depression produce inflammation markers similar to those generated by physical ailments.

The brain uses large amounts of energy. With only 2% of the body weight it consumes 20% of the available glucose and oxygen. It should also be noted that this high metabolic activity generates ROS that can damage neurons (nerve cells). Contrary to the muscles or the liver, the brain stores very little energy (glycogen). Furthermore the access to the brain by blood borne molecules including antioxidants is severely limited by the blood brain barrier (BBB). Finally, brain cells cannot regenerate and once damaged by lack of oxygen, glucose or physical injury they die. Alzheimer's disease, Amyotrophic Lateral Sclerosis, and Parkinson's disease have been shown to have an inflammation component

III-IV Immune system disorders and allergies

As we have seen, the immune system is intimately involved in the inflammation process. The immune system is composed of several organs and systems called lymphoid organs. They include the spleen and thymus. Additional components of the immune system are lymph nodes and bone marrow.
The lymphocytes (white blood cells) function as immune cells. The two major classes of lymphocytes are B-cells, which mature in the bone marrow and reside in the lymph system, and T-cells, which mature in the thymus and circulate throughout the body. B-cells are responsible for producing antibodies (immunoglobulins), which are proteins designed to recognize and tag a specific antigen (foreign molecule), while T-cells are charged with destroying antigens that are tagged with an antibody.

Several components of citrus may be able to boost the immune system. Vitamin C has been shown to be taken up by T cells and increase ability of these immune cells to destroy antigens. It may also improve the thymus gland's ability to produce infection-fighting lymphocytes. Flavonoids and beta-carotene help regulate immune cells: T and B lymphocytes, and macrophages. Flavonoids have been shown to inhibit IL-4 and IL-13 synthesis, it can be expected that the intake of flavonoids, may ameliorate allergic symptoms or prevent the onset of allergic diseases. **Therefore several citrus compounds including vitamin C and flavonoids could have a beneficial effect in boosting the immune system**.

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IV-Discussion

IV-I-Citrus antioxidant and inflammation (vitamin C, flavonoids, carotenes and limonoids)

Many inflammation problems are initiated by oxidants generated during the transformation of food into energy and the utilization of energy during exercise (oxidative stress). The body can eliminate these oxidants mostly with the help of antioxidants provided by fruits and vegetables. The production of oxidant species becomes greater with age as the ability to neutralize them decreases. Although more studies and clarification are needed in the area of citrus antioxidants there may be enough available data at present to build a marketing case for the positive effect of citrus antioxidants.

Citrus and citrus juices contain a large array of different antioxidant compounds including: vitamin C, flavonoids, carotenes and limonoids. Among these, vitamin C and flavonoids are present in large amounts.

A synergistic activity exists within citrus juices. **Studies have showed citrus juices are more effective antioxidants than each citrus component taken separately**. For example range juice was more effective than quercitin rutinoside which in turn was more effective than ascorbic acid.

Some areas that can offer a positive message will be reviewed.

IV-I-1 Co antioxidant

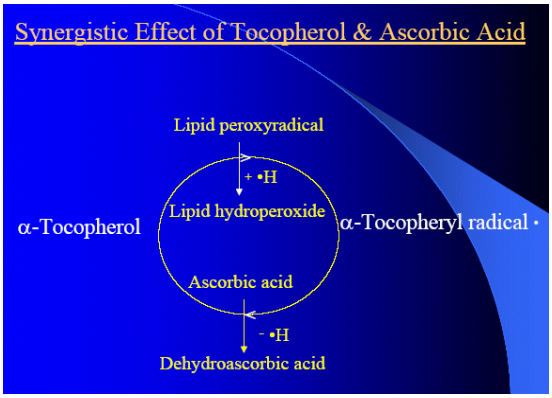


Fig.14: Interaction between Vitamin E (tocopherol) and vitamin C (ascorbic acid)

Studies have shown that antioxidants can have a synergistic effect and are much more powerful when acting together than separately.

This was originally discovered with vitamin C which was shown to be much more effective in

preventing fat oxidation (one of the main causes of CVD) when acting in conjunction with vitamin E (tocopherol) (Fig.14). Nuts, some vegetable oils, and many foods are fortified with vitamin E. **This synergistic effect could favor citrus juices when they are consumed in conjunction with other foods. It may be important to promote a partnership between citrus and other foods rich in vitamin E.**

A similar synergistic effect may exist between vitamin C and some flavonoids. Bioflavonoids and vitamin C may work synergistically to maintain blood capillary health and prevent capillary fragility. There is some *in vitro* evidence that flavonoids and vitamin C work synergistically. It has been shown that ascorbic acid acts synergistically with the flavonoid quercetin to protect cutaneous tissue cells in culture against oxidative damage induced by glutathione deficiency. However, there is a lack of evidence demonstrating that vitamin C and flavonoids work synergistically *in vivo.* **Since citrus contain various types of antioxidant, they can be expected to provide a significant protection against the different kind of oxidative species.**

IV-I-2 Neutralization of the oxidative bursts

As mentioned previously, it has been estimated that up to 2% of the consumed energy may be diverted toward the production of oxidants. This occurs in a few specific physiological steps when energy is produced and used. The consumption of citrus during these oxidative "bursts" should help neutralize some of the toxic free radicals. Furthermore many inflammatory steps generate oxidants. Some of the areas which are the most promising in reestablishing the "oxidative balance"will be reviewed.

IV-I-2-a Neutralizing the postprandial oxidative burst

The first of these steps is associated with the process of breaking up food into its elemental components (sugars, amino acids, fatty acids), after a meal. It has been called the postprandial oxidative burst. Citrus juices and fruits ingested with or after a meal may help neutralize the excess of oxidants produced during that time. **Dr Dandona and other groups have been studying the postprandial burst and could help us to determine the course of action in this area and he will be sending us research proposals for consideration.**

IV-I-2-a Neutralizing the exercise oxidative burst

During intensive exercise, the demand for energy rises quickly and our glucose reserves (glycogen) are used and if necessary fat is burned. To remain healthy it is important to be able to provide antioxidants when oxidation increases. Citrus juices and fruits rich in antioxidants may help neutralize some of the oxidative species generated during exercise. At the same time, other citrus components could also be beneficial. Exercise is certainly a time where providing some sugar would be useful in providing an extra boost of energy. Potassium in the juices could also replenish minerals lost during strenuous efforts. **Citrus juices could be promoted as exercise drinks providing energy, minerals, and antioxidants**.

IV-I-3 Slowing the aging process

Many believe that oxidants are one of the main causes of aging. We enter a spiral where more oxidants are produced as the body become less able to neutralize them. **Providing extra antioxidants with citrus may help slow the aging process although this would require extensive research.**

IV-I-4 Improving brain handling of oxidants

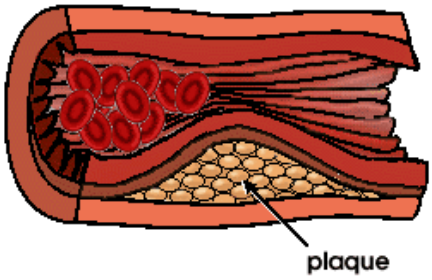
The brain is one of the main targets of the aging process. The brain constantly needs large amounts of energy, but it is protected from the rest of the body by the blood brain barrier, which prevents many molecules from crossing from the rest of the body. The condition of the brain is even more delicate since central nervous system (CNS) neurons cannot regenerate and die if deprived of oxygen and glucose. Accumulation of oxidants may be one of the main causes of brain inflammation leading to many cerebral ailments such as Alzheimer's and Parkinson's diseases**. Providing more antioxidants may prevent or slowdown some of these problems and there are reports that several citrus antioxidant flavonoids particularly tangeretin and nobeletin can cross the blood brain barrier**

IV-I-5 Limiting psychologically induced inflammation (stress, depression)

Psychological stress and depression have been shown to be associated with a rise in the inflammation of the central nervous system (CNS). Citrus compounds may play a role in limiting psychological diseases. This effect could lead to the recommendation that citrus should be ingested during period of heavy stress. This is somewhat speculative at the present.

IV-I-6 Preventing blood lipid oxidation (LDL and plaque)

Fig.15



Lipid oxidation is a major component of the inflammation process particularly in the area of cardiovascular diseases.

The formation of plaque in the blood vessels is promoted by the oxidation of LDL which are deposited in the arteries (Fig. 15)and leads to atherosclerosis (artery wall hardening), and blood vessel narrowing preventing blood flow and generating blood clots. Many CVD diseases can results from these problems including stroke, high blood pressure, infarctus and embolism. A significant number of studies have already shown that many compounds present in citrus such as vitamin C, tangeretin and nobeletin can slow blood lipid oxidation and could play a role in limiting CVDs by preventing lipid oxidation and the subsequent plaque formation . . .

IV-II- Citrus ability to decrease inflammation markers

Inflammation has been linked to an extremely broad spectrum of diseases, and the monitoring of inflammation markers is rapidly becoming the method of choice in determining the progression and

the intensity of many inflammatory diseases and particularly CVDs. Screening for markers will very soon be as widely used as measuring cholesterol levels is now. This is an extremely important area that has reached the medical community and is now spreading to the general public. Patients are now concerned with their LDL and HDL levels and in the next few years will be asking physicians to follow inflammation markers and particularly the CRP. Unfortunately, so far only one study linking citrus juices and these markers have been found. **Showing citrus compounds can decrease inflammation markers would be of great importance in establishing and proving the importance of citrus in promoting health. Inflammation is an area of medical research that is growing rapidly** and is already used for diagnostics. Figure 16 shows the present guideline values given to physicians for the levels of CRP. At this time this is the main area where research on the health benefits of citrus should be directed. A possible problem with the CRP is its lack of specificity. At present many other inflammation markers are being studied (Fig.5). It seems likely that in the next few years the use of markers will become more sophisticated as suggested in Figure 4 and each disease may be linked to a specific marker. **It would be of interest, in the next few years, to pay attention to other inflammation markers as some may they become used for specific for individual diseases.**

Figure 17 represents the title page of a new book on inflammation prevention and diet. Citrus are not very well represented in this book. If this type of diet becomes popular, efforts should be made to give citrus a significant place in the anti inflammatory struggle.

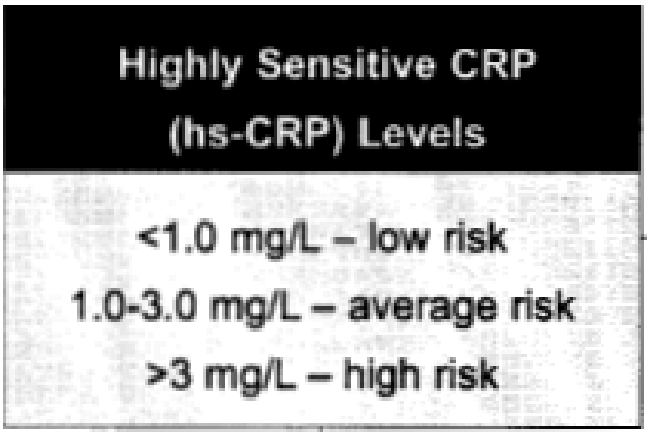


Fig:16 Guidelines for CRP levels

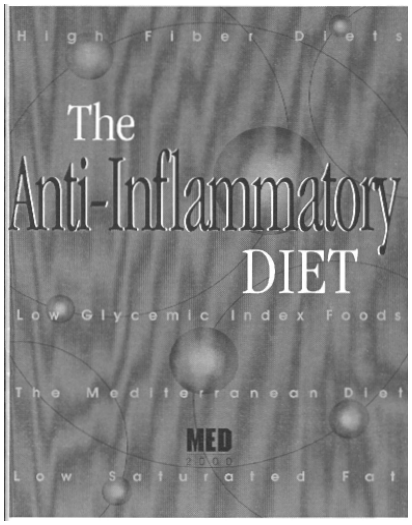


Fig17: an anti-inflammatory diet book

High blood level of the amino acid homocysteine has also been implicated with CVD . Rather than being a result of inflammation like most markers, homocysteine is a cause of inflammation. Studies have shown that homocysteine is cytotoxic and can damage many cells and organs from the

cardiovascular system to the kidneys. it seems likely that homocyteinemia has several origins, one is a genetic disorder and the other one may be due to a lack of absorption of folic acid, vitamin B6 and B12 from food. **In most cases folic acid can limit problems related to the accumulation of homocysteine** and orange juice is a good source of folic acid.

IV-III Citrus and other inflammation induced diseases

IV-III -1 Diabetes:

The ingestion of high amounts of fructose, from drinks rich in high fructose corn syrup, is very detrimental and promotes insulin resistance and some CVDs. However, studies have shown that if fructose represents less than 10% of the total carbohydrates, fructose improves liver glucose management even in the presence of insulin resistance. The presence of small amounts of fructose in meals help regulate glucose uptake and glycogen synthesis. **Citrus contain glucose and fructose in identical amounts and if ingested during meals rich in starch, that is broken down into glucose, they should help maintain the proper glucose metabolism.**

IV-III-2 Blood Pressure:

Citrus antioxidants act on blood pressure by preventing lipid oxidation and atherosclerosis but citrus juices with its high potassium content may favor the ability of the cellular Na/K pumps to remove excess sodium since these cellular pumps expel three sodium ions from cells for two potassium ions they take in (Fig.12). This is another area where citrus juices could play a role in lowering blood pressure.
The hormone angiotensin II which increases blood pressure can also generate inflammation compounds and oxidative species. An effect of citrus compounds in decreasing inflammation in the kidney could be possible.

IV-III-3 Cancer:

Both citrus antioxidants and folic acid can prevent DNA damage and may play a role in preventing mutation induced cancers. There are also many reports of the anti-carcinogenic potential of flavonoids and limonoids. In this area, results are somewhat contradictory and very difficult to prove conclusively. The main problem with this research is that it is done mainly with in vitro culture of cancer cells and the extrapolation of the results for human benefit is difficult to explain. **Of interest are recent reports on the possible beneficial effect of grapefruit juice on tobacco induced cancers. The level of the liver enzyme CYP1A2 is very high in smokers. This enzyme transforms some tobacco compounds into carcinogens. Two new studies have shown the ability of grapefruit juice to decrease the liver level of CYP1A2 in smokers. It has been suggested that this decrease in CYP1A2 could have a positive effect on tobacco induced cancer by decreasing the formation of carcinogens.** It may also be possible to study if grapefruit juice could decrease CYP1A2 in people living in areas with high air pollution (assuming that it is high). This is speculative but could deserve some attention.

IV-III-4 Immunity:

Citrus and particularly vitamin C can boost the production of T cells and raise the immunity level of the body. Citrus have been shown to help against the flu, but the data are not always extremely convincing. This is one of the best perceived beneficial effects of citrus at least to the general public.

IV-IV- Some vitamin potentials

IV-IV-1 Vitamin C:

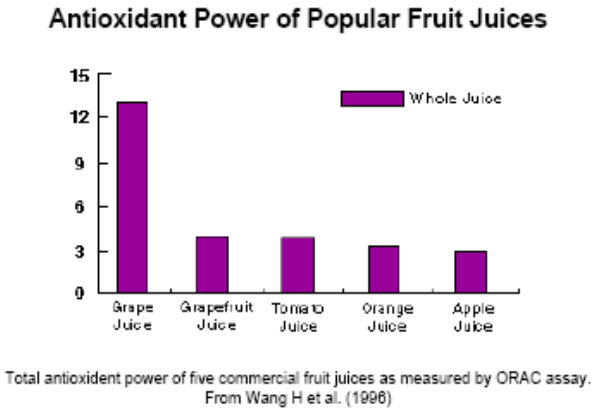
One of the most well known properties of vitamin C is its beneficial effect on collagen formation. As we age, wrinkles are largely due to the decrease of the quality of the skin collagen. **This property could be used to promote the prevention of skin degradation with age.**

IV-IV-2 Folic acid:

Folic acid has been shown be able to prevent up to 75% of neural tube defects although the role of folic acid in CNS fetal development is not clear. It has been suggested that this was due to the ability of folic acid to boost DNA synthesis. Indeed, there seems to be proofs that folate promotes the formation of nucleic acid precursors for DNA and RNA and prevent the split of DNA strands. **That could account for the beneficial effects of folic acid during periods of active cellular multiplication such as fetal development and spermatogenesis.**

IV-V Areas of potential difficulties

IV-V-1 Variation in anti-oxidative potentials of citrus components



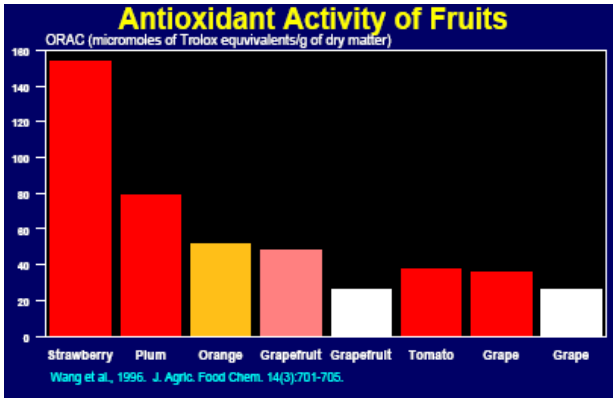


Fig: 18

One of the major problems encountered when studying the beneficial effects of citrus is the controversies attached to antioxidants in general and citrus antioxidants in particular. This is illustrated by the two graphs in Figure 16 representing data taken from the same paper by two scientists and showing that grape juice is the best or the worse antioxidant depending if taken as juice or as fruit. Some of these problems will be reviewed.

IV-V- 1-a Antioxidant can also be prooxidant.

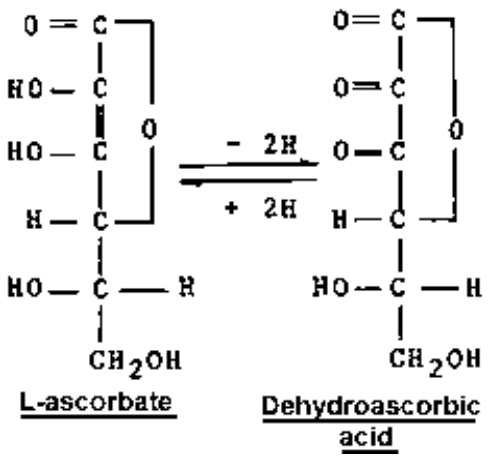


Fig. 19: Equilibrium between antioxidant and prooxidant forms of vitamin C

One major problem is that almost all antioxidants can become prooxidants under the proper conditions. An antioxidant is a molecule that is more easily oxidized than most other compounds. When oxidants are present, the antioxidant molecule is rapidly oxidized and protects the surrounding compounds. But once oxidized the antioxidant can become a prooxidant, since it may in turn oxidized another molecule to be able to go back to its original form. Figure 19 shows ascorbic acid oscillating between its antioxidant (ascorbate) and prooxidant (dehydroascorbate) forms.

IV-V- 1-b Dual role of ascorbic acid

In the body ascorbic acid has a dual activity:

1) **It acts as a vitamin (vitamin C):** A vitamin is a non nutritive compound, not manufacture by the body, provided by food and necessary for life . Vitamins function as catalysts for some enzymes to function (coenzymes). A substance that functions as a vitamin for one species does not necessarily function as a vitamin for another species. Ascorbic acid is a vitamin only for humans, gorillas, guinea pigs and fruit bats, all the other species can produce their own. Both ascorbic acid and its oxidized form dehydroascorbic acid (DHAA)acid have vitamin C properties. Vitamin C is a coenzyme for various enzymes and particularly hydroxylases involved in collagen formation and maintenance.

2) **It acts an antioxidant**: As mentioned above, ascorbic acid is an antioxidant, but DHAA is a prooxidant since the oxidized form needs to oxidize another molecule to go back to ascorbic acid. In the body this is done with the help of glutathione. This problem has been underlined recently in a paper about commercial orange juice vitamin C. It has been suggested that if an orange juice has been in contact with oxygen for a long time, in an open container, it will contain a significant amount of DHAA, it will act as a prooxidant and could damage cellular molecules once ingested. The question of DHAA formation in open container of citrus juice may need to be examined.

This also raises the question of the needed amount of ascorbic acid. The FDA requirements are for vitamin C and the amount needed to provide vitamin activity are quite small and both form can be counted as vitamin. As an antioxidant much larger amounts are needed and some could say that the amount of DHAA should be subtracted from the ascorbic acid to determine a correct antioxidant content.

IV-V- 1-c Relative antioxidant power of vitamin C and polyphenols (flavonoids)

Flavonoids have specific antioxidant properties that set them apart from molecules like vitamin C. Polyphenols have much more stable structures than vitamin C. Once oxidized the resulting free radical is stable and will no longer interact with body molecules. Several reports, mainly of in vitro, studies have shown that phenolics are better antioxidants than vitamin C. and that even in orange juice, vitamin C provides only 15% of the juice antioxidant potential. The superiority of polyphenolics has been extensively used to promote other juices particularly grape juice (fermented or not). It is not clear from the literature if these differences are physiological or due to methodological artifacts or if they depend on specific conditions. **It would be of interest to clarify the discrepancy between the antioxidant potential of vitamin C and flavonoids. Additional research in this area may be needed**

IV-V-1-d Measurement of antioxidant potential

The main cause of the discrepancy between vitamin C and flavonoids may be the lack of standardization between the various methods used to assess the antioxidant potential of a food. This may be due to the greater stability of oxidized flavonoids as compared with vitamin C. **There is a need to compare methods and establish objective standards.** This is also an area where the need to examine the potential of co-antioxidants is important since citrus contains a larger variety of these molecules than most other juices. We may want to promote the synergistic effect of various foods.

IV-V-2 Flavonoids bioavailiblity

Another important problem is the flavonoid bioavailibility. Reports on the bioavailibility of flavonoids are very controversial. In plants, flavonoids are composed of a molecule made of several

rings called a polyphenol attached to various sugars. Most studies show that only the portion without sugars (aglycone) is significantly absorbed, in the intestine. However, flavonoids are significantly degraded by the digestive tract flora with release of the aglycone. Depending on the microbial modifications, the intestinal absorption can be greatly modified, and may take place in the upper or lower intestine. Once in the blood stream flavonoids have to penetrate the organ where they may act. For example the access to the brain is very limited by the blood brain barrier. One must be careful of in vitro results on isolated cells, since the molecules may not be able to reach that organ in vivo. **To demonstrate the benefits of flavonoids it would be essential to know how much can reach the target organ.**

IV-V-3 Flavonoid requirement

Besides the bioavailibilty of flavonoids another important question is the body requirements. Again little is known in this area. There are even some indications that some polyphenols may be toxic at high doses. **Very little is known on the dose-effects of flavonoids and most other phytochemicals. More studies in this area may be necessary**.

V-Conclusion and Recommendations

Many beneficial effects of compounds found in citrus on the inflammation process have been reported in the literature. **It should be pointed out that the bulk of the published studies are on individual compounds found in citrus and not on citrus juices or fruits.**

One of the best documented is the antioxidant effect of citrus products. Protection against oxidation has beneficial effects with all inflammation related diseases. The main potential for citrus is in the area of food metabolism and energy production. However there is some controversy on the antioxidant potential of some citrus compounds that need to be resolved.

Inflammation markers are becoming very important tools in the medical community and showing that citrus can positively affect these markers will be of prime importance in the coming years, since the public will become interested in maintaining low values for these markers, in a manner similar to the way people at present control their cholesterol level. An anti-inflammation diet book is already available (Fig.17)

The main recommendations that can be made on the anti-inflammatory benefits of citrus will be summarized beginning with those having the most immediate potential to those requiring more studies.

V-I Areas where the beneficial effects of citrus compounds are well documented and where little extra research will be needed

V-I-1Antioxidant properties:

Although some controversy exists between the effectiveness of the various citrus antioxidants, this is the best documented area on citrus compounds as they relate to inflammation.

-Citrus antioxidants prevent blood lipid oxidation and may help in limiting CVD..
-Citrus help neutralize oxidative species that are some of the main promoting factors of many diseases from CVD to kidney problems and high blood pressure.

V-I-2 Folic acid

Some of the positive effects of folic acid are well known particularly on DNA.

- Folic acid promote DNA synthesis which is very important during periods of active cellular multiplication such as fetal development and spermatogenesis
- Preventing neural tube defects that was used extensively in the past. Although some controversy exists one how folic acid decreases neural tube defects in newborns
- Preventing cancer by protecting DNA
- Folic acid can, in most cases, decreases high blood homocysteine and its inflammation effects on CVD and blood pressure.

V-I-3 Influence of fructose on sugar metabolism

The presence of small amounts of fructose in meals (less than 10% of total carbohydrates) help regulate glucose uptake and glycogen synthesis. Citrus contain glucose and fructose in identical amounts and if ingested during meals rich in starch that will break down into glucose should help maintain the proper glucose metabolism.

V-II Areas where data already exist but where more research is needed

V-II -1 The role of citrus antioxidants under specific conditions when their action can be most beneficial

.When oxidants are more heavily produced
- After a meal
- After exercise (citrus as a sport drink)

By combining with other foods to promote a synergistic effect

V-II-2 Resolve antioxidant controversy

Reports that vitamin C is a much poorer antioxidant than flavonoids need to be clarified. It needs to be shown if the published data are physiologically correct or due to methodology artifacts.

FLORIDA DEPARTMENT OF CITRUS

WHITE PAPER ON INFLAMMATION AND ASSOCIATED DISEASES BENEFICIAL ROLE OF CITRUS

Paul F. Cancalon

V-II-3 Resolve the questions raised by the dual nature of ascorbic acid

Ascorbic acid acts as an antioxidant or a vitamin. This dual role has generated some confusion and raised the problem of the presence of dehydroascorbic acid in citrus juices. Some efforts should be made to clarify these questions

V-III Areas where limited data exist and where significant research is needed

V-III-1 Inflammation markers

Establishing a role of citrus in decreasing inflammation markers would be of major importance as the question of inflammation reaches the general public. CRP levels are starting to be used clinically and in the future some other makers such as the serum amyloid A protein (SAA) and fibrinogen will most likely be used very soon. Even anti-inflammation diets may become popular. Extensive research would be needed but this may have great potential.

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I- The Inflammation Process

Fig.1: From "Time" February 23, 2004

Recent medical studies have shown that a broad array of diseases, ranging from cardiovascular diseases to some forms of cancer, have in common a series of physiological steps, referred to as "Inflammation"and closely related with the immune response. It is now viewed as a major health threat and is even appearing on magazine covers (Fig.1). The concept of inflammation can be defined as an extension the processes taking place after any injury even minor, when the body reacts to any damage and repairs itself (Stvrtinova et al., 1995; Steven et al, 2002). While the injury repair process is beneficial, protracted inflammation can have very detrimental consequences, particularly since for years it may remain unnoticed There are many original causes for the inflammatory events, but pollution and the events associated with the transformation of food into the energy we use for maintaining life are among the main initiators of this pre-disease state. While food or at least its misuse, is a main cause of inflammation, many compounds that can fight inflammation are also found in food. Fruits and vegetables and more specifically citrus fruits and juices are especially rich in anti-inflammatory chemicals. In this review, the successive steps of inflammation process and the diseases they can promote will be examined. How various classes of citrus compounds may interact with the successive steps of inflammation, limiting or eliminating its effects and contribute in preventing the diseases will also be evaluated. The various steps involved in the inflamation process has been extensively described (Mullenix, 2005; Yuan ; Pecoits-Filho, 2004 ; Geisler, 2004; Baugh, 2003; Bautista, 2003; Oudijk, 2003; Ohshima, 2003; Greaves, 2002; Garcia-Moll, 1999)

There are two categories of factors capable of initiating inflammation - endogenous and exogenous. (Stvrtinova et al., 1995)

Endogenous damaging factors include immunopathological reactions, and some neurological and genetical disorders.
Exogenous factors can be divided into:

mechanical (traumatic injury),
physical (extremely low or high temperature, ionizing irradiation, microwaves),
chemical (caustic agents, poisons, venoms),
biological (viruses, microorganisms, parasites).
nutritive (deficiency of oxygen, vitamins and basic nutrients),
Cell damage that occurs during aging.

Inflammatory reactions involve a vast array of biochemical and cellular events (Fig. 2).

Fig. 2 summarizes the major steps of acute inflammation

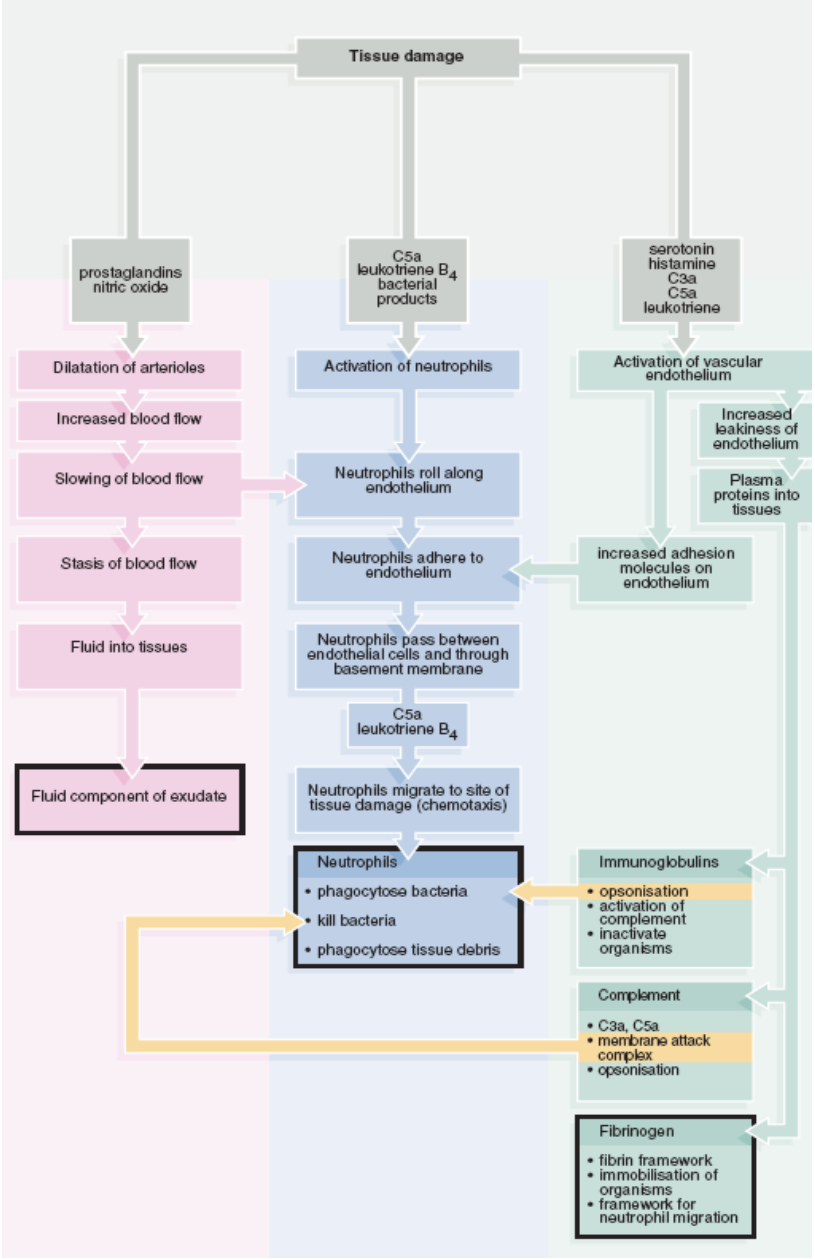


Fig.2, From : Wheater's Basic Histopathology, (Stevens et al.,2002)

The initial injury starts a web of cascading reactions and messages sometimes redundant.

The first step is a cellular influx to the inflammatory site. It is mediated by many mediator substances supporting and dispersing inflammation. These mediators are secreted by inflammatory cells upon activation, or by activated endothelial cells in blood vessels at the site of inflammation. The major

pro-inflammatory molecules responsible for early responses are called chemokines, some of the major ones are, the interleukins IL1alpha IL1-beta , IL6 , and the tumor necrosis factor TNF-alpha. The process shows a significant degree of redundancy assuring a substitution or complementation of individual components that may have been inactivated under pathological conditions.

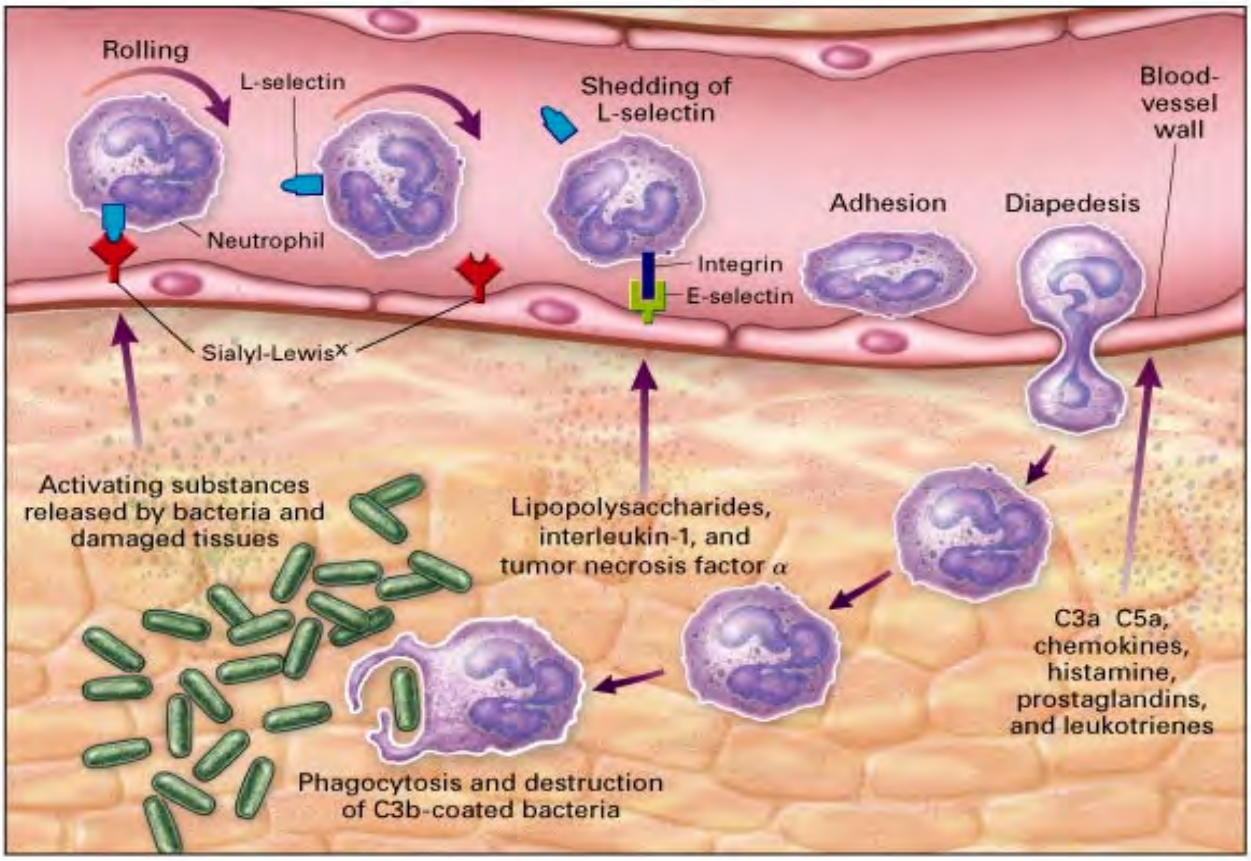
The result of each inflammatory reaction may be beneficial (defense the body against agents deranging its homeostasis) or harmful (damage to surrounding tissues).

Inflammation can be divided into **acute** and **chronic** phases

I -I Acute inflammation (Innate Immunity)

Fig.3,Steps of acute inflammation. From: www.chronicprostatitis.com/images

The acute phase is the one that comes most readily to mind when we think of inflammation, it is associated with wounds or infection and lasts only a short time. It is in many cases beneficial as it is part of the healing process. Acute inflammation lasts from a few minutes to a few days. The



classical medical signs are: heat (calor), swelling, redness (rubor), pain (dolor)

Acute inflammation is the mechanism by which the body regains its homeostasis, for this reason it has also been called ”innate immunity”.

Following an injury such as a bacterial invasion(Fig3), a complex series of events is initiated, particularly in the area close to the wound. They can be summarized in the following manner: The damaged tissue around the wound starts producing signal chemicals that attract mainly white blood cells. In turn, the white blood cells and the surrounding tissue generate the production of a cascade of new chemicals and promote the formation of new types of cells such as macrophages. These cells clean the wound and destroy invading organisms and damaged tissue by producing oxidants such as hydrogen peroxide. The tissue is then repaired and inflammation recedes.

At the local level the initial inflammatory reaction is characterized by an increase in blood flow, an enhanced vascular permeability, and the influx of different cells from the peripheral blood to the site of injury. Influx of antigen non-specific destructive neutrophil leukocytes (modified white blood cells) is one of the earliest stages of the inflammatory response. An exudation of plasma into the lesion in the early stage is observed also. At a later stage monocyte-macrophages appear at the site of injury..

There are three major steps to acute inflammation (Stevens et al.,2002).

1) Vascular dilatation

Following the onset of injury. A brief period of vasoconstriction is followed by vascular dilatation caused by a host of factors such as: histamine -a potent vasodilator, prostaglandins, bradykinins, leukotrienes (LTs) and nitric oxide. They result in an increased intravascular pressure which expels a protein-poor filtrate. (Lentsch, and Ward,2000)

2) Endothelial (capillary walls)activation

Endothelial contraction allows "pore" formation in the capillaries. As a result the vascular system becomes permeable. The increased endothelial permeability allows plasma proteins and leucocytes (white blood cells) to pass into the tissues. The process also leads to the expression of adhesion molecules on the endothelial surface which promotes(neutrophil) leucocytes to adhere to the capillary walls. (Matsukawa, and Yoshinaga, 1999)

3) Neutrophil leukocyte activation

Neutrophils are leukocytes (white blood cells) of the polymorphonuclear subgroup.

Neutrophils form a primary defense against bacterial infection. Like all the cells of the immune system , neutrophils are produced in the bone marrow and circulate in the bloodstream. However, neutrophils move out of blood vessels into infected tissue in order to attack foreign invaders. This explains the innate immunity name given to acute inflammation. Normally a serious bacterial infection causes the body to produce an increased number of neutrophils, resulting in a higher than

normal white blood cell(WBC) count. Increases in WBC is a well known symptom of inflammation. Neutrophils perform their function partially through phagocytosis of other cells and foreign substances. Neutrophils respond to chemotactic signals and leave capillaries by a complex process. the neutrophils are attracted to the site of tissue damage by chemotactic agents and migrate actively towards higher concentrations of these agents (chemotaxis).These factors include potent chemotactic cytokines called chemokines (.Schwarz, and Wells, 1999)

Many other mediators are involved. One such is a substance called interleukin-1 (IL-1), which is released by macrophages as a result of infection or tissue injury. Another is histamine, released by circulating basophils, tissue mast cells, and blood platelets.

If the inflammatory phase progresses satisfactorily, various cells (monocytes, macrophages, lymphocytes monocytes, macrophages) migrate into the affected area and destroy invading microorganisms such as bacteria through many reactive processes such as the formation of reactive oxygen species. Dead microorganisms degraded by lysosomal acid hydrolases are removed by phagocytosis. In most cases, this phase of inflammation leads to some type of healing (Gilroy et al, 2004)

I-II Acquired or Adaptive Immunity

Acute inflammation or innate immunity is the first defense against any form of insults, it does not depend on prior contact with the invading organism and is rapid. In comparison acquired (adaptive) immunity develops when the body is exposed to various antigens (foreign invading substance or microorganism)and builds a defense that is specific to that antigen. Acquired immunity is much slower and much more specific than innate immunity (Lo et al 1999)

Acquired (adaptive) immunity develops when the body is exposed to various antigens and builds a defense that is specific to that antigen. Lymphocytes, a special type of white blood cell, contain subgroups, B and T lymphocytes, that are some of the major cells involved in acquired immune responses. B lymphocytes (also called B cells) produce antibodies. T cells (and B cells) take 4-7 days after infection to become effective. T cells are lymphocytes or cells of the lymphatic system. T cells are so called because they develop in the thymus, an organ located in the upper chest just above the heart. T cells are of two types: helper T cells (which have a marker on their cell surface called CD4) and killer T cells (which have the CD8 marker on their surface). Killer T cells (also called cytotoxic T lymphocytes or CTLs) directly attack body cells that are infected with a virus, malignant or abnormal tumor cells, cells that would not normally be detected by antibodies.

Most immune responses involve the activity and interplay of both the humoral and the cell-mediated immune branches of the immune system. Furthermore, the innate and the adaptive immune systems do not act in an independent way. Activated T lymphocytes produce cytokines and some of these cytokines stimulate phagocytes to destroy infectious agents in a more efficient way; T lymphocytes help the so called B lymphocytes to produce antibodies.

As lymphocytes develop, they normally learn to recognize the body's own tissues as distinctive from tissues and particles not normally found in your body. Once B cells and T cells are formed, a few of

those cells will multiply and provide "memory" for the immune system. This allows the immune system to respond faster and more efficiently the next time you are exposed to the same antigen, and in many cases will prevent you from getting sick. Passive immunity involves antibodies that are produced in someone's body other than your own. Infants have passive immunity because they are born with antibodies that are transferred through the placenta from the mother. These antibodies disappear between 6 and 12 months of age.
Immune system disorders occur when the immune response is inappropriate, excessive, or lacking. Allergies involve an immune response to a substance that, in the majority of people, the body perceives as harmless. Autoimmune disorders (such as systemic lupus and rheumatoid arthritis) occur when the immune system acts to destroy normal body tissues. Immunodeficiency disorders (such as inherited immunodeficiency and AIDS) occur when there is a failure in all or part of the immune system.

I-III Chronic inflammation:

If the acute phase cannot be resolved and recovery does not occur the process evolves into a chronic phase. Chronic inflammation tends to occur over a longer period of time. In many cases chronic inflammation is the continuation of the acute phase, but some diseases such as autoimmune diseases start directly with chronic inflammation.

Chronic inflammation has been called the silent inflammation since it does not generate the pain associated with classic inflammation and therefore can go untreated for years. Silent inflammation is the underlying cause of heart disease, cancer, and Alzheimer's disease. Virtually every type of chronic disease has a significant inflammatory component as its underlying cause. (Brouqui et al. 1994; Devaux et al. 1997)

A non exhaustive list of such diseases include:

Allergy: Inflammatory cytokines induce autoimmune reactions

Alzheimer's: Chronic inflammation destroys brain cells

Anemia : Inflammatory cytokines attack erythropoietin production

Aortic valve stenosis : Chronic inflammation damages heart valves

Arthritis: Inflammatory cytokines destroy joint cartilage and synovial fluid

Cancer: Chronic inflammation causes many cancers

Congestive heart failure: Chronic inflammation contributes to heart muscle wasting

Fibromyalgia: Inflammatory cytokines are elevated

Fibrosis: Inflammatory cytokines attack traumatized tissue

Heart attack: Chronic inflammation contributes to coronary atherosclerosis

Kidney failure: Inflammatory cytokines restrict circulation and damage nephrons

Lupus: Inflammatory cytokines induce an autoimmune attack

Pancreatitis: Inflammatory cytokines induce pancreatic cell injury

Psoriasis Inflammatory cytokines induce dermatitis

Stroke: Chronic inflammation promotes thromboembolic events

Surgical complications: Inflammatory cytokines prevent healing

Not included in this list are the metabolic syndrome (obesity), diabetes and aging.

Inflammatory Disease	Infiltrate	Chemokine
Acute respiratory distress syndrome	Neutrophil	Interleukin-8; GRO-α, -β, -γ; ENA-78
Asthma	Eosinophil, T cell, monocyte, basophil	MCP-1, -4; MIP-1α; eotaxin; RANTES
Bacterial pneumonia	Neutrophil	Interleukin-8; ENA-78
Sarcoidosis	T cell, monocyte	IP-10
Glomerulonephritis	Monocyte, T cell, neutrophil	MCP-1, RANTES, IP-10

Inflammatory Disease	Infiltrate	Chemokine
Atherosclerosis	T cell, monocyte	MCP-1, -4; IP-10
Inflammatory bowel disease	Monocyte, neutrophil, T cell, eosinophil	MCP-1, MIP-1α, eotaxin, IP-10, interleukin-8
Psoriasis	T cell, neutrophil	MCP-1, IP-10, MIG, GRO-β, interleukin-8
Bacterial meningitis	Neutrophil, monocyte	Interleukin-8; GRO-α; MCP-1; MIP-1α, -1β
Viral meningitis	T cell, monocyte	MCP-1, IP-10

Fig.4: Main Organs affected by Inflammation. Column one shows the organ affected. Column two shows the type of cells (mainly white blood cells) that invades the inflamed organ and Column 3 shows the inflammation markers produced in the organ.

A list of organs that can be affected by inflammation is shown in Fig 4. For example lung inflammation in smokers never stops and may induce diseases such as emphysema and cancer.

The general features of chronic inflammation are an infiltration of mixed inflammatory cell infiltrate containing predominantly macrophages, lymphocytes and plasma cells (compare with the predominance of neutrophils in acute inflammation). Lymphoid cells can proliferate at the site of inflammation as well as in local lymph nodes.

The various cellular changes are:

1. Increased number of macrophages and lymphocyes
2. Plasma cells increase after about 1 week
3. Large numbers of neutrophils are not characteristic of chronic inflammation
4. Eosinophils are variably present, depending on inciting event and host response
5. Fibroconnective tissue deposition occurs later in the process and can cause tissue hardening
6. Granulation tissue (reparative) begins to replace inflammatory tissue at the lesion site
7. In successful healing, granulation tissue gives way to new parenchymal cells

Control of the development, maintenance and termination of inflammation is performed through *chemical mediators* released from damaged tissue, the inflammatory cells themselves, and from enzyme systems in blood plasma. The healing and repair processes are also *controlled by chemical interactions* between the cells and extracellular tissue elements involved. As a consequence of tissue damage, the liver produces compounds called "acute phase proteins"(Kostro et al 2001, Ley,2003, Idriss, and Naismith, 2000),.such as the C-reactive protein (CRP) (Ridker et 2004, 2004a). The various components generated during the inflammation process are called inflamation markers. They will now be reviewed .

II Inflammation markers

As mentioned previously, many new chemical species are produced during acute and chronic inflammation. All the molecules whose concentration varies during the inflammation process are potential markers of inflammation .The process involves a large number of molecules produced under genetic control in a perfectly regulated chronological order. Only a few have however been retained for routine assay, the others have not been used so far due to their fragility, short-half life or analytical difficulties. Although not a molecule, one of the oldest inflammation marker is the level of white blood cells. We have seen that many species of leukocytes multiply during the various steps of inflammation and high white blood cell count has been used for a long time as a sign of inflammation or infection.

The most widely used markers are:

The C-reactive protein, interleukin 1, interleukin 2, interleukin 2 receptor, interleukin 6, tumor necrosis factor-alpha, and tumor necrosis factor receptors 1 and 2. (Ridker at al 2004,2004a; Bautista, 2005; Shimetani, 2000; Sanchez-Margalet, 2004; Panagiotakos, 2004; Luc, 2004; Garcia-Moll, 1999)

It should be pointed out that many of these molecules appear with various types of inflammation and one has to be careful in using them to diagnose a specific ailment (Fig.4).

II-I Chemokines and cytokines

Chemokines are a good example illustrating the complexity of the inflammation process. Presently, over 50 different chemokines have been described. (Demirdag, 2003; Rudloff, 1999). They may be redundant and act synergistically or antagonistically. Cytokine include lymphokine (cytokines made by lymphocytes), monokine (cytokines made by monocytes), chemokine (cytokines with chemotactic activities), and interleukin (cytokines made by one leukocyte and acting on other leukocytes).

Chemokines have a molecular weight of 8-10 kDa (90-130 amino acids in length) and show approximately 20-50 percent sequence homology among each other at the protein level. The proteins also share common gene structures and tertiary structures. All chemokines possess four conserved cysteine residues involved in intramolecular disulfide bond formation. It is interesting to note chemokines are influence by homocysteine which could possibly interact with chemokine sulfuryl groups and alter the tertiary structure of these proteins.

These proteins bind to proteoglycans on the endothelial cell surface and within the extracellular matrix They act by binding to specific membrane receptors, which then signal the cell via second messengers, Responses to cytokines include increasing or decreasing expression of membrane proteins (including cytokine receptors), proliferation, and secretion of effector molecules. Cytokines are often produced in a cascade, as one cytokine stimulates its target cells to generate additional cytokines. (Fisher, 2004; Godessart, 200; Hedin, 2002; Heydtmann, 200; Romagnani, 2004).

Three cytokines in particular have very well-characterized activity: interleukins 1 and 6 (IL-1, IL-6) and the tumor necrosis factor alpha (TNF). These three cytokines induce the production of acute phase proteins by the liver and trigger increased hematopoiesis in the bone marrow.

Interleukin-6 (IL-6) is produced by T-cells, macrophages, and endothelial cells. It stimulates the acute-phase reaction, which enhances the innate immune system and protects against tissue damage. (Abbas, et al., 1997) It results in the release of acute-phase proteins into the blood plasma by the liver.The plasma concentrations of interleukin-6 and its hepatic byproduct, C-reactive protein,reflect the intensity of occult plaque inflammation and the vulnerability to rupture. Monocyte chemoattractant protein-1 and interleukin-8 play a crucial role in initiating atherosclerosis by recruiting monocytes/macrophages to the vessel wall, which promotes atherosclerotic lesions and plaque vulnerability. Angiotensin II and other atherogenic factors induce these cytokines in the cardiovascular tissues through the activation of transcription factors,. Conversely, HMG-CoA reductase inhibitors (statins) can inhibit these proinflammatory factors in the blood vessels.

TNF is secreted by macrophages, monocytes, neutrophils, T-cells, and NK-cells following their stimulation by bacterial lipopolysaccharides .(Idriss and Naismith,2000; Moller,2000; Wendling and Tissot,2004).

II-II Chemokine Receptors

Cytokines act on their target cells by binding specific membrane receptors. Cytokines increase the production of receptors on the target cells. Changes in chemokine receptor concentration is also a sign of inflammation.

II-III Acute-Phase Reactants

The acute phase response is a major pathophysiologic phenomenon that accompanies inflammation (Pickup et al 1997; Festa,2001)]. Despite its name, the acute phase response accompanies both acute and chronic inflammatory states.

Acute phase proteins (APR) is the generic name given to a group of approximately 30 different biochemically and functionally unrelated proteins. Acute phase proteins are synthesized predominantly in the liver. The levels of acute phase proteins in the serum are either increased (positive acute phase reactants) or reduced (negative acute phase reactants) during chronic inflammation.

The more important acute phase proteins are glycoproteins. that interact with cellular proteoglycans. C-reactive protein (CRP) and serum amyloid A protein (SAA) have a somewhat different mode of action. Increases in positive acute phase proteins may vary from approximately 50 percent with ceruloplasmin and several complement components to 1000-fold for CRP and serum amyloid A.

Other positive acute phase proteins include fibrinogen, alpha-1 antitrypsin, haptoglobin, and ferritin, while negative reactants include albumin, transferrin, and transthyretin.(Jousilahti, 2001)

II-III-1 Type-1 acute phase proteins

The acute phase reactants require the synergistic action of IIL6 and IL1 for maximum synthesis. Examples of Type-1 proteins are: C-reactive protein, serum amyloid A and alpha-1 acid glycoprotein.

C-reactive protein

The C-reactive protein is the most well known inflammation marker. (Ridker 2004,2004a, Benzaquen, 2002) CRP was first described in 1930 by Tillet and Frances, as a protein found in the blood of patients with pneumococcal pneumonia. It was named C-reactive protein because of its ability to react with the C-polysaccharide of the pneumococcus. C-reactive protein (CRP) is a nonglycosylated polymeric protein consisting of five identical subunits. It has been highly preserved throughout evolution and has been identified in a 500 million year old arthropod ,the horseshoe crab as well as humans. CRP is made in the liver and is secreted in increased amounts within 6 hours of an acute inflammatory stimulus and reaches a peak after about 50 hours and decreases thereafter. The mode of action of CRP has been elucidated. The molecule specifically recognizes phosphocholine, the hydrophilic portion of phosphatidylcholine, in cell membranes (Fig. 5). Complexation of CRP to the cell wall activates complement and stimulates macrophages and other cells to undergo phagocytosis and augments cell mediated cytotoxicity ie amplifies the immune response. The CRP acts upon blood vessels to activate adhesion molecules in endothelial cells: the intercellular adhesion molecule (ICAM-1) and the vascular cell adhesion molecule (VCAM-1). VCAM-1 is an early molecular marker of lesion-prone areas in response to experimental hypercholesterolemia. Extremely high variation in CRP levels have been reported with many inflammation associated diseases and particularly cardiovascular diseases. It is now believed that the combination of CRP level and ratio Total cholesterol/LDL is the best predictor of CVD.

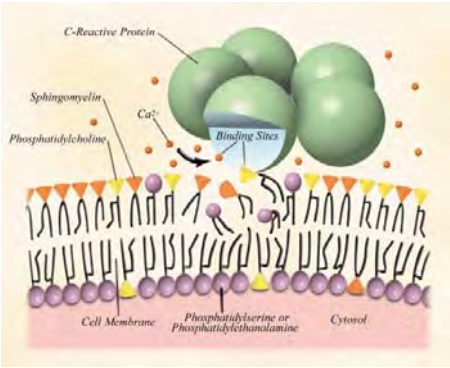


Fig.5: interaction of the c-reactive protein with cell membranes.

Serum amyloid A protein (SAA).

The amino acid sequences of SAA from different species show a high degree of homology. SAA is a 12-14 kDa protein, containing 104 to 112 amino acids. SAA can be expressed in endothelial cells, smooth muscle cells, and macrophage-derived foam cells, macrophages, and adipocytes. It is important to note that SAA isoforms are apolipoproteins a major component of high-density lipoprotein (HDL) in the blood..(Rienhoff 1990)

II-III-2 Type-2 acute phase proteins

Examples of Type-2 proteins are fibrinogen chains, haptoglobin , and alpha-2-Macroglobulin

Fibrinogen

Fibrinogen is a clotting factor produced by the liver. (Cushman, 1999; Jousilahti, 2001). When fibrinogen levels rise above normal there is an increased chance of abnormal blood clotting. If fibrinogen levels decrease below normal, a hemorrhage can result (Gan, 2004). Although the reference range used by most laboratories is 150-460 mg/dL, it is crucial to keep serum fibrinogen under 300 mg/dL. Fibrinogen is a predictor of stroke and high blood pressure (Bots et al. 2002).

The blood coagulation depends upon clotting factors that are activated following injury. A series of reactions produces a clot by converting fibrinogen to fibrin thus plugging the leak (Kohler et al. 2000). Fibrinogen also promotes platelet aggregation (Koenig 1999). Blood vessels with smooth interior walls enable platelets to flow over their surface. When a blood vessel is damaged, exposing the underlying collagen, the platelets adhere to cover the gap. Abnormal platelet stickiness increases atherosclerosis and further narrows the internal diameter of the artery (Braly 1985). In advanced plaque, fibrin is involved in the binding of LDL and the accumulation of lipids (Koenig 1999).

II-IV D-Dimer

D-Dimer is a final product of cross-linked fibrin degradation. D-Dimer is released into the circulation during the process of fibrin blood clot breakdown. D-Dimer and high molecular weight fibrin degradation products are reliable markers of pathological coagulation. Elevated level of D-Dimer was found in the blood of patients with pulmonary thromboembolism, deep vein thromboses, atherosclerosis and other cardiovascular diseases

II-V Soluble Adhesion Molecules

E-selectin, P-selectin, intracellular adhesion molecule-1 (sICAM-1), and vascular adhesion molecule-1 (sVCAM-1) are soluble adhesion molecules

II-VI Adiponectin

Adiponectin is an adipocyte-specific protein (Duncan et al., 2004), Its levels are low in obese subjects and in patients with insulin resistance and type 2 diabetes. Adiponectin appears to have anti-inflammatory and anti-atherogenic effects as well as an insulin enhancer.

II-VII Lipid-Derived Chemical Mediators

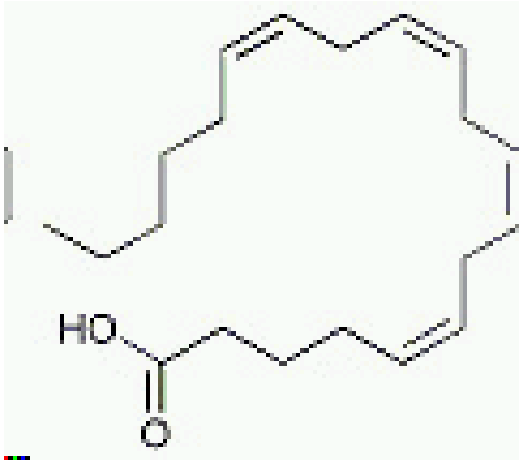


Fig.6: Arachidonic acid

Several inflamation markers are derived from arachidonic acid an essential, 20 carbon fatty acid with four double bonds. It is the precursor of molecules such as prostaglandins, leukotrienes and platelet aggregating factors (Everts et al., 2000).

Prostaglandin increases vasodilation, increases vascular permeability, and serves as chemoattractant for neutrophils

Leukotrienes increase smooth muscle contraction,and serve as chemoattractant for neutrophils and platelet-activating factors.

II-VIII Homocysteine

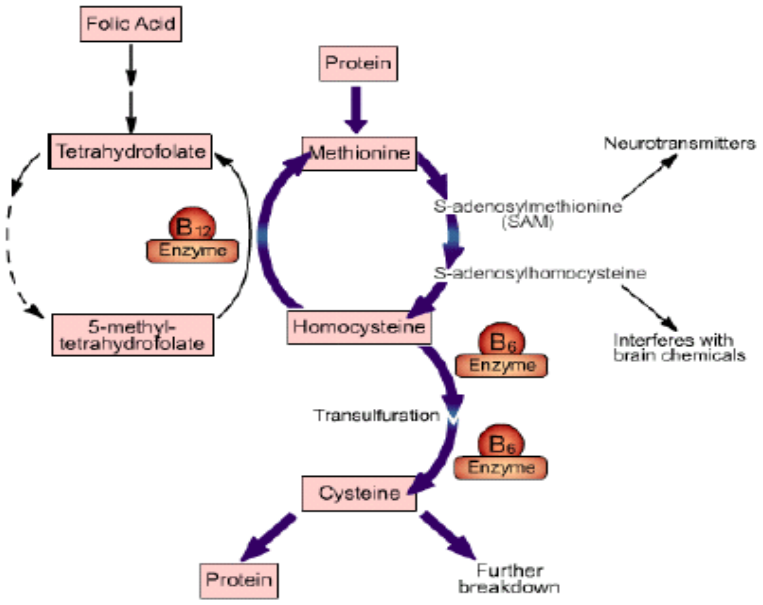


Fig.7: Transmethylation process

Homocysteine is derived from the essential amino acid methionine. Homocysteine is converted into cysteine, methionine, or S-Adenosylmethionine (Bottiglieri 1996). S-Adenosylmethionine donates methyl groups. These methylation reactions create homocysteine that must be remethylated to methionine or catabolized into cysteine. It can also be oxidized into its dimer homocystine.

Elevated homocysteine occurs in patients with nutritional deficiencies in folic acid, vitamin B12 and vitamin B6, particularly in the elderly or in those with genetic defects associated with the transmethylation process (Augoustides-Savvopoulou, 2003; Chen, 2004; Abu-Amero, 2003; Aso, 2003). The prevalence of genetic defects, associated with marginal B vitamin intake, indicates that homocysteine is a risk factor in cardiovascular disease (Guilland et al. 2003; Haynes 2002).. Elevated serum homocysteine is a sign of deficient remethylation and/or the over-production of methylated biochemical intermediates. Homocysteine is a significant factor in the oxidation process that results in atherosclerosis. Homocysteine damages the artery and then oxidizes cholesterol before cholesterol infiltrates the vessel. Although people with high blood level of homocysteine are prone to CVD and high blood pressure, it does not appear that the opposite is true and that all people with CVD have homocysteinemia. The homocysteine problem seems more confined to individuals who for genetic or nutritive reasons have an abnormal transmethylation biochemistry and taking folic acid appears to be enough, in many cases, to resolve the problem.

II-IX Circulating Heat Shock Proteins

Heat shock proteins (Hsp) were first identified in the salivary glands of Drosophila melanogaster upon application of heat shock (Ritossa, 1962). Hsp form the most ancient defense system in all living organisms. These proteins act by helping in the refolding of misfolded proteins and assisting in their elimination if they become irreversibly damaged. (Eggert-Kruse, 2002; Zbytek, 2003)

Hsp 60 is associated with early cardiovascular disease. The phylogenetically conserved nature of heat shock proteins (Hsp) has led to the proposition that they may provide a link between infection and the inflammatory component to vascular disease.Serum Hsp 70 levels predict the development of atherosclerosis in subjects with established hypertension.

II-X Conclusion

For a long time, elevated levels of white blood cells have been used as an inflammation marker. More recently, health professionals have started to monitor some of the inflammation markers. Described above and particularly: Cytokines, the C-reactive protein, the serum amyloid A protein (SAA) and fibrinogen

III- Factors Promoting Inflammation

There is a tendency at least in the popular health press to reverse the cause-effect relationship. The site " Recovery Biomedical Medicine" provides a list of the "disease conditions that have their origins in inflammation of the tissues". It includes among many, back pain, tendinitis and sport related injuries. Obviously, inflammation does not cause sport injuries, but the opposite. "It should read disease conditions that cause inflammation of the tissues". Inflammation is not the original cause of diseases. As mentioned earlier, inflammation is the reaction of the body to any factor that disturbs its homeostasis. Inflammation represents the body's efforts to repair damage, sometimes successfully and sometimes not. When acute inflammation is successful, equilibrium is reestablished and no disease occurs. If the homeostasis cannot be reestablished because the original cause does not stop (autoimmune diseases, overeating etc.)or if the recovery processes are not successful, the inflammation mechanisms remain active and lead to chronic inflammation, organ damage and diseases.

Most often the causes of inflammation are synergistic and rarely act independently of one another. The major causes of inflammation were described in the introduction

Three of the most important are: The oxidative process, the metabolic syndrome and aging.

III-I Oxidative process

III-I-1 Free Radicals and oxidative species

Reactive Oxygen Species (ROS) is a term collectively describing radicals (superoxide anion, hydroxyl radical, and peroxy radicals) and other non-radical reactive oxygen derivatives such as hydrogen peroxide and singlet oxygen, as well as carbon, nitrogen, and sulfur radicals.

Table I: Major radical species

Radicals		**Non-Radicals**	
Hydroxyl	$OH^{\bullet}$	Peroxynitrite	ONOO
Superoxide	$O_2^{\bullet}$	Hypochloric acid	HOCl
Nitric Oxide	$NO^{\bullet}$	Hydrogen Peroxide	H_2O_2
Thyl	$RS^{\bullet}$	Singlet Oxygen	$^1)_g$ ($^1O_2$)
Peroxyl	$RO_2^{\bullet}$	Ozone	O_3
Lipid peroxyl	$LOO^{\bullet}$	Lipid peroxide	LOOH

Free radicals are chemical species which contains an unpaired electron in its outermost shell of electrons. This is an extremely unstable configuration, and within a fraction of a second, free radicals react with other molecules or radicals to achieve the stable configuration of 4 pairs of electrons in their outermost shell.

Non-radical species indicate molecules that are easily converted to free radicals or are powerful oxidizing agents. One of the most familiar example is ozone O_3. Ozone is a molecule that breaks down into oxygen O_2 and the free radical O (native oxygen) which cannot remain in this form and immediately reacts with any molecule in its vicinity and therefore is a very powerful oxidant. (Cross et al. 1987, Packer 1994, Halliwell, 1998;1999a,b; Halliwell and Guttenridge 1989, Eunok and Min 2005, Lee et al 2005, Lee et al 2004, Min and Boff 2002)

III-I-2 Generation of free radicals and oxidative species

Exogenous origin

Free radicals can be generated by environmental stimuli such as ionizing radiation (from industry, sun exposure, cosmic rays, and X rays), ozone and nitrous oxide from car exhaust.

Endogenous origin

A major generator of oxidative species is the energy generation process. Cells generate energy aerobically by oxidizing food during ATP production by the mitochondria. The electrons extracted from the food are eventually used to reduce molecular oxygen (O) to water. The cytochrome c oxidase-catalyzed reaction involves transfer of four electrons to oxygen. One to two percent of total

oxygen consumption may, be converted to superoxide anion radical (O_2-). The formation of superoxide anion radical leads to a cascade of other ROS. It has been shown that significant amounts of oxidative species are produced during the digestive process.

As already mentioned, inflammatory processes produce free radicals during the destruction of invading organisms. Two enzymes, catalase and glutathione (GSH) peroxidase, convert H_2O_2 to H_2O + O_2, and in neutrophils H_2O_2 is converted by myeloperoxidase (MPO) to hypochlorous acid (HOC1). HOCl is a strong oxidant that acts as a bactericidal agent in phagocytic cells.

Free radicals react with substrates such as lipids, proteins, and DNA. Oxidation of these biomolecules can damage them, disturbing normal functions and may contribute to a variety of diseases.(Eunok and Min 2005, Lee et al 2005,Henle and Linn,1997, .Inoue and Kawanishi 1995)

III-I-3 Oxidative Stress

Oxidative stress occurs when the generation of ROS in a system exceeds the systems ability to neutralize and eliminate them(.Sies, 1991) The imbalance can result from a lack of antioxidant capacity caused by disturbance in production and distribution, or by an overabundance of ROS from an environmental, biochemical or behavioral stressor. This excess ROS can damage the cells

The organs most susceptible to oxidative damage are those closely associated with the various oxidation processes:(Sies, 1991; Mukherjee, 2003; Bowler, 2004; Son, 2004)

1) The organs in contact with the environment: The pulmonary, the eye and epithelial systems exposed to high levels of oxygen, and to a large number of pollutants.

2) All the organs associated with the production and distribution of energy: The biological activities involved in large fluctuations in the production and use of energy can induce an oxidative stress, particularly the postprandial oxidation of food into immediately usable or stored energy molecules as well as intense exercise activity when large amounts for energy are released and processed. Many organs are involved in this activity.

-The gastrointestinal tract

-The cardiovascular system where fuel (food) and oxidant (oxygen) are in close contact. This results in all the cardiovascular diseases such as myocardial infarction, stroke and atherosclerosis

-The energy controlling organs: liver, pancreas.

-Heavy users of energy such as: The striated muscles. The cental nervous system (CNS). The brain exhibits an intense metabolic activity but does not store significant amounts energy and furthermore is protected from exogenous molecules by the blood brain barrier and therefore has a low level of antioxidants. Brain inflammation may lead to Alzheimer's Disease and Parkinson's Disease

3) The reproductive system which include the sperms, but also every cells DNA such can be extensively damaged by ROS producing error in duplications that can lead to the development of malignant cells (Cross et al, 1987; Halliwell et al, 1992 , Halliwell,1997,1992 1999, 2000, 2001; Halliwell and Whiteman,2004)

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III-I-4 Cellular Mechanism of Oxidative Damage

Reactive oxygen species, in particular the hydroxyl radical, can react with all biological macromolecules(lipids, proteins, nucleic acids and carbohydrates).The initial reaction generates a second radical,which in turn can react with a second macromolecule to continue the chain reaction.

Lipid Peroxidation:

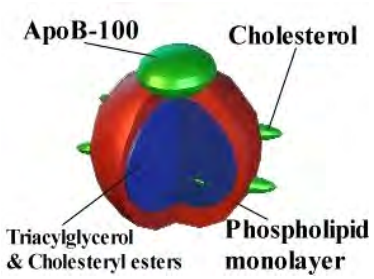

Fig.8 Lipoprotein

Cholesterol and other lipids travels through the blood attached to proteins. Lipoproteins are large complexes of molecules that transport lipids (primarily triglycerides and cholesterols) through the blood (Bowen, P. and Borthakur 2004; Lee et al, 2004,2005; Mattsson et al, 2005). Apolipoproteins are proteins on the surface of the lipoprotein complex that bind to specific enzymes or transport proteins on the cell membranes. This directs the lipoprotein to the proper site of metabolism. Lipoproteins are either high density or low density, depending on how much protein they have in relation to lipids(Hersberger and von Eckardstein ,2003).

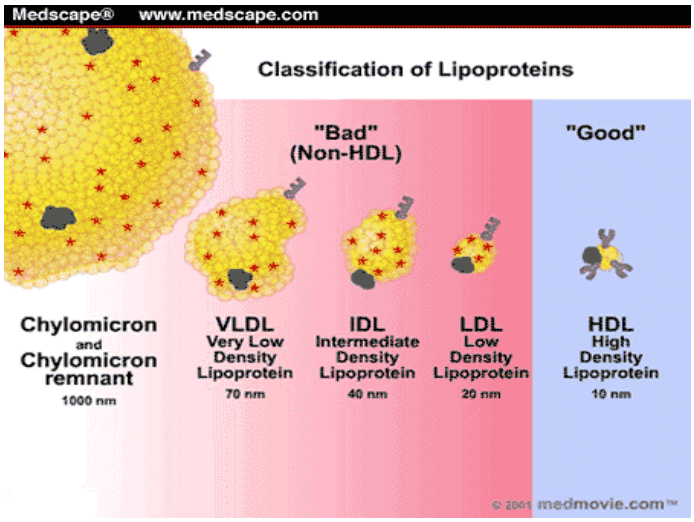

Fig.9: Various types of lipoproteins

The small lipoproteins contain relatively more proteins than lipids cholesterol and since proteins are denser than lipids, they are called high-density lipoproteins (HDL) and also “good” cholesterol. HDL helps remove cholesterol from the body by binding with it in the bloodstream and carrying it back to the liver for disposal. A high level of HDL cholesterol appears to lower your risk of developing heart disease and stroke. An HDL level of more than 60 mg/dl is very good while a level lower than 40 mg/dl is viewed as dangerous.

Lipoproteins with more fat than protein are called low-density lipoproteins (LDL). Low-density lipoprotein cholesterol is sometimes called "bad" cholesterol. A normal level of LDL should be below 130mg/dl. LDL cholesterol collects inside the walls of the arteries and often contributes to the formation of plaque. The process of LDL deposition is significantly increased by oxidation. (Stocker and Keaney, 2004; Takahashi et al.,2005).

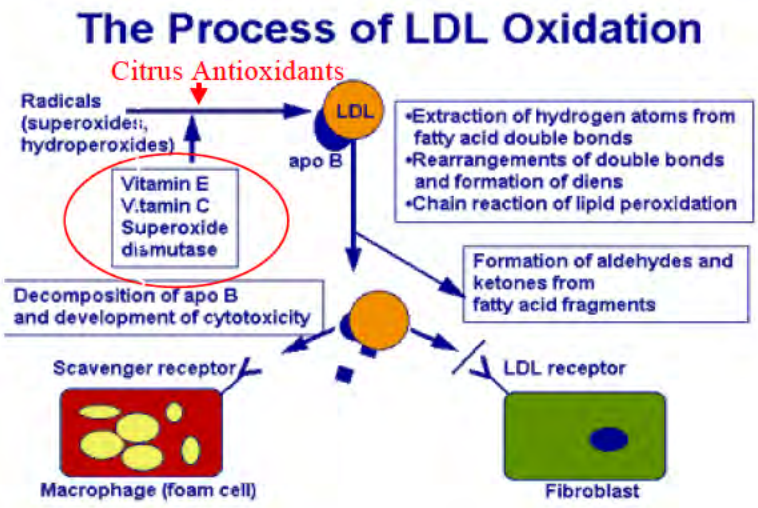

Fig. 10: Development of lipid oxidation

Oxidation involves the lipid moiety of LDL in a chain reaction mechanism (Salvayre R F and Negre-Salvayre, 2004) . In the initial phase, free radicals preferentially attack highly oxidizable polyunsaturated fatty acids. Removal of hydrogen atoms from a polyunsaturated fatty acids initiate the process of lipid peroxidation. Since a radical is also produced in the process, it is a chain reaction. It spreads to other molecules includeing cholesterol and phospholipids. By-products formed during oxidation of LDL lipids, which may have biological activity, react with amino acid residues of the LDL protein backbone with the consequent modification of the cellular chemical and immunological properties. Oxidation alters the apolipoprotein B of oxidized LDL which are then recognized by the macrophage scavenger receptor and are then more readily taken up by

macrophages. The process of oxidative metabolism of LDL initiates many cascades of oxidative events, generating an array of oxidant and other molecules that profoundly influence the atherogenic process. (Massaeli and Pierce 1995; Griffin,1999:Kojo,2004)

They include:

1) Chemotactants for T-cells and monocytes.

2) Endothelial cell adhesion molecules.

3) Monocyte chemotactic protein 1.

4) Macrophage colony stimulating hormone.

5) Interleukin-1 which stimulates smooth muscle cell proliferation.

6) Immunogenic epitomes that evoke immune responses.

7) Cytokines released by CD4+ cells in atheroma plaques.

8) Products that impair nitric oxide-mediated coronary vasorelaxation.

9) Oxysterols that are highly toxic to endothelial cells.

10) Tissue factors that initiate coagulation.

11) Insoluble toxic lipid-protein adducts.

Another major target of lipid oxidation is arachidonic acid, which is transformed into prostaglandins (PGF), that have been characterized as inflammation markers. Their level in plasma or urine is an index of in vivo free radical generation and oxidative lipid formation. There is very strong evidence from animal studies that PGFs increase in plasma and urine as a result of oxidative stress, particularly in smokers.(Bowen, 2004; Szapary, 2003; Mattsson Hulten, 2005)

Protein Oxidation:

Oxidative damage can affect proteins giving rise to protein carbonyl derivatives, via a variety of mechanisms that include fragmentation and amino acid oxidation (Kinobe, 2004; Hitchon, 2004; Kunjathoor et al., 2004). Protein oxidation has major deleterious effects on normal functioning of organisms. 2-Oxohistine and nitrotyrosine are thought to be indicators of protein oxidation induced by peroxyl radical and peroxynitrite, respectively.

DNA damage:

Oxidative damage to DNA is a result of the interaction of DNA with ROS, in particular the hydroxyl radical (Aruoma and Halliwell,1998). Hydroxyl radicals produce a multiplicity of modifications in DNA. Oxidative attack by OH radicals on the deoxyribose moiety will lead to the release of free bases from DNA, generating strand breaks. The C4-C5 double bond of pyrimidine is particularly sensitive to attack by OH radical, generating many types of oxidative pyrimidine damage .This may lead to mutations. These oxidative modifications to DNA may cause mutations, preferentially at G-C base pair interactions and an increase in G to T transition. As a conclusion, these mutations may lead to the development of various types of cancer (Henle and Linn, 1997, Inoue S, Kawanishi, 1995)

III-II Metabolic Syndrome, pre-diabetes and diabetes

III-II-1 Energy generation and control system

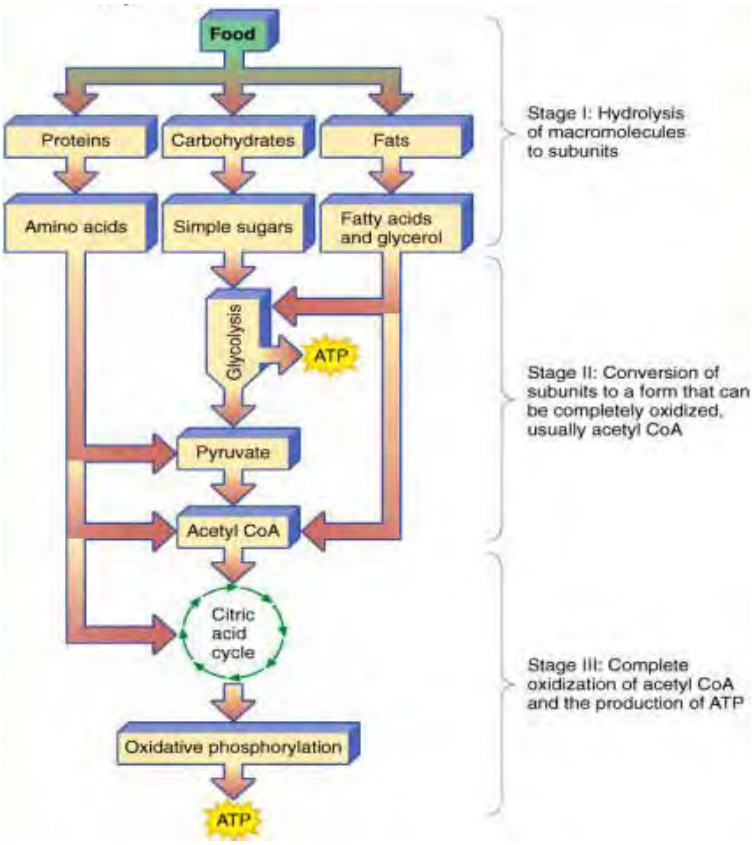

Fig. 11: Process of energy generation from food

The various types of food provide very different amounts of energy. Proteins generate very little fuel in humans. All carbohydrates either simple or complex sugars eventually enter the glycolysis energy pathway often after transformation into glucose. Glucose can be stored in the liver and the muscles as the polymer glycogen (about 500g in the entire body). This is a transient form of storage that is quickly used and replaced during exercise Fats are the main source of energy in the body. They are energy dense and as we are all aware can be stored in large amounts for a long time . It can be calculated that lipids can provide 9 calories/g whereas carbohydrates give only 4 calories/g.

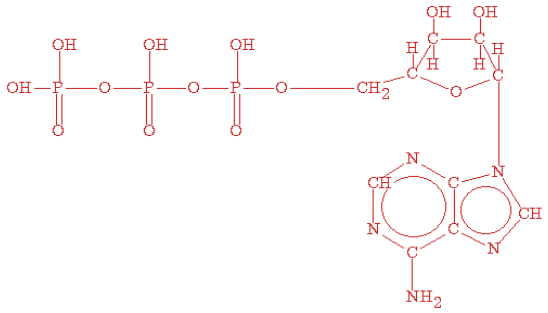

Fig.12: Adenosyl triphophate (ATP): End production of the energy generation process

All three types of food, lipids (fats), carbohydrates (sugars, starches) and proteins are eventually catabolized by mitochodria into energy in the form of adenosyl triphophate (ATP). The universal cell fuel, ATP provides the energy necessary for biochemical reactions. The energy is stored in the three phosphate bonds. When the third phosphate group of ATP is removed by hydrolysis, a substantial amount of free energy is released. (7.3 kcal per mole.)

ATP + H_2O ÷? ADP + P_i

ADP is adenosine diphosphate. P_i is inorganic phosphate. (Metzler,2003)

III-II-2 Sugar intake

Sugars are among the foods responsible for initiating the metabolic syndrome. All the sugars present in food are eventually incorporated in the glucose energy production pathways. During transport storage and utilization, glucose is under the control of two pancreatic hormones insulin and glucagon. The insulin glucagon interactions are shown in figure 13.

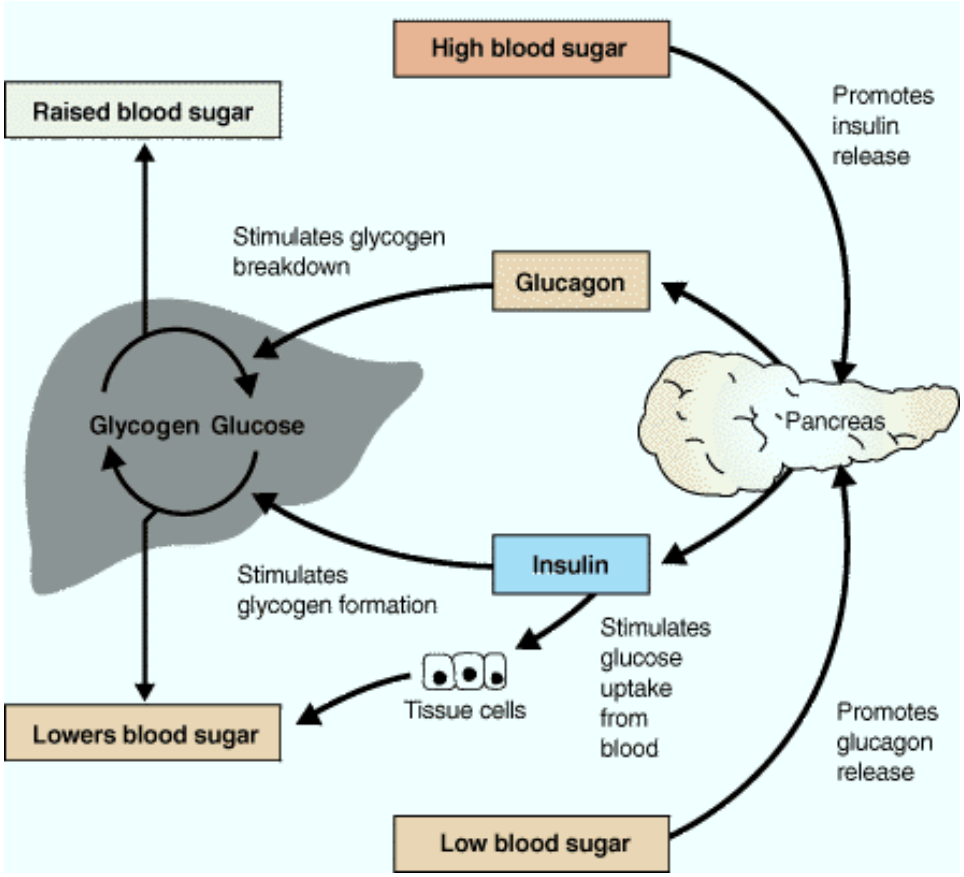

Fig. 13**:** Interaction between insulin and glucagon

Insulin's role is to control the distribution and storage of glucose after a meal when blood glucose level is high, and therefore maintains blood glucose at a proper level. Excess glucose is stored as a

glucose polymer similar to starch (glycogen). Glycogen is the readily available stored energy and is rapidly depleted during exercise since only about 500g of glycogen can be stored in the liver and in the muscle. The remaining excess energy is transformed into fat, a very concentrated form of energy, that can be stored indefinitely in very large amounts, as we are all aware. Insulin acts by interacting with receptors located on the organs using glucose. If the amount of glucose in the blood is constantly too high the receptors become less and less sensitive and need higher and higher levels of insulin to respond and initiate glucose storage (Fig.14). This condition is called pre-diabetes or insulin resistance. It leads to type 2 diabetes when insulin can no longer manage glucose properly and it remains constantly high in the blood. Diabetes can promote many other health problems and particularly all the cardiovascular diseases (CVD) as well as blindness and gangrene.

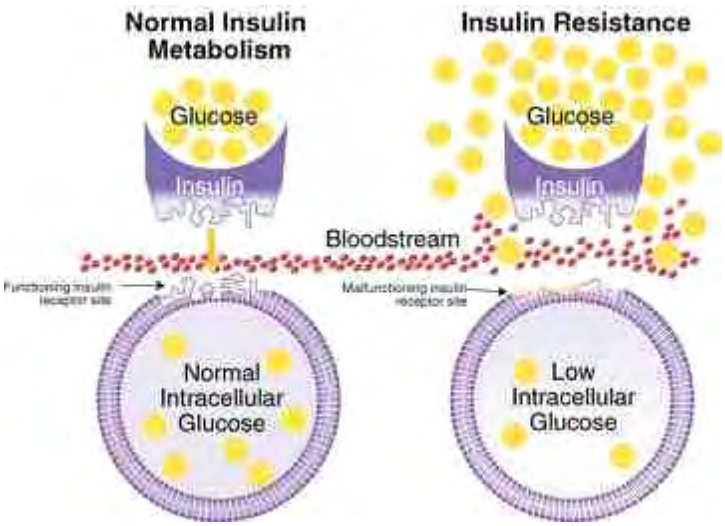

Fig. 14**:** Insulin resistance occurs when the insulin receptors become insensitive and do not allow glucose to move from the bloodstream into the cells

Glucagon activity is the opposite of that of insulin. It releases glucose from storage when needed between meals, stops liver glycogen synthesis, increases liver glycogen breakdown and stimulates

gluconeogenesis (synthesis of new glucose by the body) in the liver to further increase the circulating blood glucose concentration.

Fruits and particularly citrus contain three main sugars; glucose, fructose and sucrose (a disaccharide made of glucose and fructose). During digestion sucrose is split into fructose and glucose and fructose is mainly incorporated into the glucose metabolism. The introduction of high fructose corn syrup has drastically increased the amount of fructose ingested by the US population. It is believed that exposure of the liver to large quantities of fructose leads to rapid stimulation of lipid production and accumulation, which in turn contributes to reduced insulin sensitivity and hepatic insulin resistance and nutritive inflammation (Elliot et al. 2002). The consumption of high amounts of fructose is view as a main contributor to insulin resistance (Basciano et al.2005). However it has also been shown that in smaller quantities fructose, less than 10% of the total sugar intake, can have a beneficial effect in controlling blood glucose level (Hawkins et al.,2002). This may be of importance for citrus juices which contain glucose and fructose in similar proportions. Therefore, during a meal rich in starch, which produces large quantities of glucose, citrus could be absorbed and provide the small amount of fructose necessary to improve sugar management.

The body transforms the food we ingest. It sends it to all the organs as they need it and stores it either for short or long terms. A very complex series of processes are necessary to deal with the energy distribution. Two main hormones, produced by the pancreas, insulin and glucagon control the energy distribution process.

Table II: Hormones associated with the glucose metabolism

HORMONE	Source	Target tissue	**Action**
Glucagon	Pancreas	Liver	Stimulates glycogen breakdown
Insulin	Pancreas	Liver and Muscle	Stimulates glycogen synthesis

The islets of Langerhans within the pancreas are composed of various cell types. The alpha cells secrete glucagon, which accelerates release of stored glucose in the liver (glycogenolysis). The beta cells secrete insulin. The delta cells secrete somatostatin, which regulates release of both glucagon and insulin.

Insulin stimulates uptake of glucose into both muscle and liver and stimulates increased glycogen synthesis in both muscle and liver once the stores are saturated. Excess glucose is diverted to the synthesis of fats. When there is no dietary glucose intake (between meals), circulating glucose concentration must be maintained. The pancreas secretes more glucagon and less insulin.

Glucagon stops liver glycogen synthesis, increases liver glycogen breakdown and stimulates gluconeogenesis (synthesis of new glucose by the body) in the liver to further increase the circulating blood glucose concentration .

Maintaining the energy balance is a very complex task and many problems can occur. One of the major group of diseases associated with malfunctioning of the energy regulation is diabetes.

III-II-3 Diabetes and insulin resistance

All forms of diabetes are linked to the abnormal functioning of insulin. The main types of diabetes will be quickly reviewed.(Flier, 1983;Czech and Seals, 1984; Campbell 2005, : Amer 2005)

III-II-3-a Diabetes insipidus (DI)

(DI) is a disease characterized by excretion of large amounts of severely diluted urine, which cannot be reduced when fluid intake is reduced. It denotes inability of the kidney to concentrate urine. DI is caused by a deficiency of antidiuretic hormone, or by an insensitivity of the kidneys to that hormone.

III-II-3-b Diabetes mellitus Type I

Type I diabetes, sometimes referred to as insulin-dependent diabetes mellitus (IDDM) has also been called Juvenile Onset Diabetes Mellitus, Insulin Dependent Diabetes. For reasons not clearly understood, the body attacks its own insulin-producing tissue, the beta cells of the islets of Langerhans in the pancreas. When too many of these cells are destroyed, there is not enough insulin produced and the patient becomes diabetic and must take insulin injections.

III-II-3-c Diabetes Mellitus Type II

Type II comprises about 85% of all diabetes and is viewed as an inflammation generated by the constant influx of glucose. It is characterized by insulin resistance as body cells do not respond appropriately when insulin is present. With insulin resistance, muscle, fat, and liver cells do not use insulin properly. The pancreas tries to keep up with the demand for insulin by producing more. Eventually, the pancreas cannot keep up with the body's need for insulin, and excess glucose builds up in the bloodstream. Many people with insulin resistance have high levels of blood glucose and high levels of insulin circulating in their blood at the same time. Type 2 diabetes can be brought on by poor diet, obesity and genetics factors.

Many ailments result from type 2 diabetes. Longer-term complications include cardiovascular disease, heart attacks, atherosclerotic diseases, renal failure, retinal damage with possible blindness, nerve damage and even gangrene.

III-II-3-D Prediabetes

Before developing type II diabetes, most people have "pre-diabetes"(Moller, 2005). They have a blood glucose level higher than normal (between 100 and 126 mg/dl) but not yet high enough to be diagnosed as diabetes. Diabetes is typically diagnosed when fasting blood glucose levels are 126 mg/dl or higher. People with pre-diabetes also have a higher risk of heart disease. Many people with insulin resistance and high blood glucose have excess weight around the waist, high LDL (bad) blood cholesterol levels, low HDL (good) cholesterol levels, high levels of triglycerides (another fat in the blood), and high blood pressure, all conditions that put the heart at risk. This combination of problems is referred to as the metabolic syndrome, or the insulin resistance syndrome (formerly called Syndrome X).

III-II-4 Metabolic Syndrome

The metabolic syndrome is a concept more than a disease (Ridker 2004,2004a; Moller, 2005). The overwhelming cause of the syndrome is the absorption of excessive amounts of food and some

genetic factors. It regroups a series of physiological symptoms associated with abnormal distribution and control of energy. To be affected by the metabolic syndrome a person must show three or more of following symptoms:

-Central obesity (excessive fat tissue in and around the abdomen) waist measurement of more than 40 inches for men and more than 35 inches for women.

-Atherogenic dyslipidemia. High levels of triglycerides (150 mg/dL or higher, low levels of HDL or "good" cholesterol (below 40 mg/dL for men and below 50 mg/dL for women).

-Insulin resistance or glucose intolerance.

-Prothrombotic state (e.g., high fibrinogen or plasminogen activator inhibitor in the blood).

-Raised blood pressure (130/85 mmHg or higher).

Patients with the metabolic syndrome increase the likelihood of developing harmful changes including:

1) Damage to the lining of coronary and other arteries, a key step toward the development of heart disease or stroke .

(2) Changes in the kidneys' ability to remove salt, leading to high blood pressure, heart disease and stroke .

(3) An increase in triglyceride levels, resulting in an increased risk of developing cardiovascular disease.

(4) An increased risk of blood clot formation, which can block arteries and cause heart attacks and strokes.

(5) A slowing of insulin production.

III-III Aging and psychosomatic diseases

III-III-1 Aging

There are many theories explaining the aging process. One such theory proposes that aging is a genetically-controlled process and that ideally humans may be expected to live for about 120 years. However many other factors have been shown to have an influence including heredity, environment, cultural influences, diet, exercise, past illnesses, and many other factors. The most significant changes occur in the heart, lungs, and kidneys. Connective tissue changes, becoming increasingly stiff. This makes the organs, blood vessels, and airways more rigid. Waste products accumulate in tissue with aging. Aging cells are less able to eliminate metabolic intermediates such as different free radicals, aldehydes, ketones, This results in a serious defect in the integrity and physiological homeostasis of cells and tissue. As humans grow older, systemic inflammation can inflict degenerative effects throughout the body and the level of many inflammation markers described previously has been seen to increase (Gavrilov and Gavrilova, 1999,2001; Santoro and Mancini, 2002, Investti,2002)

In aged people with multiple degenerative diseases, the inflammatory marker, C-reactive protein, is often sharply elevated, indicating the presence of an underlying inflammatory disorder (Bogden, 1999;Muthuvel et al, 2000;Lee et al. 2004, 2005; Joseph et al. 2005). When a cytokine blood profile is conducted on people in a weakened condition, an excess level of one or more of the inflammatory marker, TNF-a, IL-6, IL-1(b), or IL-8, is usually found (Lee et al., 2004,2005)

III-III-2 Physical and physiological stress

A second very important mediator of inflammation is stress. There are two kinds of stress, physical and *psychic*, and both cause inflammation. Physical stress are caused by trauma, wounds, infections. Mental or psychic stress affect brain function and can also be very detrimental. Stress induces the production of cortisol. The release of cortisol allows our muscles to be fueled for action. However, when a high level of cortisol circulates in the body for long periods of time, it is toxic to our organ systems. It will destroy brain cells, elevate blood sugar (resulting in diabetes), and cause damage to the immune system.

IV- Organs targeted by inflammation and resulting diseases

All tissues can be affected by inflammation. Since organs are made of a combination of one or more types of tissue, most organs can be affected by inflammation (Stvrtinova , 1995; Stevens, 2002). It

should be pointed out that inflammation remains rarely associated with a single organ. One example is cardiovascular diseases where diabetes and CVD are closely intertwined.

There are four basic types of tissue. Connective tissue supports other tissues and binds them together. This includes bone, blood, and lymph tissues in addition to the tissues that give support and structure to the skin and internal organs. Epithelial tissue provides a covering. The skin and the linings of the various passages inside the body are made of epithelial tissue. Muscle tissue includes two types of tissue. Striated muscles, such as those that move the skeleton (also called voluntary muscle). Smooth muscles, such as the muscles that surround the stomach. Nerve tissue is made up of nerve cells (neurons) and is used to carry messages to and from various parts of the body. The brain and the spinal cord constitute the central nervous system (CNS). The part of the vertebrate nervous system made of the nerves outside the central nervous system including the sympathetic and parasympathetic nervous systems is called the peripheral nervous system(PNS). The PNS can regenerate after an injury but the CNS cannot.

IV-I Organs associated with the Energy Maintenance System

IV1-1 The digestive system

The digestive system is a series of joined organs from the mouth to the anus. The mouth and the stomach break down the food physically and enzymatically into sugars, lipids and amino acids. All of the digested nutrients are absorbed through the intestinal walls. The waste products are propelled into the colon and eliminated.

Many microorganisms and compounds in the food can induce inflammation of the digestive system. They have been identified as gastritis, colitis, ileitis, hepatitis, and cholecystitis. The various parts of the gastrointestinal tract can also be affected by cancers often initiated by the food ingested. Crohn's disease is an auto-immune disease where the body reacts by attacking the intestine.

IV-I-2 The lungs

The main role of the lungs is to transfer oxygen from the air to the red blood cells and to dispose of the carbon dioxide, the end product of food oxidation. Being in direct contact with environment the

lungs are submitted to all air born inflammatory molecules including all allergens , industrial and car generated oxidants and carcinogens as well as tobacco smoke. Exercise and stress have also been shown to increase lung ROS.

The deleterious effects of lung inflammation have been extensively documented for emphysema, asthma and lung cancer. Lung inflammation has been demonstrated by the release of several markers such as cytokines (Wilson,2000). Tobacco smoke has been shown to induce an increase in the hepatic level of CYP1A2. This enzyme transforms tobacco chemicals into carcinogens (Le Marchand,2002).

IV-1-3 The cardiovascular system

The cardiovascular system is made up of the heart and the blood vessels. The arterial system sends the oxygenated blood as well as the elementary food components (sugar, amino acids and lipids) to the various organs and the venous system brings back the catabolic waste. The cardiovascular system is very sensitive to inflammation and most of the diseases that can afflict it are related to inflammation.

IV-1-3 -a Atherosclerosis

Arteriosclerosis is a generic term for a number of diseases in which the arterial wall becomes thickened and loses elasticity (Libby,2001; Mullenix etal,2003);. Possible causes of damage to the arterial wall are free-radical reactions, elevated levels of oxidized serum cholesterol, triglycerides, fibrinogen, insulin, high blood pressure, obesity, physical inactivity, tobacco smoking and diabetes (Fortmann et al, 2004)

The build-up of deposits on artery walls is called plaque. Plaques are a thickening of the innermost layer of the arterial wall caused by the accumulation of low density lipoprotein (LDL) . Not only is the amount of LDL in the bloodstream important, so is its size. Small LDLs penetrate the artery wall easier than large LDLs, so they are more easily deposited into the artery wall. The small LDLs are also more vulnerable to oxidation than the larger LDL particles. (Stevens,2002; Aikawa and Libby,2004)

Oxidized LDL are a major contributor of the development of atherosclerosis through the following steps (Fig.15)(Stocker and Keaney:, 2004).

1. By direct cytotoxic actions on endothelial cells. (This induces endothelial injury and contributes to the pathogenesis of atherosclerosis).
2. By increasing chemotactic stimulus for monocytes. (These monocytes are attracted to the arterial intima where atherogenesis begins. Monocytes then differentiate into macrophages),
3. By transforming macrophages into foam cells. (Production of foam cells is a starting point in atherogenesis ,but their presence is also typical for advanced atherosclerotic lesions, which are prone to rupture, producing clinical complications such as myocardial infarction and stroke).
4. By enhancing the proliferation of various cell types, e.g., endothelial cells, monocytes, and smooth muscle cells. (Proliferation and migration of cells leads to a thickening within the intima (innermost) layer and a marked occlusion of the vessel).

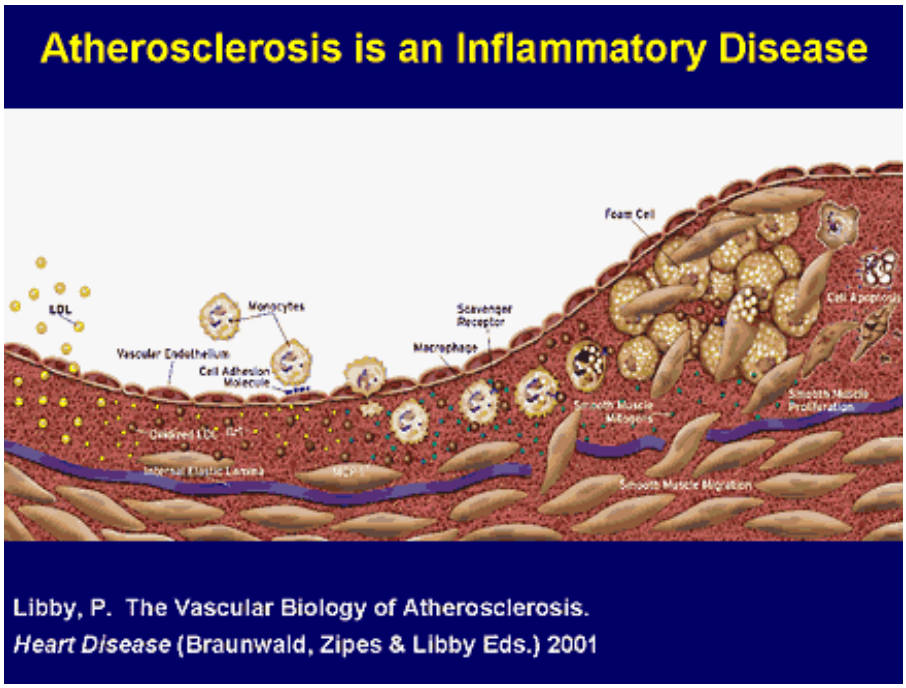

Fig. 15: Atherosclerosis generation

These plaques may grow so large that they obstruct the flow of blood through the blood vessel (Fig.16). In more advanced cases calcium is also deposited and the plaques become very hard and

lose their elasticity. Complications occur if the plaque surface becomes damaged and cause fragments of the plaque to break away. The dislodged plaque material can travel to other parts of the body (e.g., brain, heart, kidneys, and legs), resulting in serious injury to tissues and organs principally by blocking blood flow through smaller arteries (Stevens et al,2002).

Arteries are seldom totally blocked by atherosclerotic plaque. Atherosclerosis results in heart attacks and stroke in two ways. First, it may it reduce the lumen to such an extent that little or no blood can get through to the heart. Second, atherosclerotic plaque can trigger the formation of blood clots, which form on top of the plaque and block the artery. Wherever there is plaque, two things can occur:

1. There can be bleeding (hemorrhage) into the plaque.
2. A blood clot (thrombus) can form on the plaque surface.

If either of these occur, a blood clot may be formed that blocks the flow of blood through the arteries. Depending upon where the blood clot, that is formed, lodges, the result can be a heart attack (a blood clot in one of the coronary arteries) or a stroke (a blood clot in one of the cerebral arteries).

(Pearson 2002).

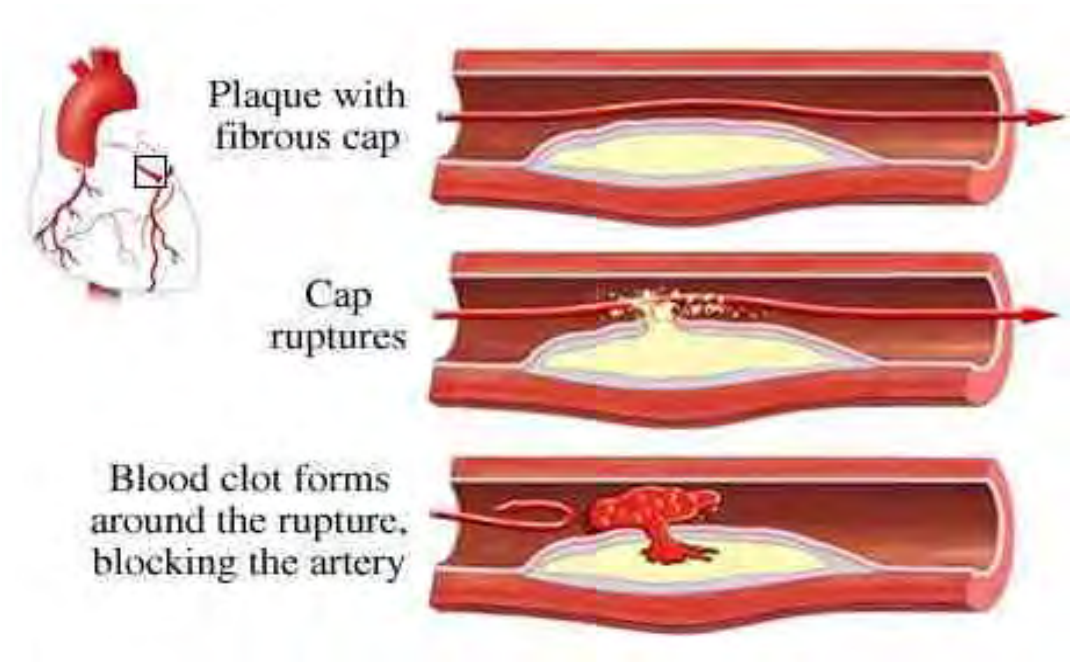

Fig. 16: Plaques generation

From http://www.mountauburn.caregroup.org

IV-1-3 -b Inflammatory Cytokines and Cardiovascular Disease (CVD)

Changes occurring during the development of atherosclerosis result in major modifications of the inflammation marker composition (Massaeli and Pierce,1995; Fortman et.,2004). Plaque inflammation induces a rise in plasma concentrations of interleukin-6 and C-reactive protein, monocyte chemoattractant protein-1 and interleukin-8 play a crucial role in initiating atherosclerosis by recruiting monocytes/macrophages to the vessel wall, which promotes atherosclerotic lesions and plaque vulnerability. Angiotensin II and other atherogenic factors induce these cytokines in the cardiovascular tissues. Conversely Interleukin-10 exerts anti-inflammatory effects on the cardiovascular tissues, possibly by deactivating proinflammatory cytokines and inducible nitric oxide synthetase.

The modulation of these inflammatory cytokines may be useful in the prevention of atherosclerosis and in predicting future cardiovascular events (Fig.17). Significant research has been done in the last few years to correlate CVD and inflammation markers. Higher levels of CRP increase the risk of stroke, heart attack, and peripheral vascular disease (Rifai et al 2001a,b;Benzaquen, L.R.,et a (2002). Stroke patients with the highest CRP levels were 2-3 times more likely to die within a year than patients with low levels. CRP levels increase during systemic inflammation and tests to determine the levels of this protein in the blood can help assess the risk of cardiovascular disease (Lindahl et al, 2000;Packard at al 2000). Some evidence suggests that blood levels of CRP may be a stronger predictor of heart.

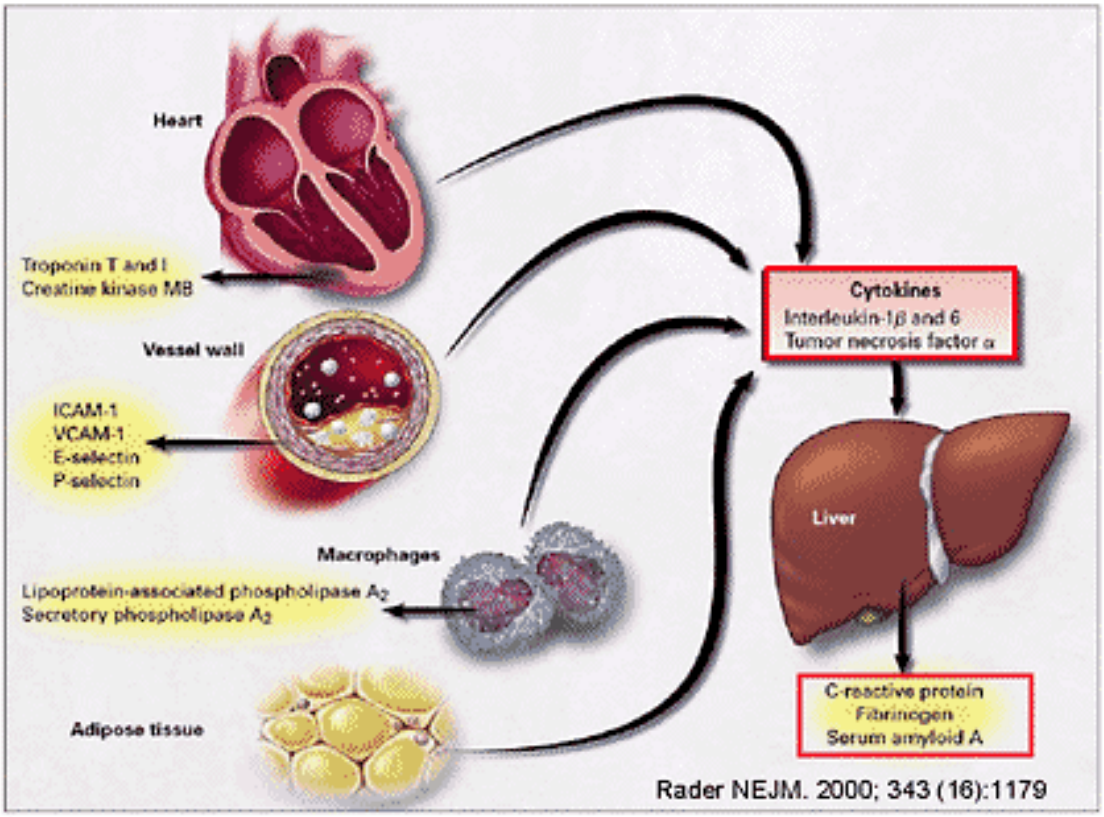

Fig.17: Inflammation markers mode of action

disease than levels of LDL-C (Ridker et al.,1998,2000a,b,2002, 2004a,b;). High levels of CRP have predicted future coronary events in patients with stable CHD. Studies have shown that high CRP levels predict the risk of future heart attack, stroke, peripheral arterial disease and vascular death in people that have no other signs of cardiovascular disease (Ridker 2001; Ridker et al. 2000; Ridker et al. 1998, Mendall et al. 2000). High CRP has also been associated with increased vascular events in people with acute ischemic heart disease, stable angina, and a history of heart attack (Ridker 2001).. Responses to rising levels of CRP may include disruption of existing plaque resulting in a blood clot. CRP levels can predict future coronary events in healthy individuals. Increased monocytes (white blood cells critical in early plaque development) and macrophages (mononuclear phagocytic cells capable of scavenging and ingesting dead tissue and degenerated cells) are present

in atherosclerosis, particularly at points of plaque rupture. It appears that CRP and several other inflammatory markers may be elevated many years prior to a coronary event.

The CRP is not only a marker but has very specific effects on the development of CVD. CRP acts upon blood vessels to activate adhesion molecules in endothelial cells.(The intercellular adhesion molecule (ICAM-1) and the vascular cell adhesion molecule -VCAM-1). VCAM-1 is an early molecular marker of lesion-prone areas in response to experimental hypercholesterolemia. In humans, ICAM-1 and VCAM-I expression are increased in the endothelium of atherosclerotic plaque. CRP affects the activity of LDL, which contributes to the process of increasing atherogenesis. CRP has been shown to promote the uptake of LDL by macrophages (Braley 1985; Zwaka et al. 2001). Once filled with lipids the macrophages develop into foam cells, which discharge their fats into the blood vessel walls.

Homocysteine (Verhoef,,1996; Van Oort and Floor 2003; Aso et al. 2003)) is a significant factor in the oxidation process that results in atherosclerosis. Homocysteine damages the artery and then oxidizes cholesterol before cholesterol infiltrates the vessel.

IV-1-2-c Thrombosis

A serious complication of plaque is thrombosis. It is defined as a blood clot that forms inside the blood vessel or cavity of the heart. Thrombosis usually starts when a plaque ruptures. The blood clots on the damaged area and if the clot becomes big enough it may impair or stop blood flow The end result of thrombosis is an obstruction of the blood flow. These thrombi may remain static in the vessel. However, clots can also become mobile. An embolism is a blood clot that forms in the blood vessel in one part of the body and travels to another part. If a clot travels from a lower extremity vein to the lungs, the result is a pulmonary embolism and/or a pulmonary infarction (lung cell death). Similarly, if a clot moves from the heart or the carotid artery to the brain, it causes a stroke. If a clot travels to a position that occludes, or blocks, the coronary artery, it can develop into a heart attack (myocardial infarction).

An inflammation marker, fibrinogen plays a major role in blood clotting (Geisler & Bhatt, 2004).. Fibrinogen is produced in the liver by pro-inflammatory cytokines called interleukin-1b, interleukin-6, and tumor necrosis factor alpha (TNF-a) (Ridker et al. 2000). A clot is produced by converting fibrinogen to fibrin. This protein forms a network of fibers that traps blood cells and platelets, which under normal conditions plugs the leak (Kohler and Grant, 2000) When fibrinogen levels rise above normal there is an increased chance of abnormal blood clotting.

IV-1-2-d Stroke

A stroke occurs when the blood supply to a part of your brain is interrupted or severely reduced and brain tissue is deprived of oxygen and nutrients. Within a few minutes to a few hours, brain cells begin to die.(Bots et al.,2002)

Ischemic stroke

About 80 percent of strokes are ischemic strokes. They occur when blood clots or other particles block arteries to your brain and cause severely reduced blood flow (ischemia). This deprives the brain cells of oxygen and nutrients, and neurons are rapidly destroyed..

Thrombotic stroke.

This type of stroke occurs when a blood clot (thrombus) forms in one of the arteries that supply blood to your brain.

IV-1-2-e Blood Pressure

High blood pressure is a major risk factor for stroke, heart attack, heart failure and kidney failure.

Two forms of high blood pressure have been described--essential (or primary) hypertension and secondary hypertension.

Essential hypertension accounts for 95% of hypertension. The cause of essential hypertension is multifactorial, and, the effect of several factors combine to produce hypertension by increasing resistance (lack of elasticity) in the tiny arteries that are most distant from the heart (peripheral arteries or arterioles). This increased peripheral arteriolar stiffness is present in those individuals whose essential hypertension is associated with genetic factors, obesity, lack of exercise, overuse of salt, and aging.

Secondary hypertension is largely due to kidney abnormality .

Genetic Factors. A number of genetic factors or interactions between genes play a major role in essential hypertension.

Abnormalities in the Angiotensin-Renin-Aldosterone System (Izzzo et al. 2005). Malfunction in the genes regulating the angiotensin-renin-aldosterone system may be implicated in some blood pressure

problems. This system (see below) influences many aspects of blood pressure control, including blood vessel contraction, sodium and water balance.

Inherited Abnormalities in the Sympathetic Nervous System. Abnormalities of the autonomic nervous system that controls heart rate, blood pressure, and the diameter of the blood vessels may be one of the causes of high blood pressure.

Insulin Resistance and Diabetes Type 2.(Czech and Yu, 1984, Amer, 2005) Hypertension is strongly associated with diabetes, both type 1 and 2. Kidney damage is generally the cause of high blood pressure in diabetes type 1. Obesity and insulin resistance are the factors associated with hypertension in type 2 diabetes,

Obesity. Obesity has a number of possible effects leading to hypertension. It may decrease the ability of insulin to dilate blood vessels, and it may also causes changes in the kidney and abnormal handling of sodium.

Low Levels of Nitric Oxide. Nitric oxide affects the smooth muscles cells that line blood vessels. It helps keep them relaxed and flexible. It may also help prevent blood clotting. Low levels of nitric oxide may be an important factor in essential hypertension (Huyn et al, 2002).

Sodium (Na) levels also play a major role in the modulation of blood pressure. Sodium is the most abundant extracellular ion and has a strong influence in controlling fluid and electrolyte balance. Sodium is the only cation that exerts significant osmotic pressure. Therefore, high internal sodium levels will increase the osmotic pressure and influence both blood volume and blood pressure (Yoshida et al. 1999)

Potassium (K) on the other hand has great influence in limiting blood pressure. Potassium facilitates sodium excretion. Potassium is the counter ion sodium in the cellular Na+/K+ Pump (Fig 18). The pump is energy driven and uses ATP as a power source. For every ATP molecule used, three Na+ ions are pumped out of the cell and two K+ ions are pumped into the cell. Therefore a high level of extracellular K will favor Na elimination from the cells. Potassium may also have vasodilative properties (Smith,2003, Macdonald and Stuthers,2004).
The benefits of reducing dietary sodium and increasing potassium are additive, as the dietary ratio seems to be more important than the total dietary content of either ions.

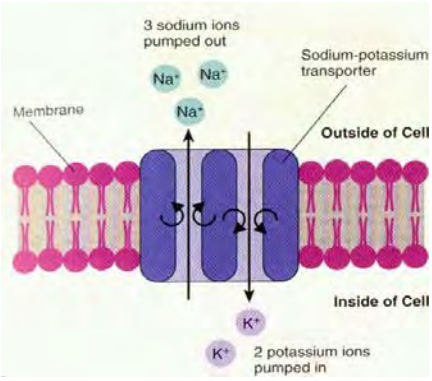

Fig 18: Na/K transmembranal transport

When blood pressure falls (for systolic, to 100 mm Hg or lower), the kidneys release the enzyme renin into the bloodstream. Renin splits angiotensinogen, a large protein that circulates in the bloodstream, into pieces. One piece called Angiotensin I is further cut by angiotensin-converting enzyme (ACE) into angiotensin II. Angiotensin II (Ang II), causes the muscular walls of small arteries (arterioles) to constrict, increasing blood pressure. Angiotensin II also triggers the release of the hormone aldosterone from the adrenal glands. Aldosterone causes the kidneys to retain salt (sodium) and excrete potassium (Fig.19). The sodium causes water to be retained, thus increasing blood volume and blood pressure. Besides its activity in regulating blood pressure, Ang II starts an inflammatory process with the production of Reactive Oxygen Species, adhesion molecules, chemokines, as well as other mediators. Ang II and interleukin 6 (IL-6) induce other pro-inflammatory compounds and superoxide anions. Thus, the induction of IL-6 by Ang II may represent one important mechanism by which Ang II could be involved in the development of atherosclerosis.

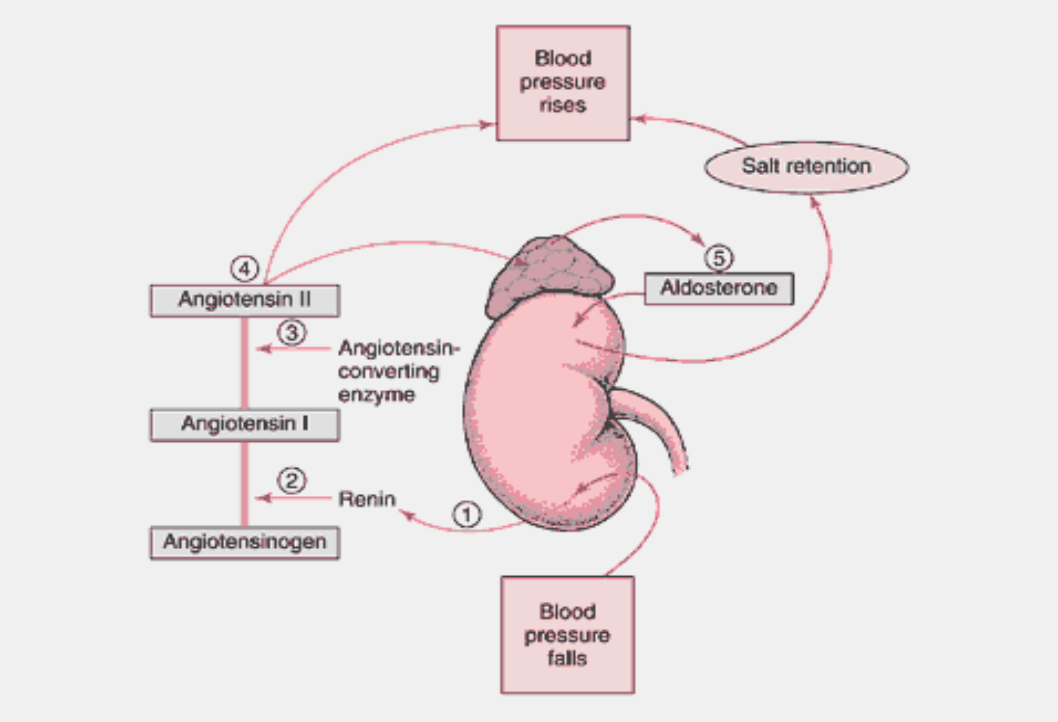

Fig.19: Hormonal control of blood pressure

IV-3 CNS and cognitive systems

The nervous system is divided into two parts:
The peripheral nervous system (PNS), composed of the somatic and automonic nervous sytems (Cancalon,1990). The PNS neurons have the ability to regenerate their axon after an injury. The central nervous system (CNS) is constituted of the brain and the spinal cord. CNS neurons cannot regenerate and die once damaged.

IV-3-1 CNS and inflammation mediated diseases

The brain uses large amounts of energy. With only 2% of the body weight it consumes 20% of the available glucose and oxygen. It should also be noted that this high metabolic activity generates ROS that can damage neurons. Contrary to the muscles or the liver, the brain stores very little energy (glycogen). Furthermore the access to the brain by blood molecules is severely limited by the blood brain barrier (BBB). The endothelial cells of the BBB fit tightly together and many molecules

cannot pass out of the bloodstream.. Substances in the blood that gain rapid entry into the brain include glucose, the main source of energy, certain ions that maintain a proper medium for electrical activity, and oxygen for cellular respiration. Small fat-soluble molecules, like ethanol, pass through the BBB.

The BBB has several important functions:

1 Protects the brain from "foreign substances" in the blood that may injure the brain.

2 Protects the brain from hormones and neurotransmitters present in the rest of the body.

3 Maintains a constant environment for the brain.

Therefore the access to the brain is limited but it needs a constant delivery of energy. If glucose and oxygen becomes unavailable the CNS neurons can die very rapidly. Cytokines circulating in the blood affect CNS function through a variety of pathways. One of these pathways is by being transported directly across the blood-brain barrier (BBB). Cytokine transport across the BBB, however, is complex (Aarli,2003).. Not all cytokines are transported and, for those which are, transport rates differ among cytokines, among brain regions, with physiological circumstances, and with disease.(Youdim,2004)

IV-3-1-a Brain Inflammation

Dementia is a general term for diseases involving nerve cell deterioration. It is defined as a loss in at least two areas of complex behavior such as language, memory, visual and spatial abilities, and judgment that are severe enough to interfere with daily living. Dementia, the most serious form of age-associated mental impairment, is often a slow, gradual process that may take months or even years to become noticeable. Symptoms vary depending on which areas of the brain are affected.

Neurological diseases such as dementia manifest most commonly in the elderly. Any one or a combination of the following factors can cause age-associated cognitive dysfunction:

1. The damaging effects of chronic free radical exposure.

2.The damaging effects of chronic inflammation causing injury to both cerebral blood vessels and neurons.

3.Changes in lifestyle and diet leading to nutrient deficiencies.

4. Decreases in oxygen available to brain cells because of impaired circulation due to pathology or lifetime habits (e.g., smoking, drinking, bad diet, or stress).

5.Declining energy output of brain cells.

6.Essential fatty acid deficiencies.

7.Suppressing free radicals

.

Brain neurons are particularly vulnerable to the effects of free radical oxidation because of their high-energy production.. The destructive effects of excess free radical activity have been implicated in many disease processes, including Alzheimer's disease and Parkinson's disease. Antioxidants neutralize free radicals and help prevent some of the damage associated with normal brain aging(McGeer and McGeer,1999).

Free recall, recognition, and vocabulary were significantly related to vitamin C and beta-carotene levels. The levels of these antioxidants were found to be significant predictors of cognitive function even after adjusting for possible confounding variables (Perrig et al. 1997). A study determined that low folate levels are not only associated with cognitive deficits, but that patients treated with folic acid for 60 days showed a significant improvement in both memory and attention efficiency (Fioravanti et al. 1997). Higher intake of vitamins A, C, E, and B complex were shown to be significantly related to better performance on abstraction and visio-spatial tests (La Rue et al. 1997).

Elevated homocysteine levels have been linked to poorer cognitive function. Studies show that vitamin B6 and folate taken at higher than recommended dosages reduced blood levels of homocysteine (McCaddon et al., 1998,1002a,b; Miller,1999). One study revealed that less-than-optimal levels of vitamin B6, B12, and folic acid lead to a deficiency of S-adenosylmethionine (SAMe). SAMe deficiency can cause depression, dementia, or demyelinating myelopathy (a degeneration of the nerves) (Abou-Saleh et al. 1986).

IV-3-1-b Alzheimer's disease

Alzheimer's disease is a progressive neurodegenerative brain disease that is characterized by the development of extracellular amyloid plaques and intraneuronal neurofibrillary tangles. These senile plaques of beta-amyloid peptide and neurofibrillary tangles eventually lead to a loss of synapses and ultimately neuronal death. Both neurofibrillary tangles and beta-amyloid senile plaques are due to protein abnormalities. The beta-amyloid peptide present in the core of senile plaques is a 42-amino acid chain produced by cleavage of a larger protein known as amyloid precursor protein (APP).. The damage begins when the beta-amyloid becomes concentrated in senile plaques and an inflammatory

reaction with ongoing oxidative stress and free-radical damage starts. There is evidence to support the hypothesis that free-radical damage is a central cause in Alzheimer's disease (Christen 2000). The brain lesions present in the brains of Alzheimer's disease patients are typically associated with attacks by free radicals (for example, damage to DNA, protein oxidation, lipid peroxidation, and advanced glycosylation end products). Alzheimer's disease has been linked to mitochondrial anomalies affecting cytochrome c oxidase. These anomalies may contribute to the abnormal production of free radicals. Free radical scavengers (such as vitamin E, and C) have produced promising results in Alzheimer's disease.

Alzheimer's disease generates a rise in the levels of the acetylcholinesterase, resulting in acetylcholine depletion. This contributes to loss of memory and loss of attentiveness. Decrease in the level of other brain neurotransmitters by at least 50% impairs signal transduction between neurons and brain functions.(Calderon-Garciduenas, 2004). Beta-amyloids initiate an inflammatory response involving cytokines and prostaglandins that develops around beta-amyloid in the neuron. Inflammation is considered to be an underlying cause of Alzheimer's disease, primarily because beta-amyloid is an inflammatory protein (Hull 1996; McGeer et al. 1999). C-reactive protein has been shown to be associated with Alzheimer's disease (Iwamoto et al. 1994).

IV-3-1-c Amyotrophic Lateral Sclerosis (ALS)

The ALS is caused by a genetic defect in superoxide dismutase, an antioxidant enzyme that continuously removes superoxide free radical. A defective glutamate transport system has been proposed as a mechanism for ALS . A study showed the plasma levels of glutamate in ALS patients to be as much as 70% higher as compared to controls. Defects in mitochondrial DNA have also been proposed as a causative mechanism in sporadic ALS (Beal 1998,1999).

IV-3-1-d Parkinson's disease

Parkinson's disease is a degenerative central nervous system disorder of unknown origin affecting mainly older patients (Glanze 1996). However, the disease may occur in younger persons, particularly following inflammation of the brain (encephalitis) or from poisoning by carbon monoxide, metals, particularly aluminum (Cooper 1991; Farina et al. 1994). The condition results from several factors. One of the possible causes is the death of dopamine-producing neurons in the substantia nigra. Discoveries have led to the suggestion that Parkinson's disease may arise as a

combined consequence of the ongoing aging process coupled with environmental exposures that accelerate the process of nigral cell death. Parkinson's disease has been observed in virtually all ethnic groups. This observation suggests that genetic factors may possibly have an important role in disease development (Shults et al 1998, 1999).

IV-3-1-e Aging and Free-Radical Damage

Many theories have attempted to explain aging, It is thought that human genes are programed for a life span of about 120 years. Chronic inflammation is viewed as one of the main causes preventing us from reaching this optimal age. As we grow older, the ability to absorb and use antioxidants decreases, while at the same time our ability to generate energy deteriorates and the production of oxidants increases. Although all organs are affected by aging the brain is one of the main targets of the senescence process. Decrease in the efficiency of the energy handling mechanisms is believed to be a major cause of brain aging. The decline in respiratory processes and the increase of oxidation processes can be considered the root cause of brain aging and degeneration. Increasing respiratory processes would increase oxygen delivery to the neurons, improve neuronal glucose metabolism, and antioxidant defenses, and generally increase efficiency and functioning of the brain. (Youdim, 2004)

IV-3-1-f Aging and homocysteine

Disruption of the transmethylation process may also be involved in brain degeneration. The end results of faulty transmethylation is an accumulation of homocysteine. It has been proposed as a possible marker for the early detection of cognitive impairment in the elderly. Several studies have found elevated homocysteine levels in Alzheimer's patients (Gottfries et al. 1998; McCaddon et al. 1998; 2001a,b).

Serum homocysteine levels were found to be significantly higher and serum folate and vitamin B12 levels were lower in patients with Alzheimer's disease as compared with control(Clarke et al. 1991).Similar results were obtained by Joosten et al (1997) and Miller (1999). Vitamin B12 deficiency is common in the elderly and may be a risk factor for Alzheimer's disease (Regland et al. 1991).

IV-3-1-g Psychosomatic diseases and inflammation

Psychosomatic diseases refer to problems related to both the mind and the body. Somatogenic diseases occur when the mind increases the body's susceptibility to some diseases. Psychogenic

diseases are associated with emotional stress which by itself lowers the body's immune system (Couraud et al. 1997).

It has also been reported that physiological problems such as stress and depression can induce inflammation in the body (Abou-Saleh and Coppen ,1986) . Reports have shown that people suffering from depression produce inflammation markers similar to those generated by physical ailments. In response to psychological stress or certain physical stressors, an inflammatory process may occur by releasing neuropeptides, or other inflammatory mediators from sensory neurons and activating mast cells or other inflammatory cells. Central neuropeptides, particularly corticosteroid releasing factor, initiate a systemic stress response by activation of neuroendocrinological pathways such as the sympathetic nervous system, hypothalamic pituitary pathway, and the renin angiotensin system, with the release of the stress hormones (i.e., catecholamines, corticosteroids, growth hormone, glucagons, and renin). These, together with cytokines induced by stress, initiate the induction of acute phase proteins. Central nervous system norepinephrine may also induce the APR perhaps by macrophage activation and cytokine release. Repeated episodes of acute or chronic psychogenic stress may produce chronic inflammatory changes which may result in atherosclerosis in the arteries or chronic inflammatory changes in other organs as well. People with high levels of anger or depression have high blood levels of C-reactive protein. Studies in patients with depressive disorder were shown to have high level of c-reactive protein, IL-6, and TNF-alpha.

Cytokines have been implicated as a contributing factor for mood disorders such as depression. A relationship between cytokines and depression has been shown (Panagiotakos et al.2004):

1) Proinflammatory cytokines (interleukin-1, interleukin-6, tumor necrosis factor-alpha) and bacterial endotoxins elicit sickness behaviors and symptoms of anxiety/depression that may be attenuated by chronic antidepressant treatment.

2) Cytokines induce neuroendocrine and central neurotransmitter changes reminiscent of those implicated in depression, and these effects are exacerbated by stressors,.

3) Severe depressive illness is accompanied by signs of immune activation and by elevations of cytokine production or levels.

4) Immunotherapy, using interleukin-2 or interferon-alpha, promotes depressive symptoms that are attenuated by antidepressant treatment.

It is argued that cytokine synthesis and release, elicited upon activation of the inflammatory response system, provoke neuroendocrine and brain neurotransmitter changes that are interpreted by the brain as being stressors and contribute to the development of depression.

IV-3-2 Spinal neural tube defects (spina bifida)

Neural tube defects (NTDs) is the main spinal cord disease occurring during fetal development. . Within the first month after conception, the central nervous system develops from a sheet of nerve cells that must close on itself to form the neural tube that will become the brain and spinal cord. Failure at the brain level is fetal but not at the spinal cord level. This case is called Spina bifida and accounts for half of all neural tube defects. It results in the the spinal cord protruding through the backbone and failing to develop normally. Children born with spina bifida are severely handicapped. It has bee shown that folic acid can prevent up to 75% of neural tube defects. Folate plays a central role in the formation of nucleic acid precursors for DNA and RNA, which are essential for widespread and sustained cell division, a central feature of fetal development. It is likely that this effect of folic acid is due to its role in nucleic acid synthesis and/or its role in the metabolism of homocysteine to methionine. Increasing folate tissue concentrations might overcome a metabolic deficiency of the vitamin in the production of nucleic acids, and possibly proteins, at the time of neural tube closure. It should also be mentioned that an alternative view is that folic acid do not prevent the occurrence of neural tube defects, but may instead selectively increase the abortion rate of affected fetuses (Brent et al. 2000, Brouwer et al. 2000; Gross 2001).

V- Beneficial effects of citrus antioxidants, vitamins and other phytochemicals

V-I Estimation of the antioxidant potential of foods

The estimation of antioxidants in food was recently reviewed at the 2nd International Congress on antioxidants (2005). For review see also Rice-Evans2000, Prior and Coa,2000; Huang et al 2005. Depending on the reactions involved, these assays can be classified into two types (Prior and Coa, 2000; Huang et al 2005):

1) Assays based on hydrogen atom transfer (HAT) reactions.
2) Assays based on single electron transfer (SET) reactions.

Table III: antioxidant assays

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Table 1. In Vitro Antioxidant Capacity Assays

assays involving hydrogen atom transfer reactions	ORAC (oxygen radical absorbance capacity)
$ROO^{\bullet} + AH \rightarrow ROOH + A^{\bullet}$	TRAP (total radical trapping antioxidant parameter)
$ROO^{\bullet} + LH \rightarrow ROOH + L^{\bullet}$	Crocin bleaching assay
	IOU (inhibited oxygen uptake)
	inhibition of linoleic acid oxidation
	inhibition of LDL oxidation
assays by electron-transfer reaction	TEAC (Trolox equivalent antioxidant capacity)
$M(n) + e$ (from AH) $\rightarrow AH^{\bullet+} + M(n-1)$	FRAP (ferric ion reducing antioxidant parameter)
	DPPH (diphenyl-1-picrylhydrazyl)
	copper(II) reduction capacity
	total phenols assay by Folin–Ciocalteu reagent
other assays	TOSC (total oxidant scavenging capacity) (*90*)
	inhibition of Briggs–Rauscher oscillation reaction (*91*)
	chemiluminescence (*92*)
	electrochemiluminescence (*93*)

HAT-Based Assay represented by the ORAC assay, involves peroxyl radicals as the oxidant and provide

useful information on radical chain-breaking capacity. The majority of HAT based assays involve a competition reaction scheme, where antioxidants and substrates compete for thermally generated peroxyl radicals. These assays include inhibition of induced-LDL auto-oxidation, oxygen radical absorbance capacity (ORAC), total radical trapping antioxidant parameter (TRAP), and crocin bleaching assays.

SET-Based Assay. SET based assays measure the capacity of an antioxidant to reduce a single electron oxidant which changes color when reduced. The degree of color change is correlated with the sample's antioxidant concentration. SET-based assays include the total phenols assay by Folin-Ciocalteu reagent (FCR), Trolox equivalence antioxidant capacity (TEAC), ferric ion reducing antioxidant power (FRAP), "total antioxidant potential" assay using a Cu(II) complex as an oxidant. The assays are summarized in Table III(From Huang, et al. 2005**).**

The pH value has an important effect on the reducing capacity of antioxidants. At acidic conditions, the reducing capacity may be suppressed due to protonation on antioxidant compounds, whereas in basic conditions, proton dissociation of phenolic compounds would enhance a samples reducing capacity. Sugar is known to reduce metal ions such as Cu(II) (Fehling's reagent). It should be noted that the total phenols assay by FCR is conducted at rather basic conditions (pH 10, necessary for phenols to dissociate protons). Simple phenols (e.g., C_6H_5OH, the phenolic group in tyrosine) react with FCR, although they are not effective radical scavenging antioxidants. Therefore there may not necessarily be a good correlation between the "total phenols content"and the radical scavenging antioxidant capacity of a sample. The total phenols assay by FCR is carried out in aqueous solution. For lipophilic antioxidants, this assay in its current form is not applicable. Therefore, there is an immediate need for a modified FCR for lipophilic samples (Serafini and DelRio, 2004). Serafini (2005) applied various criteria to review the pro and con of the various methods used to determine AO capacity (Table IV).

Serafini (2005) suggested that the total phenols assay by FCR be used to quantify an antioxidant's reducing capacity and the ORAC assay to quantify peroxyl radical scavenging capacity. To comprehensively study different aspects of antioxidants, validated and specific assays are needed in addition to these two commonly accepted assays (Coa and Prior, 1998; Pror and Coa, 1999;Cao, 1998; Sanchez-Moreno,2002, Prior et al, 2003).

Table IV. From Serafini (2005) (ranked by quality: 0 very bad, 5 very good)

	Simplicity	Complexity of instrumentation	Physiological Conditions	With hydrophilic AO	with hydrophobic AO	# studies
TEAC	5	5	3	5	0	4
FRAP	5	5	0	5	0	4
ORAC	3	4	5	5	2	3
TRAP	4	4	5	5	0	3
Luminol	4	1	3	4	0	1

V- II Absorption and bioavailibility

V-II - a Polyphenols

Flavonoids comprise the most common group of plant polyphenols. More than 5000 different flavonoids have been described (Aherne,et al.,2002) They are usually subdivided into six major subclasses of flavonoids are as follow:(Slanina and Taborska, 2004, Walle, 2004).
The Flavones (apigenin, luteolin), Flavonols (quercetin, myricetin), Flavanones (naringenin, hesperidin, catechins) or Flavanols (epicatechin, gallocatechin), Anthocyanidins (cyanidin, pelargonidin) and Isoflavones (genistein, daidzein).

Most of the flavonoids present in plants are attached to sugars (glycosides), although, they are also found alone (aglycones). Interest in the possible health benefits of flavonoids has increased due to their antioxidant and free-radical scavenging activities (Scalbert and Williamson, 2000, Halliwell et al. 2005). Some studies support a protective effect of flavonoid as free-radical scavenging, inhibition of cellular proliferation, as well as antibiotic, antiallergic antidiarrheal, antiulcer, and anti inflammatory agents in cardiovascular disease and cancer. Other studies demonstrate no effect and a few studies suggest potential harm depending on specific circumstances (Ross and Kassum 2002, Manach et al, 2004, Erlund,2004, Felgines et al, 2000). The associations between polyphenol intake and health were extensively reviewed at the 1st International Conference on Polyphenols and Health held in 2004 with the proceedings published in 2005.

Citrus phenolic compounds have been reviewed in great detail by Berhow et al (1998). Flavonoids include many polyphenolics such as flavonoid glycosides ,including neohesperidin and naringin, hesperidin, rutoside, sinensetin, polymethoxylated flavones such as nobiletin, tangeretin, numerous hydroxycinnamates and at least 44 different flavone glycosides (Horowitz and Gentilli,1997). Other common flavonoids include catechin, epicatechin, myricetin, quercetin (quercetin is consistently the most active of the flavonoids), and kaempferol. Other polyphenolic compounds, specifically phenolic acids that are powerful antioxidants include gallic acid, p-hydroxybenzoic acid, caffeic acid, ferulic acid, and ellagic acid. Manthey and Guthrie (2002) examined the anti-inflammatory and anti-carcinogenic properties of citrus flavonoids.

The health benefits of any food compound depends greatly on its bioavailibily. It must first be able to cross into the blood stream and to have access to the target organs. Very few estimations of dietary intake of flavonoids are available. Kuhnau(1976) estimated daily intake of flavonoids in the United States to be between 1 and 1.1 g/day, depending on the season. The author assumed that the aglycones only could pass freely into the blood stream from the gut wall, and that no enzymes were secreted in the gut that could cleave the glycosidic bonds (Kuhnau, 1976). .

Recent studies, however, have demonstrated that the bioavailability of specific flavonoids is much higher than previously believed. Hollman et al. (1995,1996,1997,1999,2000,2001) examined in vivo the extent of absorption of quercetin, quercetin rutinoside, or pure quercetin aglycone. The authors found that the absorption of orally administered quercetin aglycone was approximately 24%; whereas the absorption of quercetin glycosides was 52%, suggesting that the glycoside moiety actually enhanced absorption. The bioavailability of other flavonoid compounds has also been explored by Felgines et al. (2000) who reported that naringenin, the predominant flavonone found in grapefruit, and mainly found in a glycoside form (naringenin-7-rhamnoglucodise and naringenin-7-glucoside), was efficiently absorbed by rats.

The bioavailability of quercetin glycosides has been extensively studied, because dietary quercetin is mostly present as its glycoside form. The small intestine acts as an effective absorption site for glucose-bound quercertin. Glucosides are effectively absorbed in the small intestines because small intestinal cells have been shown to possess a glucoside-hydrolyzing activity and their glucose transport system is capable of participating in the glucoside absorption. A study using a cultured cell model for intestinal absorption explains that the hydrolysis of the glucosides accelerates their absorption in the small intestine (Murota and Terao,2003). The small intestine is also the site for metabolic conversion of other flavonoids as it possesses enzymatic activity of glucuronidation and sulfation. Modulation of the intestinal absorption and metabolism may be beneficial for regulating the biological effects of dietary quercetin. The absorption of quercetin glucoside and quercetin rutinoside were examined . The bioavailability of the rutinoside was only 20% of that of the

glucoside. The authors concluded that quercetin glucoside is actively absorbed from the small intestine, whereas quercetin rutinoside is absorbed from the colon after deglycosylation (Hollman et al 1999).

Naringin is a major flavonoid found in grapefruit. Naringenin is the aglycone and a metabolite of naringin. Naringenin was administered intravenously and orally to rabbits, and naringin was administered orally only. The concentration of naringenin in serum prior to and after enzymatic hydrolysis was determined by HPLC. The results showed that the absolute bioavailability of oral naringenin was only 4%, whereas after taking the conjugated naringenin into account, it increased to 8%(Hsiu et al 2003)

Bioavailability studies of flavanones or flavanone-containing foods[1]

Source	No. of subjects	Dose	T_{max}	Plasma concentration	AUC	Urinary excretion	Elimination half-life	Ref
			h	*µmol/L*	*µmol · h/L*	*% of intake*	*h*	
Orange juice	5	110 or 220 mg eq hesperetin	5.4–5.8	0.46–1.28	4.19–9.28	4.1–6.4		48
Orange juice	5	22.6 or 45 mg eq naringenin	4.6–5	0.06–0.2	0.43–1.29	7.1–7.8		48
Orange juice	8	126 mg eq hesperetin	5.4	2.2	10.3	5.3	2.2	49
Orange juice	8	23 mg eq naringenin	5.5	0.64	2.6	1.1	1.3	49
Grapefruit juice	5	199 mg eq naringenin	4.8	5.99	27.7	30.2	2.2	49
Tomato paste	5	3.8 mg naringenin	2	0.12				50
Pure compound	6	500 mg pure naringin				4.0		51
Pure compound	1	500 mg pure naringin				4.8		52
Pure compound	1	500 mg pure hesperidin				3.0		52
Grapefruit juice	4	325 mg eq naringenin				6.8		52
Orange juice	4	44 mg eq hesperetin				24.4		52
Grapefruit juice	6	7.2 mg naringin/kg bw				8.9	2.6–2.9	53
Grapefruit juice	2	214 mg naringin/d for 1 wk				14d		54

[1] T_{max}, time to C_{max}; AUC, area under the curve; eq, equivalents; bw, body weight.

Table V : Distribution of citrus flavonoids and metabolites (Manach et. 2005)

An extensive review of polyphenols bioavailabilty was published by Manach et al (2005) and a summary is shown in Table V. The bioavailability of polyphenols was reviewed by the authors (Manach et al.1998,2003,2004a,b;2005a,b) with particular focus on intestinal absorption and the influence of chemical structure (eg, glycosylation, esterification, and polymerization), food matrix, and excretion back into the intestinal lumen. The role of microflora in the catabolism of polyphenols and the production of some active metabolites were also investigated. Mechanisms of intestinal and hepatic conjugation (methylation, glucuronidation, sulfation), plasma transport, and elimination in bile and urine were also described. Pharmacokinetic data for the various polyphenols need to be compared. Studies on the identification of circulating metabolites, cellular uptake, intracellular metabolism with possible deconjugation, biological properties of the conjugated metabolites, and specific accumulation in some target tissues are the most important factors when the health benefits

of phytochemicals are examined. Again, the most abundant polyphenols in our diet are not necessarily those that have the best bioavailability profile.

Flavanones represent a group of polyphenols, including glycosides of hesperetin present in citrus and glycosides of naringenin present in grapefruit.. The C max values for flavanone metabolites were measured 5 h after the ingestion of citrus fruits (Table V). This is the time required for hydrolysis of the rhamnoglycosides hesperidin, naringin, and narirutin by the microflora, before absorption of the released aglycones in the colon. Aglycones are absorbed more rapidly. Bugianesi et al (2002) showed that C max was reached as early as 2 h after the ingestion of tomato paste, which contains naringenin aglycone. However, natural foods rarely contain significant amounts of flavanones in the aglycone form. Plasma metabolites of flavanones have not yet been identified. Monoglucuronides of hesperetin were shown to be the major forms present in plasma after ingestion of orange juice, but the sites of glucuronidation on the hesperitin molecule are still not known (Mao,1999). Microbial metabolites such as *p*-hydroxyphenylpropionic acid, -coumaric acid, p-hydroxybenzoic acid, and phenylpropionic acid were produced with in vitro incubation of naringenin with human microflora (Rimm et al 1996 ; Fremont et al, 1998 ; Paolini et al,1999) and were also found in rat urine (Rimm et al 1996). The same types of microbial metabolites were detected for hesperetin (Rusznyak and Szent-Gyorgyi, 1936; Ross, 1998). Therefore, microbial metabolites are also present in human plasma.

The total urinary excretion of conjugated flavanones accounted for 8.6% of the intake for hesperidin and 8.8% for naringin. Plasma concentrations reached 1.3–2.2 umol/L hesperetin metabolites with an intake of 130–220 mg given as orange juice (McAnlis 1997, Mao 1999) and up to 6 umol/L naringenin metabolites with 200 mg ingested as grapefruit juice (McAnlis 1997). The metabolites present in blood, resulting from digestive and hepatic activity, usually differ from the native compounds. The plasma concentrations of total metabolites ranged from 0 to 4 mumol/L with an intake of 50 mg aglycone equivalents, and the relative urinary excretion ranged from 0.3% to 43% of the ingested dose, depending on the polyphenol. Gallic acid and isoflavones are the most well-absorbed polyphenols, followed by catechins, flavanones, and quercetin glucosides, but with different kinetics. The least well-absorbed polyphenols are the proanthocyanidins, the galloylated tea catechins, and the anthocyanins..

Small intestinal absorption ranges from 0 to 60% of the dose and elimination half-lives (T1/2) range from 2 to 28h. Absorbed flavonoids undergo extensive first-pass Phase II metabolism in the small intestine epithelial cells and in the liver. Metabolites conjugated with methyl, glucuronate and sulfate groups are the predominant forms present in plasma. Most citrus flavonoids are absorbed 6h after ingestion at the colon level mostly as glucuronides. The beneficial effects of these compounds depends upon their further uptake and disposition in tissues and cells.

These studies show that a least three parameters are important for the determination of the oxidative potential of a food.

1) The bioavailaibilty, that is how much actually of a compound crosses the digestive tract and reaches the blood stream.

2) The biotransformation of the original compound by the body into metabolites and the oxidative potential of the metabolites.

3) The ability of these metabolites to penetrate a specific organ where they can act as antioxidants.

V-II- b Ascorbic acid

Gastrointestinal absorption of ascorbic acid occurs through an active transport process, as well as through passive diffusion. At low gastrointestinal concentrations of ascorbic acid active transport predominates, while at high gastrointestinal concentrations active transport becomes saturated, leaving only passive diffusion.

Vitamin C with bioflavonoids: The effect of bioflavonoids on the bioavailability of ascorbic acid has been examined in two studies. In one study synthetic ascorbic acid in a blend containing natural citrus bioflavonoids (in the ratio of bioflavonoids to ascorbic acid of 4:1), proteins, and carbohydrates, was more slowly absorbed and 35% more bioavailable than synthetic ascorbic acid alone, based on plasma levels of ascorbate and 24-hour urinary excretion of ascorbate. In the other study, there was no difference in the bio-availability of 500 mg of synthetic ascorbic acid and that of a preparation vitamin C andbioflavonoids, where the ratio of bioflavonoids to ascorbic acid was 0.05:1. (Vinson and Bose,1998, Vinson et al,2002)

V-III Ascorbic acid as vitamin C or as antioxidant and radical scavenger

V-III-1 Dual role of ascorbic acid

Intracellular Non-enzymatic Function **Quenching of reactive oxidants** **Neutrophils, monocytes activation**
Extracellular- Non-enzymatic Function **Prevention of LDL oxidation** **Quenching of reactive oxidants** **Iron absorption in gastrointestinal tract**
Function Uncertain, tissue contains high concentrations
Host defense **B lymphocytes, T lymphocytes, platelets**
Endocrine **Adrenal cortex, testes, ovaries, pancreas**
Vision **Lens Retina**

Vitamin C (Ascorbic Acid and Dehydroascorbic Acid)	*Biochemical Function*
Intracellular Enzymatic Function	
Proline hydroxylase (EC 1.14.11.2)	**Collagen synthesis**
Procollagen-proline 2-oxoglutarate 3-dioxygenase (EC 1.14.11.7)	**Collagen synthesis**
Lysine hydroxylase (EC 1.14.11.4)	**Collagen synthesis**
Gamma-butyrobetaine, 2-oxoglutarate 4-dioxygenase (EC 1.14.11.1)	**Carnitine synthesis**
Trimethyllysine-2-oxoglutarate dioxygenase (EC 1.14.11.8)	**Carnitine synthesis**
Dopamine beta-monooxygenase (EC 1.14.17. 3)	**Catecholamine synthesis**
Peptidyl glycine alpha-amidating monooxygenase (EC 1.14.17.3)	**Peptide amidation**
4-Hydroxyphenylpyruvate diooxygenase (EC 1.13.11.27)	**Tyrosine metabolism**

Table VI: Ascorbic acid metabolic activity as an enzyme and as an antioxidant

In the body ascorbic acid has a dual activity:

1) It acts as a vitamin (vitamin C): Vitamins are a class of essential nutrients that cannot be synthesized (either at all or in sufficient quantities) by a given animal (a vitamin for one species does not necessarily a vitamin for another species) and must be provided by food to maintain good health. Humans require 13 different vitamins. Vitamins function as catalysts for some enzymes to function (coenzymes). Ascorbic acid is a vitamin only for humans, gorillas, guinea pigs and fruit bats, all the other species can produce their own. Both ascorbic acid and its oxidized form dehydroascorbic acid (DHAA)acid have vitamin C properties. Vitamin C is a coenzyme for various enzymes and particularly hydroxylases involved in collagen formation and maintenance. Ascorbic acid is required for at least eight intracellular enzymatic reactions, and for other intracellular nonenzymatic reactions. In the precursor of collagen, procollagen, every third amino acid is a glycine and it contains a high frequency of two amino acids not found in any other proteins -hydroxyproline and hydroxylysine. These latter two amino acids are converted from proline and lysine, respectively, after the procollagen molecule has been synthesized. The hydroxylation of proline and lysine in procollagen is carried out by the enzyme prolyl hydroxylase using vitamin C as a cofactor. Without ascorbic acid, scurvy occurs and is fatal if untreated. In conclusion, vitamin C is required in the synthesis of collagen in connective tissue, neurotransmitters, steroid hormones, carnitine, conversion of cholesterol to bile acids and improvement of iron bioavailability. It has been implicated in the health of gums, skin, bones, eyes, calcium absorption, before and after surgery for healing, treating burns, fighting infections, fighting scar formation, detoxification, preventing fatigue, breaking fevers and treatment of mumps.

2) Ascorbic acid also acts as an antioxidant, but in this case DHAA is a prooxidant since the oxidized form needs to oxidize another molecule to go back to ascorbic acid. In the body this is done with the help of glutathione and NADPH. Ascorbic acid is a biological reducing agent, it is also linked to prevention of degenerative diseases - such as cataracts, certain cancers and cardiovascular diseases.
Extracellular ascorbate functions to quench radicals. Dehydroascorbic acid formed in this fashion is recycled intracellularly.

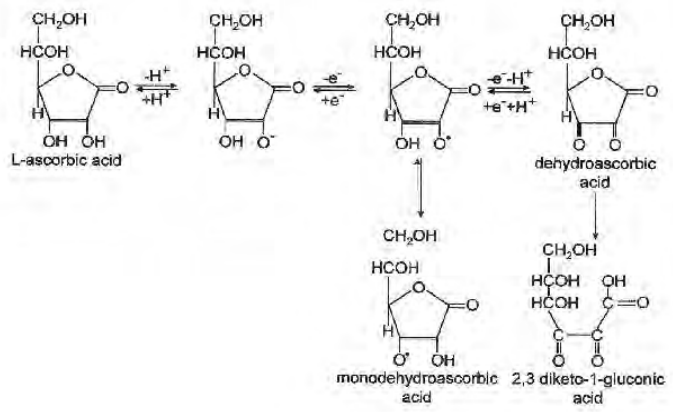

Fig.20: Ascorbic acid oxido-reduction

The dual activity of ascorbic acid raises the question of the amount needed. The FDA recommended dietary allowance (RDA) for vitamin C is 60 mg. The RDA is based on three parameters.

1) The prevention of the deficiency disease scurvy.

2) The urinary excretion of vitamin C. The hypothesis is that once urinary excretion occurrs, body stores of the vitamin are saturated.

3) Catabolism. Catabolism has been estimated to be approximately 60 mg daily; ingestion of 60 mg would maintain a body pool size of 1500 mg.

Thus, the RDA is based on preventing signs and symptoms of scurvy, achieving the urinary threshold for vitamin C excretion, and replacing catabolized vitamin C.

The dose of ascorbic acid necessary to insure maximal benefits has been a subject of controversy. It ranges from the recommended 60mg/d to up to 5g/d promoted by L. Pauling.. Many studies have shown that once the blood is saturated with ascorbic acid further intake will result in rapid excretion. Hallfrisch et al (1994)reported that peak plasma concentrations of vitamin C occurred at intakes of approximately 215 mg/day in women and 345 mg/day in men. Further increases in plasma vitamin C did not occur with higher intakes.. However, one of the most complete studies was published by Levine et al (1996). The authors demonstrated that vitamin C steady-state plasma concentrations as a function of dose displayed sigmoid kinetics. The steep portion of the curve occurred between the 30- and 100-mg daily dose, the current RDA of 60 mg daily was on the lower third of the curve. The first dose beyond the sigmoid portion of the curve was 200 mg daily, and complete plasma saturation occurred at 1000 mg daily. It was concluded that the current RDA of 60 mg daily should be increased to 200 mg daily, which can be obtained from fruits and vegetables. Several studies have pointed out that some groups have been shown to need higher amounts of vitamin C. The elderly, alcohol consumers, diabetics and smokers, for example, tend to be low in vitamin C(Gaby et al, 1991; Schectman, 1991, Levine, 1999).

V-III-2 Beneficial effects of citrus Vitamin C

V-III-2-a Citrus Ascorbic acid as an antioxidant

As seen previously, an antioxidant can be defined as a molecule that can counteract the damaging effects of oxygen in tissues. Although the term applies to molecules reacting with oxygen, it is often applied to radical scavengers that protect from any free radical (molecule with unpaired electron).

The various types of antioxidants include:
1. Hydrogen donating compounds

- Singlet oxygen quenchers
- Metal chelators
- Enzymes

2. Oxygen scavengers and reducing agents

Ascorbic acid is the most prevalent citrus antioxidant. It is an effective antioxidant because it can itself be oxidized more easily than other molecules. Ascorbic acid can donate a hydrogen atom to a free radical molecule ($R^{\cdot}$) thereby neutralizing the free radical while becoming an ascorbate radical itself which as seen previouslycan be regenerated from dehydroascorbic acid. The role of ascorbic acid is particularly important during the oxidative processes. Once a superoxide radical is formed, the enzyme superoxide dismutase catalyzes its conversion to O_2 and H_2O_2 (Galley and Thiollet,1993). Ascorbate, non-enzymatically, also converts superoxide to H_2O_2 but is oxidized in the process to the ascorbate free radical and dehydroascorbate. As seen previously, the ascorbate free radical and dehydroascorbate are reduced back to ascorbate either by NADH (catalyzed by semidehydroascorbate reductase and forming NAD) or reduced glutathione (GSH) (catalyzed by dehydroascorbate reductase and forming oxidized glutathione (GSSG)) . Some of the peroxide can be converted to oxygen and water by catalase but most will be destroyed by a glutathione-requiring enzyme system. GSH (catalyzed by glutathione peroxidase) reduces the peroxide to water but in the process is oxidized to GSSG. The resulting GSSG is reduced by NAD(P)H (catalyzed by glutathione reductase).

V-III-2-b Synergistic properties of ascorbic acid

The antioxidant properties of ascorbic acid have been shown to greatly increase by interacting with other antioxidants present in citrus juice or other foods.

co-antioxidant effect

Sixty years ago, Golumbic and Mattill (1941) observed that while ascorbic acid (vitamin C) alone has little effect in preventing lard oil from oxidation, the combination of ascorbic acid with tocopherol (vitamin E) gave rise to a strong synergistic antioxidative effect. These authors concluded that the role of ascorbic acid was to preserve tocopherol. This behavior of ascorbic acid is termed a co-antioxidant effect. The synergistic effect of vitamin C and E is of major importance and should be taken into consideration during food preparation(Bowry, et al, 1995).
The synergistic prperties of ascobic acid are not limited to vitamin E. Recently, Chen et al (2005)reported a major synergistic effect between ascorbic acid and food. Flavonoids from almond skins (ASF) act synergistically with vitamins C and E to enhance hamster and human LDL resistance to oxidation. Combining ASF with vitamin E or ascorbic acid produced a 250 fold increase in the AO effect as compared with the sum of two acting separately (Fig21).

ASF possess antioxidant capacity and interact with vitamins E and C in a synergistic manner to protect LDL against oxidation in vitro. Of the five identified flavonoids in the ASF, all appeared in plasma and liver after oral administration. These flavonoids and related compounds in the ASF slightly enhanced the resistance of hamster LDL against ex vivo Cu2+ -induced oxidation, and their

antioxidant capacity was amplified with the in vitro addition of vitamin E. Because almonds represent one of the richest dietary sources of vitamin E, further research is warranted examining the relations between the natural ingredients in this whole food and their potential association with health benefits, such as a reduction in the risk of cardiovascular disease. This may be particularly important in the case of citrus juices taken with other foods for example during a breakfast.

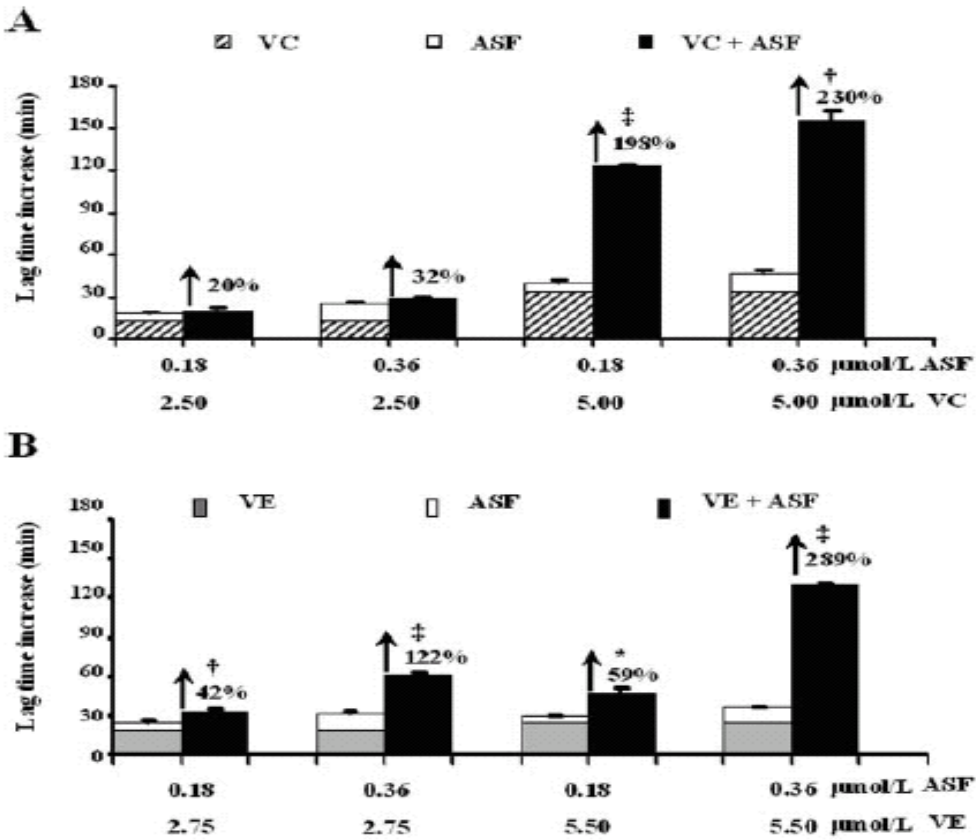

FIGURE 3 The synergistic effect of ASF and vitamin C or E (VC or VE) on the lag time of human LDL oxidation in vitro. LDL (182 μmol/L) was oxidized by 10 μmol/L Cu^{2+} with the addition of ASF, VC or VE, or ASF + VC or VE. Values are means ± SE, $n = 3$. Lag time of control (no added ASF, VC, or VE) = (*A*) 45.5 ± 0.7 min and (*B*) 47.7 ± 1.5 min. Bars are stacked to illustrate the calculated additive effect of the 2 treatments. The percentage value above the solid bar indicates the observed synergy greater than the calculated sums of the individual treatments. Symbols indicate different from the calculated sums: $^{*}P \leq 0.05$, $^{\dagger}P \leq 0.01$, $^{\ddagger}P \leq 0.005$.

Fig. 21: Synergistic effect of food (from Chen et al-2005)

Antioxidant synergy within citrus juices

A synergistic activity was also seen within citrus juices. Vinson (2005)looked at the quality of compounds as AO. He used in vitro methods (Methods in Enzymology) to examine the oxidation of LDL by cupric ions and determined the IC 50 (50% LDL oxidized). The study showed that there was a synergistic effect of citrus juice as compared to the effect of each citrus component taken separately (Fig. 22). Orange juice was more effective than quercitin rutinoside which in turn was more effective than ascorbic acid. This shows that the whole juice is more effective than its components taken separately.

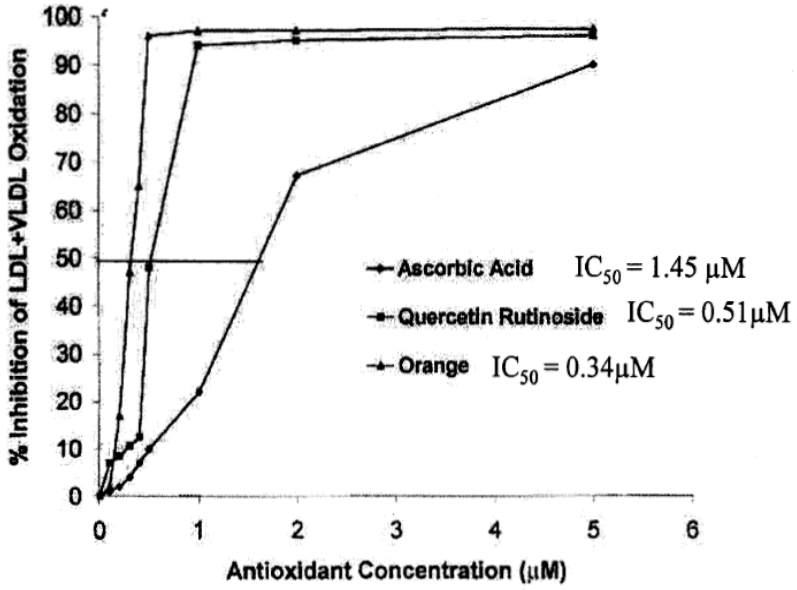

Fig.22 : synergistic effect of orange juice components
From Vinson J. 2nd Intern Congress on Antioxidant methods (2005)

It can be concluded that the components of orange juice act synergytically making the juice more effective than its components used separately.

V-III-3 Physiological effects of vitamin C

V-III-3- a Vitamin C and cardiovascular diseases (CVD)

Vitamin C intake has been associated with a reduced incidence of cardiovascular disease in several epidemiological studies (Levine et al, 1996)). Many studies have shown beneficial effects of high doses of ascorbic acid on endothelial-dependent vasodilation(Carr an Frei1999; May 2000). In four studies, 1,000-2,000 mg of oral ascorbic acid or 25 mg per minute of ascorbic acid infusion increased vasodilation 45-220% in patients with CVD. Similar results were found in patients with diabetes, hypercholesterolemia, and hypertension, as well as in healthy adults.(Carr an Frei1999; May 2000; Duffy et al, 2000). Ascorbic acid improves both endothelial function and insulin sensitivity in patients with CVD (Hirashima 2000) The mechanism by which ascorbic acid improves vasodilation has been attributed to the vitamin's ability to promote the synthesis and/or prevent the oxidation of the endothelial-derived relaxing factor, nitric oxide (NO).(May 2000). Some beneficial effects of ascorbic acid supplements on thrombotic risk of CVD and impaired endothelial function in women with pre-eclampsia have also been reported.(Chambers et al, 2001)Another study of the effect of vitamins C and E on 3-year progression of carotid atherosclerosis in Finnish adults showed significant benefit from combined vitamin C and E supplementation, but not with either vitamin C or E alone.(Salonen et al. 2000).

Vitamin C has multiple actions that can reduce the risk of heart disease (Hercberg et al. 1998). Diaz et al (1997) have found that 500 mg daily can significantly reduce blood pressure. In a study 38 people with mild to moderate hypertension, 500 mg daily of vitamin C supplements reduced blood pressure by almost 10 percent in just one month. In a separate study, Fotherby et al (2000), found that 500 mg daily of vitamin C reduced blood pressure by an average of 2 mmHg in 40 elderly men and women after three months.

In a randomized, double-blind, placebo-controlled study conducted to evaluate the effect of vitamin C supplements on blood pressure in hypertensive subjects, the mean blood pressure was reduced by about 10% in subjects taking 500 mg/day of vitamin C for a one-month period (Duffy et al.1999).

Stroke

As mentioned previously, Jessa Netting summarized several studies that associated vitamin C with decrease risk of strokes (2000). Over a twenty-year period, Yokoyama et al (2000)measured vitamin C content in the blood of 880 Japanese men and 1,241 women. They compared the results with the incidence of death or disability from strokes during this same period among this population. People with the highest levels of vitamin C in their blood were the least likely to have a stroke. Those with elevated levels of vitamin C also ate the most fruits and vegetables. It is not yet clear if other factors play a role in the results. In a similar study, Gale et al. (2000) also found that elderly people with the highest vitamin C intake, the equivalent of only an orange a day, had the least risk of dying from stroke. But other studies have not found such clear association.

Blood lipids

At the molecular level, it appears that the effects of vitamin C on CVD are mainly due to ascorbic acid's ability to prevent blood lipid oxidation (Jialal et al. 1990; 1995; Shidfar, 2003) . As seen previously, atherosclerotic plaques can reduce the blood's flow through an artery and. plaques rupture causes blood clots to form that can block blood flow or break off and travel to another part of the body. The oxidized low-density lipoproteins (LDLs) are implicated in the pathogenesis of atherosclerosis, and dietary antioxidants are thought to protect the vasculature against LDL-induced cytotoxicity. However, Vinson (2005) proposed that the water soluble vitamin C may be less effective in preventing LDL oxidation than hydrophobic compounds such as polyphenols which can bind to LDL.

Hallfrisch et al (1994) examined the relationship between cholesterol and vitamin C and the possible role of vitamin C in CVD. The authors showed that after adjustment for age, sex, obesity, and smoking, high plasma vitamin C was directly associated with high HDL cholesterol.. This was shown in a double-blind placebo-controlled study.120 healthy males and females aged 50+ received either vitamin c, vitamin E, a combination of C and E or a placebo for 75 days to ascertain whether such intervention lowers serum lipid levels. Those subjects receiving vitamin C had a significant decline in cholesterol, LDL and triglyceride and an increase in serum HDL, compared to the placebo group (Shidfar, 2003). In a comparable study, the USDA and the National Institute on Aging studied the effect of vitamin C intake on total cholesterol levels and its fractions. The researchers found that in a population with vitamin C intake well above the RDA of 60 mg/day, and with adequate concentrations of plasma vitamin C, there was a significant positive association between high levels of plasma vitamin C and high levels of HDL cholesterol. After adjustment for age, sex, obesity, and smoking, high plasma vitamin C was directly associated with high HDL cholesterol. Similarly, Brown and Goodman (1998)showed that the supplementation of vitamins A, C and E lowered the risk of death from CVD. They also concluded that diabetics, smokers and those with hypertension would benefit from taking vitamin C. In a different study the progression of atherosclerosis was followed in 520 participants. Those participants who were deficient in vitamin C had more benefit from the supplementation in terms of cholesterol levels and slowing progression of heart disease. The use of both vitamin E and vitamin C provided some benefit from the progression of heart disease and helped to improve cholesterol levels (Salonen et al. 2003).

The role of ascorbic acid in protecting HDL from oxidation was demonstrated in-vitro by incubating HDL at 37 degrees C with cupric ions in the absence or presence of vitamin C. In the absence of vitamin C, lipid oxidation in HDL began immediately and proceeded rapidly. Cholesteryl linoleate declined to a minimum, whereas lipid oxidation products increased to near-maximal levels within 1 hr. Vitamin C retarded initiation of lipid oxidation for at least 4 hrs under the same conditions. The ability of vitamin C to preserve the cardioprotective antioxidant function of HDL was also assessed. HDL preincubated with Cu2+ for 2 h in the absence of vitamin C lost antioxidant activity (45.4 +/- 6.2% inhibition of LDL oxidation compared with 93.2 +/- 3.6% for native HDL, $P < 0.05$). The addition of vitamin C (50-200 micromol/L) during preincubation of HDL with Cu2+, however, resulted in no significant loss of HDL antioxidant activity (77.3 +/- 0.3 to 89.8 +/- 5.4% inhibition of LDL oxidation, $P > 0.05$ compared with native HDL). The results demonstrate that vitamin C

inhibits lipid oxidation in HDL and preserves the antioxidant activity associated with this lipoprotein fraction (Ling et al, 2002).

Because LDL oxidative modification may vary within atherosclerotic lesions, Siow et al (1999) examined the effects of defined, oxidatively modified LDL species on human arterial smooth muscle cell apoptosis and the cytoprotective effects of vitamin C. Moderately oxidized LDL (0 to 300 microg protein/mL), which has the highest content of lipid hydroperoxides, induced smooth muscle cell apoptosis within 6 hours, whereas native LDL and mildly and highly oxidized LDL had no effect. Moderately oxidized LDL increased cellular DNA fragmentation and release of fragmented DNA into the culture medium. The results suggest that moderately oxidized LDL, with its high lipid hydroperoxide content, rather than mildly or highly oxidized LDL, causes apoptosis of human smooth muscle cells and that vitamin C supplementation may provide protection against plaque instability in advanced atherosclerosis.

The effect of vitamins C and B6 therapy on some lipid metabolic aspects was studied in normal and alloxan diabetic rats. Long term administration of vitamin C (30 mg/kg/day) and vitamin B6 (14 mg/kg/day) induced significant reduction in serum total cholesterol triglycerides and serum LDLcholesterol levels decreased significantly after vitamin C treatment. Serum HDL cholesterol was markedly increased. Furthermore vitamin C and B6 supplementation reduced lipid peroxidation in normal and diabetic groups. The results suggest that the therapeutic doses of vitamin C and B6 in long term therapy increased LDL metabolism and produced hypochlosterolemia in male rats. On the other hand, vitamin C and B6 had a beneficial effect on cardiovascular risk profile (El Sweidy et al. 1996). Easwaran et al (2002) selected forty two hyperlipidemic patients to assess the impact of intakes of antioxidants vitamin E and vitamin C on lipid profile, plasma alpha-tocopherol and plasma ascorbic acid levels. These findings indicated the beneficial effects of vitamins E and C both as food (almond and orange juice) and in synthetic form. A combined intake of vitamins E and C had better results than consuming vitamin E or C alone.

Antioxidants can increase the resistance of LDL to oxidation and hence reduce its atherogenicity. Nagyava et al (2003) assessed how a modest dietary supplementation with a mixture of antioxidant nutrients changed antioxidant status and LDL oxidizability in healthy volunteers. In conclusion, a modest dietary supplementation with a mixture of antioxidant nutrients favorably affected plasma antioxidant status and malondialdehyde levels, and increased the resistance of LDL to oxidation in healthy volunteers (Brown and Goodman,1998). Whalley et al (2003) studied 82 non-demented old people and, using MRI, derived measures of grey and white matter and intracranial volumes. Controlling for sex and intracranial volume, we related grey and white matter volumes to plasma concentrations of vitamins C, B12, folate, homocysteine, cholesterol, triglycerides, high density and low density lipoproteins,. The results showed that lower grey matter volume was associated with lower plasma vitamin C and higher homocysteine, cholesterol and LDL. These data are consistent with the putative benefits of dietary vitamin C and folate intake and the role of cholesterol in age related neurodegeneration.

The studies on beneficial effects of vitamin C, by itself, in combination with other vitamins and antioxidants or with food such as fruit and fruit juices, on the cardiovascular system appear to be the

best documented, the least controversial and the most scientifically sound. Through its anti-oxidative ability ascorbic acid can prevent or limit blood lipid oxidation and play a role in the prevention of cardiovascular diseases. However, the question the hydrophylicity of ascorbic acid has been viewed as a drawback for prevention of lipoprotein oxidation (Vinson, personal communication). It has been hypothesized that hydrophobic antioxidants would bind lipoproteins and provide a better protection than molecules remaining in solution.

V-III-3- b Vitamin C and the elderly

Reports have shown that vitamin C levels appear to decline in the elderly. The mechanism responsible for vitamin C transport in the gut and all other tissues is a Sodium-dependent Vitamin C Transporter known as SVCT. Two isoforms of this protein, SVCT1 and SVCT2, are known to exist in humans. It has been hypothesized , that the decline in age-related vitamin C levels is due to a loss of vitamin C transport, primarily due to changes in SVCT activity. Aging Affects Vitamin C Status (Michels et al, 2003)

Adequate vitamin C intake may lower the risk of arteriosclerotic cardiovascular disease, but little is known about its influence on the progression of atherogenic disease in the elderly. Okamoto examined whether ascorbic acid intake was associated with serum lipids, apolipoprotein A-1 (ApoA1) and apolipoprotein B (ApoB), in 680 Japanese elderly persons. There were no significant gender differences among mean serum lipids and apolipoprotein concentrations and intakes of macronutrients. Ascorbic acid intake had a significant positive association with serum concentrations of high-density cholesterol and ApoA1, but an inverse association with serum concentrations of low-density cholesterol and ApoB, after adjusting for age, body mass index, total energy, and macronutrients. AsA intake was strongly inversely related to ApoA1/ApoB. This study also showed that an increased ascorbic acid intake could play an important role in lipid composition and could be of potential importance in the genesis and prevention of atherogenic disease in the elderly (Okamoto, 2002).

It is hypothesized that a reduction in the level of ascorbic acid with age leads to an impairment in the quenching of free radicals by antioxidants, which in turn increases the risk of succumbing to age-associated disorders. Healthy volunteers of both sexes at different age group were selected from the geriatric community (Muthuvel et al. 2000). They were subjected to vitamin C supplementation. Lipids, lipid peroxidation, enzymatic, and nonenzymatic antioxidant status were gauged at the end of 30, 60, and 90 days. The activities/levels of enzymatic and nonenzymatic antioxidants were found to be low in the older humans, whereas the lipid peroxidation status was found to be high. Supplementation of vitamin C lowered the levels of lipid peroxides, cholesterol, triacylglycerol, and phospholipids and increased the activities of enzymatic antioxidants in the geriatric population. A substantial rise in the levels of nonenzymatic antioxidants such as vitamins C, E, and A were also observed From these observations, the authors concluded that ascorbic acid normalizes lipid peroxidation and partially restores the antioxidant status.

The beneficial effects of vitamin C supplementation seen in the general population may be even be stronger in older people since with age the ability to absorb ascorbic acid decreases.

V-III-3- c Diabetes

The tissues and organs of diabetics may be deprived of vitamin C, requiring them to consume more of the nutrient than does the average person. Vitamin C must compete with glucose to reach the tissues and organs through a common cellular transport system. An insufficient supply of insulin also can inhibit the transport of vitamin C to cells that require insulin for their glucose uptake. The cellular uptake of vitamin C ascorbic acid, is promoted by insulin and inhibited by hyperglycemia. If a rise in plasma ascorbic acid is uncoupled from insulin replacement in insulin-dependent diabetes mellitus (IDDM) then the degree of hyperglycemia could account for "tissue scurvy" in IDDM. Leukocyte ascorbic acid is lower in IDDMs compared with non-diabetics when vitamin C consumption is adequate and our data suggest that this is a variable component of the pathophysiology of IDDM. The complications of diabetes mellitus are believed to result from either the intracellular accumulation of sorbitol or the nonenzymatic glycoxidation of proteins or both. With respect to the abnormal cellular accumulation of sorbitol, vitamin C supplementation has been shown to be effective in several studies of adults with diabetes. The situation regarding the prevention of protein glycoxidations by supplementation is presently unclear. The roles of ascorbic acid as an aldose reductase inhibitor and a water soluble antioxidant in body fluids are potentially very important as adjuncts to tight glycemic control in the management of diabetes. Tissue saturation and maximal physiologic function in IDDM may require supplemental vitamin C intake (Cunningham, 1998).

Timimi et al (1998) examined if vitamin C improves endothelium-dependent vasodilation of forearm resistance vessels in patients with insulin-dependent diabetes mellitus. Endothelium-dependent vasodilation is impaired in patients with diabetes mellitus. Oxidatively mediated degradation of endothelium-derived nitric oxide contributes to abnormal endothelium-dependent vasodilation in animal models of diabetes mellitus. Vitamin C was shown to selectively restore the impaired endothelium-dependent vasodilation in the forearm resistance vessels of patients with insulin-dependent diabetes mellitus. These findings indicate that nitric oxide degradation by oxygen-derived free radicals contributes to abnormal vascular reactivity in humans with insulin-dependent diabetes mellitus.

V-III-3- d Bones and cartilages

Vitamin C has also been implicated in collagen formation and in the maintenance of bone mass..
Osteoporosis occurs most often in older women (Morton et al. 2001), in part because estrogen appears to help protect against bone loss. In several studies of postmenopausal women and a mixed population, vitamin C intake was correlated with bone mineral content or bone density. Gaby and Singh (1991)showed that ascorbic acid intake at moderate doses is important and safe for bone maintenance, and therefore a factor in mitigating or delaying osteoporosis. Vitamin C has also been reported to play a role in arthritis. W hen mice with arthritis and inflammation in their paws received

vitamin C for 20 days, the treatment reduced arthritic swelling, increased their pain tolerance and decreased polymorphonuclear leukocyte infiltration(Davis, 1990).

V-III-3- e Immunity

Vitamin C assists the immune system in two of its primary functions to rid the body of foreign invaders and to monitor the systems for any sign of tumor cells. It accomplishes these vital tasks by stimulating the production of white blood cells, primarily neutrophils, which attack foreign antigens such as bacteria and viruses. It also boosts the body's production of both antibodies and interferon, the protein that helps protect us from viral invaders and cancer cells (Kronhausen et al. 1989)

V-III-4- f Cancer

Studies suggest that vitamin C's antioxidant mechanisms may help to prevent cancer. It combats the peroxidation of lipids, which has been linked to the aging process and degeneration. Gaby and Singh (1991) reviewed several studies. One study of elderly people found that 400 mg of vitamin C per day (for a one-year period) reduced serum lipid peroxide levels. Vitamin C can also work inside the cells to protect DNA from the damage caused by free radicals. Several studies report vitamin C reduced the level of potentially destructive genetic alterations or chromosome aberrations. Finally, vitamin C can reduce the development of nitrosamines from nitrates, chemicals that are commonly used in processed foods. Once formed, nitrosamine can become a carcinogen. But in several human studies, in which the subjects consumed a nitrosamine precursor, the urinary levels of nitrosamines were significantly reduced by vitamin C. Three animal studies also support the preventive effects of ascorbic acid on nitrate-induced cancer. In all three cases, the formation of tumors was inhibited, suppressed or reduced in frequency in the animals treated with vitamin C (Dzhioev et al. 1988; Dittrich et al,1998; Shidfar, 2003, Viasenko et al,1998).

V-III-3-g Conclusion: Vitamin C and health

The uptake of vitamin C is essential for human.

The daily required intake to maintain a good health, ranges between 200 and 500 mg. Certain groups such as diabetics and the elderly may need a larger amount.

Fruits and vegetables particularly citrus can provide vitamin C as well as many other compounds such as flavonoid anti-oxidants that potentiate the affect of ascorbic acid.

The best documented effect of ascorbic acid is on the cardiovascular system. By preventing the oxidation of blood lipids vitamin C appears to play a major role in limiting or preventing atherosclerosis and possibly stroke.

Vitamin C may also have an effect in potentiating the immune system and as a consequence may have a moderating action on flu and colds and allergies. An effect on the immune system has also been implicated in reinforcing the natural defenses against cancer and possibly AIDS

Ascorbic acid may promote bone formation and limit osteoperosis.

V-IV Flavonoids and polyphenols

Citrus phenolic compounds have been reviewed in great details by Augustin et al (2005) and Horowitz and Gentilli (1977). Citrus flavonoids include many polyphenolics such as flavonoid glycosides,including neohesperidin and naringin, hesperidin, rutoside, sinensetin, polymethoxylated flavones such as nobiletin, tangeretin, , numerous hydroxycinnamates and at least 44 different flavone glycosides (Paul,1995;2000, Berhow et al,1998). Other common flavonoids include catechin, epicatechin, myricetin, quercetin , quercetin is consistently the most active of the flavonoids, and kaempferol. Other polyphenolic compounds, specifically phenolic acids that are powerful antioxidants include gallic acid, p-hydroxybenzoic acid, caffeic acid, ferulic acid, and ellagic acid. Manthey et al (2001,2002) reviewed the anti-inflammatory and anti-carcinogenic properties of citrus flavonoids

V-IV-1 Flavonoids: Antioxidant and radical scavenger

Many studies have shown flavonoids to be effective antioxidants (Maron, 2004, Chenier 2005). They have been shown to be able to to scavenge peroxyl radicals, alkyl peroxyl radicals, superoxide hydroxyl radicals, and peroxynite in aqueous and organic environments.

Flavonoids and polyphenols in general have specific antioxidant properties that set them apart from other types of molecules like vitamin C (Collins 2005). Once oxidized ascorbic acid, remains in equilibrium between oxidized and reduced form and can move in either direction according to the local conditions. Phenolics on the other hand have much more stable structures. Their antioxidant capacity is determined by their structure. When acting as an antioxidant polyphenols generate free radicals that do not initiate another free radical due to the delocalization of radical electrons within the benzene ring. The resulting antioxidant free radical is not subject to rapid oxidation due to its stability.

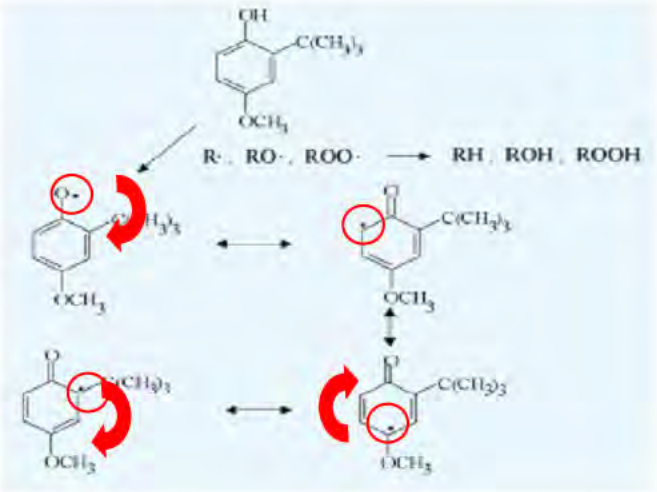

Fig.23: Phenol oxidation

Differences in the antioxidant potential of various polyphenols is due in part to the position of hydroxyl groups. In particular the ease with which a hydrogen atom from an aromatic hydroxyl group can be donated to a free radical and the ability of an aromatic compound to support an unpaired electron is due to its conjugated system.

The antioxidant activity of polyphenols is also influenced by molecular substitutions. Quercetin glycosides activity is lower than quercetin aglycone in an artificial membrane system. This may imply that glycosidation weakens the antioxidant activity of flavonoids. This decrease may be caused by increased blocking of the phenolic groups responsible for radical scavenging and metal chelation and possibly to a decrease in accessibility of the membranes owing to the large glycoside group. Reaction rate constants in organic media for several flavonoids exceed that of vitamin E. Suggested reasons for this include flavonoids having a more extended conjugated system to support an unpaired electron, two or more reactive OH groups, and less stearic hindrance at the site of abstraction. Similar results have shown that many aglycones, including quercetin, luteolin, myricetin, and kaempferol, have greater antioxidant capacity than do the conjugated flavonoids, such as quercetin-3-glucoside, quercitrin, and rutin.
These differences in the oxidative mechanisms of vitamin C and polyphenols may explain the lower oxidative potential reported for ascorbic acid in in vitro analyses. Vinson et al (2002)
measured the polyphenol content of citrus juices by an oxidation-reduction colorimetric method (Folin) using catechin as the standard. The order was tangerine juice > grapefruit juice > orange juice. The antioxidant contribution of ascorbic acid was measured by the difference in Folin reactive content following removal by ascorbate oxidase. Ascorbate contributed 56 to 77% of the antioxidant content of orange juice, 46% of the single tangerine juice measured, and 66 to 77% of grapefruit juice. It is not completely clear if the differences noted in in vitro methods are physiological or due to methodological artifacts. Liu (2003) reported that the vitamin C in apples with skin accounts for only 0.4% of the total antioxidant activity, suggesting that most of the antioxidant activity of fruit and vegetables may come from phenolics and flavonoids. The additive and synergistic effects of phytochemicals in fruit and vegetables are responsible for their potent antioxidant and anticancer activities, and the benefit of a diet rich in fruit and vegetables is attributed to the complex mixture of phytochemicals present in whole foods. This study points out the importance of ingesting juices or fruits as compared to the single product

Flavonoids may also exert antioxidant abilities through protection or enhancement of endogenous antioxidants. Numerous flavonoids have been shown to minimize oxidative stress by inducing glutathione S-transferase (GST), an enzyme shown to protect cells against free-radical damage caused by hydrogen peroxide (Fiander et al 2000). Some flavonoids, including quercetin, myricetin, and fisetin, were shown to cause statistically significant increases in GST-specific activity.

V-IV -2 Flavonoid hydrophobicity and lipid oxidation

The flavonoids hydrophilicity may also explain some of their antioxidant properties. In contrast to vitamins C which is concentrated in the aqueous phase and vitamin E which binds to the cellular

membrane phospholipid bilayer, flavonoids can insert themselves between the two phases and may trap chain-initiating radicals at the interface of the membranes, thus preventing the progression of the radical chain reaction. In addition to free-radical scavenging properties, some flavonoids can chelate transition metal ions responsible for the generation of reactive oxygen species and therefore inhibit the initiation of the lipoxygenase reaction (McAnlis et,1997).

Studies by Jeon et al (2001,2002) in rabbits on a high cholesterol diet found those receiving supplementation with the citrus bioflavonoid naringin increased plasma antioxidant capacity similar to that of two cholesterol-lowering drugs. Naringin has also been found to reduce the aortic fatty streak areas in rabbits fed a high cholesterol diet (Lee et al, 1998, 2001). The polymethoxylated flavones tangerin and nobiletin, were found in macrophage studies to reduce plasma concentrations of LDL, and to inhibit macrophage foam-cell formation (Whitman,2005). Tangeretin, may also be able to modulate apoB-containing lipoprotein metabolism, including reducing apoB secretion, suggesting a role in treating hypertriglyceridemia. (Kurowska and Manthey, 2004). . Vinson (2005) examined the polyphenol quality in the juices analyzed by using the inhibition of lower density lipoprotein oxidation promoted by cupric ion, an in vitro model of heart disease. Quality decreased in the following order: orange juice > grapefruit juice > tangerine juice. In orange juice, polyphenols accounted for 84-85% of the antioxidant quality. In a hamster model of atherosclerosis, citrus juices were able to significantly inhibit atherosclerosis and lowered cholesterol and triglycerides.

V-IV-3 Physiological effects of flavonoids

A list of possible positive effects of flavonoids include:

Lower cancer risk. (Lambert et al, 2005 , Duthie and Crozier, 2000) A high intake of such flavonoids as polyphenols and quercetin is linked to lower rates of stomach, pancreatic, lung, and possibly breast cancer. Taking the flavonoid genistein, a phytoestrogen that acts as a weak form of the hormone estrogen, may help prevent breast cancer and other hormone-related cancers, including prostate cancer, because it binds with estrogen receptors in the body's cells.

At the molecular level recent studies have suggested that dietary flavonoids may protect free-radical–induced damage to DNA by a mechanism other than direct free-radical scavenging. Results have shown that flavonoids can reduce the incidence of single-strand breaks in double-stranded DNA as well as residual base damage.

Reduce the risk of stroke and heart disease. As seen previously flavonoids may prevent lipid oxidation and minimize CVD. Bohm et al. (1998) and Manach et al (2005) reviewed the literature and showed that a diet rich in flavonoids, may help prevent blood clots and blocked arteries, significantly reducing the chance of death from stroke or heart disease.

Protect against age-related vision disorders, such as cataracts and macular degeneration. Studies showed that quercetin inhibited the accumulation of sorbitol in the eye's lens a contributing factor in the development of cataracts. Flavonoids may also help combat cataracts and prevent macular degeneration by improving blood circulation in the eye.

Relieve hay fever, sinusitis, and asthma symptoms. (Denny et al, 2003)Quercetin's proven anti-inflammatory properties may help the body counter allergic reactions to pollen. Quercetin also seems to reduce inflammation in the lungs and other air passages, making breathing easier.

Alleviate inflammatory skin conditions. Some flavonoids anti-inflammatory properties can help treat skin irritations.

Reduce inflammation in the joints and muscles common to fibromyalgia, gout, and rheumatoid arthritis. Because of their anti-inflammatory properties flavonoids may improve these conditions.

Decreases the risk of infection. Because flavonoids boost immunity, they may help the body fight off illnesses and may speed recovery time.

V-IV-3-a Cardiovascular and Cerebrovascular Disease

The ability of polyphenols to prevent lipid oxidation gives them great potential to limit CVD. The main citrus polyphenols involved in CVD protection are hesperetin and naringenin found in the fruit tissue and peel largely as their glycosides, hesperidin and naringin and two polymethoxylated flavones, tangeretin and nobiletin. In vivo (Kurowska and Manthey, 2004) and in vitro (Wilcox et al 2001, Kurowska et al, 2004))studies indicated that citrus flavonoids may reduce the occurrence of cardiovascular disease through their ability to reduce hepatic production of cholesterol containing lipoproteins (Wilcox et al 2001) and hence reduced total plasma cholesterol concentrations.

Hertog et al. (2002) studied patients taking average flavonoid intake of 26 mg/day. After 5 years, the authors reported that high intake of flavonols was associated with a decreased risk of coronary heart disease (CHD) mortality. In a 10-year follow-up of the same group of elderly men, Hertog et al. (1993,1997a,b) reported a relative lower risk for a coronary event among men in the highest tertile of flavonoid intake compared with men in the lowest tertile. They also reported a decreased risk of all cause mortality with increasing flavonol intake. However, a study of coronary heart disease in US male health professionals aged 40–75 found no significant association with flavonoid intake (Rimm et al, 1996).

Stroke

Fewer of these studies have explored flavonoid intake and risk of stroke. In the Zutphen study (Keli et al, 1996)), there was an inverse association with increasing dietary flavonoid consumption . Tea consumption, an important source of flavonoid intake, was associated with a decreased risk of stroke.

Blood pressure

Hesperidin, was studied in spontaneously hypertensive rats and normotensive rats, with diets containing 30 mg/d/kg body weight for 25 weeks (Ohtsuki, K., et al. ,2002). Hypertensive rats that received the hesperidin had decreased blood pressure and heart rate, suggesting anti-hypertensive effects of the compound.

V-IV-3- b Antiproliferative Activity and Cancer

There has been many studies examining the anticarcinogenic effect of flavonoids. Results appear to be controversial with many opposite conclusions. Whereas the lack of an association may be real, other explanations could include a lack of an adequate measure to assess flavonoid intake and the multitude of factors affecting flavonoid content in foods and bioavailability (see above:V- II Absorption and bioavailibility).

In an extensive review, Lopez-Lazaro (2002)examined the effect of flavonoids on the various phases of cancer development and the relationship between molecular structure and activity. Franke et al. (1998) investigated the effects of dietary flavonoids on inhibiting neoplastic transformation induced by 3-methylcholanthrene in mouse fibroblasts. They examined several flavones, flavonols, flavanones, catechins, and isoflavones and found that the flavonones hesperetin and hesperedin and the catechins exhibited the highest levels of inhibition of transformation. The isoflavones demonstrated the weakest inhibition in this cell culture model.

Wenzel et al. (2000) evaluated how flavones, affect proliferation, differentiation and apoptosis in a human colon cancer cell line. In particular, they evaluated the effect on the expression of cell-cycle and apoptosis-related genes in the cell line. They reported major changes in mRNA levels of specific genes including cyclo-oxygenase-2, nuclear transcription factor kappaB, and bcl-X. Further, there was high selectivity for apoptosis in the transformed cells. The authors concluded that flavones could have some chemopreventive properties. A study from Finland of 9959 men and women reported an inverse association between the intake of flavonoids and the incidence of all cancers combined with the association being most apparent for lung cancer.(Knekt 1996,1997).

However, results were not always positive. Specific flavonoids have been shown to cause single- and double-strand DNA breaks in cultured human cells (Duthie and Crozier, 2000, Strick et al, 2000). This suggests that not all cells undergo apoptosis in the presence of flavonoids.. Hertog et al. (1993) reported no association between flavonol or flavone intake and total cancer mortality or mortality from specific cancers. Arts et al. (2001) evaluated catechin intake and the incidence of epithelial cancer and did not find any clear positive or negative results.

V-IV-3-c Immunity

Some studies have also shown an influence of polyphenols on immunity. Park et al (2005)examined the passive cutaneous anaphylaxis-inhibitory activity of the flavanones isolated from the pericarp of Citrus unshiu (Family Rutaceae) and the fruit of Poncirus trifoliata (Family Rutaceae). Naringenin, hesperetin and ponciretin potently inhibited IgE-induced beta-hexosaminidase release. Among the flavanones examined, naringenin was the most potent and intraperitoneally administered naringenin (5 mg/kg) was 70% more effective than control. The authors claimed that the inhibitory activity of naringenin was comparable to that of a commercially available antiallergic drug. However, their glycosides, hesperidin, naringin and poncirin, did not inhibit the in vitro release of beta-hexosaminidase from the RBL-2H3 cells. When the flavanone glycosides were administered to rats, the aglycones, but not the flavanone glycosides, were excreted in urine. This suggests that flavanone glycosides can be activated by intestinal bacteria, and may be effective toward IgE-induced atopic allergies. Similarly, when hesperidin was incubated with human intestinal microflora, its main metabolite was hesperetin, The antiallergic activity of hesperidin and its metabolite hesperetin were investigated. Hesperidin did not inhibit the histamine release from RBL-2H3 cells induced by IgE. However, its metabolite hesperetin potently inhibited the histamine release from RBL-2H3 cells

induced by IgE and the PCA reaction. The inhibitory activity of hesperetin was found to be comparable to that of commercially available antiallergic drug, and to potently inhibit prostaglandin E2 production in lipopolysaccharide-stimulated RAW 264.7 cells. Hesperetin weakly inhibits cyclooxygenase 2 enzyme activities. These results suggest that hesperidin may be a prodrug, which is metabolized to hesperetin by intestinal bacteria.

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V-IV-3-d Joint Inflammation

Polymethoxylated flavonoids provide antioxidant protection and suppress inflammation (Manthey et al (2001). Several flavonoids, including tangeretin, sinensetin, and nobiletin, have been shown to suppress PGE-2 and matrix metalloproteinase production in joint cells, suggesting a chondroprotective effect of citrus. More specifically, the flavonoid nobiletin has been identified as a novel anti-inflammatory agent that has the potential to inhibit the degradation of articular cartilage in osteoarthritis and rheumatoid arthritis (Ibrahim et al, 1990).
Quercitin is very effective at inhibiting expression of the inflammatory cytokines tumor necrosis factor-alpha and IL-10 (Cho et al, 2003). Nobiletin has also been found to interfere with numerous inflammatory cytokines, including interleukin-1 beta and interleukin-6(Liu and Kubes,2003)). The authors assumed that the anti-inflammatory effects are comparable to those seen with powerful anti-inflammatory steroids such as dexamethasone. Tangeretin, another citrus flavonoid, has been found to offer complementary effects against inflammation (Datla, 2001).

V-IV-3-e Antiestrogenic properties of flavonoids

Some flavones and flavonols (apigenin, kaempferol, and naringenin) act through estrogen-receptor mediated mechanisms (Adlercruetz et al. 1993) and have been shown to have antiestrogenic effects similar to those of the isoflavones in breast cancer cell cultures (Miksicek,1995).

V-IV-3-f Neuroprotective effect of flavonoids

Neuroprotective effects of tangeretin was examined in the 6-hydroxydopamine lesion rat model of Parkinson's disease . Following the chronic oral administration significant levels of tangeretin were detected in the hypothalamus, striatum and hippocampus (3.88, 2.36 and 2.00 ng/mg, respectively). (Juergenliemk, 2003. Dakta et al,2001).

V-IV-3-g Conclusion

Flavonoids may possess specific properties that could benefit human health. However, in vivo and in vitro data have produced conflicting results. Many of these opposite results may be in part due to the lack of knowledge of dose-effect response of flavonoids, particularly what amounts of flavonoids could be beneficial or become harmful. Bioavailibilty and distribution of metabolites in many cases have not clarified. The data from epidemiological studies regarding flavonoids in human health have generated opposite results. Recent clinical studies using synthetic flavonoids showed the need to

proceed with caution in using flavonoid supplements, since little is known of effects at high doses. Watjen et al.(2005) showed that high level of flavonoids can induce DNA breaks and a prooxidant effect of high quercitin levels was reported by Choi et al.(2003). Citrus juices, on the other hand, contain several flavonoids at levels that have been shown to be positive for various aspects of human health..

V-V Carotenoids

Carotenoids are a group of fat-soluble pigments. Carotenoids have been shown to have antioxidant activity in the human body (Rock, 1997;Russell, 1998; Palace,1999, Herceberg, 2005). Carotenoids "quench" singlet oxygen primarily by a physical mechanism, in which the excess energy of singlet oxygen is transferred to the carotenoid's electron-rich structure (Krinsky,1998). Because this is a physical mechanism the carotenoid structure is unchanged, and the carotenoid remains intact to protect against further singlet oxygen. Some carotenoids are converted into vitamin A and are known as "provitamin A" carotenoids. The most common are beta-carotene and alpha-carotene (Garcia-Closas, 1999; Dixon, 1994,1996,1998).

V-V-1 Beneficial effects of carotenoids

V-V-1-a Antioxidant properties of carotenoids

Due to their antioxidant effects, it has been suggested that certain carotenoids might protect against atherosclerosis by preventing oxidative damage to serum cholesterol (Kritchevsky,1999). Simin et al,2001). However, carotenoids are much less effective in protecting against damage to serum cholesterol than is vitamin E. The antioxidant activity of carotenoids may help protect against macular degeneration and cataracts and may protect against heart disease. Carotenoids from dietary sources have been shown to have a beneficial effect against heart disease. Because of their antioxidant activity, it has been suggested that beta-carotene and other carotenoids might protect against atherosclerosis by preventing oxidative damage to serum cholesterol (Hughes,1999,2000;Simin et al,2001).

V-V-1-b Carotenoids, immunity and cancer

Research shows that carotenoids can help strengthen the immune system. Carotenoids have been shown to provide anti-inflammatory benefits, which can strengthen the immune system (Bendich,1999).

Preliminary studies suggest that a diet rich in carotenoids offers protection against certain forms of cancer by reducing oxidative or free radical damage(Nishino, 1998; Cooper et al,1999; Slattery 2000;Tonolio,2001). However, studies have shown that supplementation with isolated synthetic beta-carotene does not reduce the risk of lung cancer and may even increase that risk in smokers. (Gerster,1993, Cooper,1999; Hughes, 1997,1999). Recently, Pattison et al (2005) showed that orange juice B-cryptoxanthin may reduced the risk of inflammatory disorder such as rheumatoid arthritis.

V-V-1-c Conclusion

In conclusion, dietary carotenoids have been shown to have beneficial health properties. However, some studies suggest that isolated carotenoids may not have positive health effects, particularly as procancer compounds(Paolini et al.,1999). It is not absolutely clear if the health benefits of foods rich in carotenoids are due to this class of compounds or other elements present in the food.

V-VI Limonoids

Limonoids are oxygenated triterpenes having a 4,4,8 trimethyl - 17 furanyl steroid skeleton present in Rutaceae (Waterman and Grundon, 1983, Berhow et al 2000). The citrus limonoids occur as limonoid aglycones and limonoid glucosides (Paul,1995,2000). Thirty limonoid aglycones and 17 limonoid glucosides have been isolated from citrus and its closely related genera. Nomilin is most likely the initial precursor of all the known limonoids in citrus.. Limonoid aglycones are converted to non-bitter 17ß-D-glucopyraonoside derivatives such as limonin 17ß-D-glucopyranoside during maturation. Limonoid glucosides are major secondary metabolites and accumulate in the fruit tissues and seeds in significant quantities.

As seen previously phytochemicals to be effective much reach the body's various organs. Limonin glucoside is the most abundant of the limonoid glucosides in citrus and it bioavailiblity was examined by Manners et al (2003). Limonin glucoside was administered to four groups of human subjects in concentrations ranging from 4 to 30 times the level of total limonoid glucosides present in orange juice. Blood samples were analyzed from the presence of limonin, the limonoid produced through the removal of the glucose from limonin glucoside. Analysis results showed the presence of limonin in the blood, with the greatest amount occurring six hours after consumption.

V-VI-1 Beneficial effects of limonoids

V-VI-1-a Antioxidant activity

Limonoids have been shown to have antioxidant properties. The oxidant capacity of citrus was reviewed by ChongDe et al. (2005) and Yu et al. (2005). This activity is maximal in limonoid aglycone when the fruits are unripe (Paul,1995,2000; Berhow,2000). But the limonoid glucosides which form when the fruit matures have also been shown to possess antioxidant activity but not as high as the aglycons. Four limonoid glucosides, limoin 17ß D-glucopypranoside (LG), obacunone 17ß D-glucopyranoside (OG), nomilinic acid 17ß D-glucopyranoside (NAG), and deacetylnomilinic acid 17ß D-glucopyranoside (DNAG) were tested for superoxide radical (O_2–)-quenching activity. All 4 scavenged O_2– as measured by inhibition of pyrogallol decomposition in a spectrophotometric assay. Quenching by NAG in particular was similar to equivalent concentrations of vitamin C.

V-VI-1-b Antiproliferation activity and cancer

The effects of limonoid specific form of cancer have been reviewed in several studies (Miller, 2004; Poulose,2005). Limonin, nomilin, 12,hydroxyamdorastatin, and isofraxinellone are limonoids or their derivatives that have been shown successful in treatments with in vitro bioassays on human tumor cell lines. These limonoids have been found to induce increased activity of the detoxifying enzyme

glutathione-S- transferase. The increased enzyme activity was correlated with the ability of these compounds to inhibit chemically induced carcinogenisis in laboratory animals. Administration of nomilin to mice reduced the incidence and number of forestomach tumors induced by benzo pyrene (BP), a potent epoxide former.. Addition of nomilin to the diet at various concentrations inhibited BP induced mice lung tumor formation. This was attributed to the limonoid's inhibition of the formation of BP-DNA adducts in the lung.
The apoptotic effect of limonoids against human breast cancer cells was studied using flow cytometry. Dose-dependant inhibition effects of limonoids against six human cancer cell lines were observed. Limonoid mixtures exhibited a higher inhibition effect against human breast cancer cells than individual limonoids at the same concentration level, indicating that a synergetic or additive effect may be present. Apoptosis was the primary cause of cell death (Tanaka, 2000;Miller, 2004) . The authors concluded that citrus limonoid glucosides are toxic to SH-SY5Y cancer cells. Cytotoxicity is exerted through apoptosis by an as yet unknown mechanism of induction. Individual limonoid glucosides differ in efficacy as anticancer agents, and this difference may reside from structural variations (Tian,2001).

Limonoids have been shown to inhibit the growth of estrogen receptor-negative and -positive human breast cancer cells in culture. The antiproliferative activity of limonoids (obacunone 17 beta-D-glucopyranoside, nomilinic acid 17 beta-D-glucopyranoside, limonin, nomilin, and a limonoid glucoside mixture) was tested against a series of human cancer cell lines. The human cancer cell lines included leukemia (HL-60), ovary (SKOV-3), cervix (HeLa), stomach (NCI-SNU-1), liver (Hep G2), and breast (MCF-7). The growth-inhibitory effects of the four limonoids and the limonoid glucoside mixture against MCF-7 cells were significant, and the antiproliferative activity of the different citrus limonoids was also dose and time dependent. (Tanaka et al, 2000)

V-VII Folic acid

Folic acid, is one of the most abundant vitamin in citrus. Dietary folic acid is a mixture of polyglutamates. Folates are initially deconjugated in the cells of the intestinal wall to the monoglutamate form. Folic acid deficiency has been implicated in a wide variety of disorders from Alzheimer's disease to atherosclerosis, (Vermeulen et al,2000; Rimm et al, 1998) heart attack (Verhoef et al, 1996), stroke, osteoporosis, cervical and colon cancer, depression, dementia, cleft lip and palate, hearing loss, and of course, neural tube defects (Gross et al. 2000; Brent et al, 2000, Brouser et al,2000).

When all these results are examined, it can be summarized that folic acid is mainly involved with two main biochemical precesses, nucleotide synthesis and metabolism of the essential amino acid methionine and its associated transmethylation reactions

V-VII-1 Folic acid and nucleic acid synthesis

Folic acid is involved in the synthesis of adenine and thymine, two of the four nucleic acids that make up our genes, DNA and chromosomes. Folic acid participates in DNA synthesis and therefore folate deficiency may lead to increased uracil incorporation in DNA. Increased uracil incorporation in DNA can lead to disruption of the integrity of DNA. Folate deficiency may also result in diminished DNA

repair A central feature of fetal development is widespread and sustained cell division. Folate plays a central role in the formation of nucleic acid precursors, such as thymidylic acid and purine nucleotides,. which they play an important role in the synthesis of purines and pyrimidine bases, in the synthesis of transfer RNA. The requirement for folate increases during times of rapid tissue growth being essential for rapid cell division. from fetal development to sperm genesis. A major importance has been given to the role of folic acid in preventing spinal neural tube defects (spina bifida). Since folate plays a central role in the formation of nucleic acid precursors for DNA and RNA, it is likely that this effect of folic acid is due to its role in nucleic acid synthesis. Increasing folate tissue concentrations may promote the production of nucleic acids and proteins at the time of neural tube closure. It should however also be mentioned that folic acid may not prevent the occurrence of neural tube defects, but may instead selectively increase the abortion rate of affected fetuses (Gross et al. 2000; Brent et al, 2000, Brouser et al,2000, Wong et al 2002).It should also be mentioned that folic acid has been reported as having a positive effect on spermogenesis (Wong et al. 2002, Ebisch et al.2003).

V-VII-2 Folic acid and cardiovascular diseases

A folic acid deficiency has been clearly linked to an elevated level of homocysteine, a sulfur-containing amino acid. High homocysteine levels, in turn, have been linked to cardiovascular disease and other diseases (Moghadasian et al, 1997; McCully et al, 1998; van Oort et al, 2003).
Hyperhomocysteinemia is associated with cardiovascular disease, cerebrovascular disease and carotid artery stenosis in adults. Folic acid can lower homocysteine blood levels by converting homocysteine to methionine. A high intake of folate has been associated with a lower risk of coronary events. There is evidence that hyperhomocysteinemia is a risk factor for coronary heart disease independent of other known risk factors (hypercholesterolemia, hypertension, diabetes, smoking). Folic acid can lower homocysteine blood levels, but the mechanism by which hyperhomocysteinemia might increase the risk of vascular disease is unclear. Homocysteine may promote atherogenesis through endothelial dysfunction and oxidative stress. Elevated homocysteine levels may result in increased oxidation of low-density lipoprotein

VI-Discussion

VI-I Citrus antioxidant and inflammation (vitamin C, flavonoids, carotenes and limonoids)

The excess of oxidants of environmental or physiological origin is at the root of many inflammation ailments. Fruits and vegetables and particularly citrus, contain large amounts of antioxidants which have the potential to limit many of the inflammation processes. Despite extensive research in the area of antioxidants many questions have not been fully clarified.

VI-I-1 Measurement of antioxidant potential

Significant discrepancy exits between the reported antioxidant potentials of various fruits. This is in large part due to the lack of standardization between the many methods used to assess the antioxidant potentials. There is a need to compare methods and establish objective standards for the determination of antioxidant concentrations. It should also be pointed out that most studies are done in vitro and may not reflect the in vivo situation. The activity of an antioxidant reaching an organ may be very different from that measured in the compound in the original food, since in most cases it is a metabolite and not the starting molecule that affects a specific target.

VI-I-2 Relative antioxidant power of vitamin C, polyphenols (flavonoids) and other citrus compounds

Flavonoids have specific antioxidant properties that set them apart from molecules like vitamin C. Polyphenols have much more stable structures than vitamin C. Once oxidized the resulting free radical is stable and will no longer interact with body molecules. Several reports, mainly of in vitro, studies have shown that phenolics are better antioxidants than vitamin C and that even in orange juice, vitamin C provides only 15% of the juice's antioxidant potential. The superiority of polyphenolics has been extensively used to promote many juices. It is not clear from the literature if these differences are physiological or due to methodological artifacts or if they depend on specific conditions. It would be of interest to clarify the discrepancy between the antioxidant potential of vitamin C and flavonoids. Additional research is needed to assess the relative antioxidant values of each class of citrus compounds.

VI-I-3 Antioxidant bioavailiblity

The bioavailibility of flavonoids has not been completely elucidated. In plants, flavonoids are composed of a molecule made of several rings called polyphenols attached to various sugars. Most studies show that only the portion without sugars (aglycone) is significantly absorbed in the intestine. Flavonoids are significantly degraded by the upper or lower intestine flora which release the aglycone. Depending on the microbial modifications, the intestinal absorption can be greatly modified. Some studies have shown that among some of the fruits with a high in-vitro antioxidant activity, only a very percentage is able to penetrate the body and therefore the physiological activity may be much less than expected. Once they cross the intestine, flavonoids have to penetrate the organ where they may act. For example the access to the brain is very limited by the blood brain barrier. Often, antioxidants are chemically modified particularly in the kidneys and the liver and new metabolites are generated. Only some of these metabolites that may reach a specific target. The effective in-vivo antioxidant of a food is that of the final metabolite penetrates an organ or a cell and many of these physiological processes are unknown.

VI-1-4 Dual role of ascorbic acid

The effectiveness of ascorbic acid needs also to be clarified. In the body ascorbic acid has a dual activity:

1) **It acts as a vitamin (vitamin C):** Both ascorbic acid and its oxidized form dehydroascorbic acid (DHAA)acid have vitamin C properties. Vitamin C is a coenzyme for various enzymes and particularly hydroxylases involved in collagen formation and maintenance and in some immune activities.

2) **It acts an antioxidant**: As mentioned above, ascorbic acid is an antioxidant, but DHAA is a prooxidant since the oxidized form needs to oxidize another molecule to go back to ascorbic acid. In the body this is done with the help of glutathione. The question of DHAA formation in citrus juice may need to be examined.
This also raises the question of the needed amount of ascorbic acid. The FDA requirements for vitamin C represent the amount needed to provide vitamin activity and prevent scurvy. However, the amounts of ascorbic acid needed to provide antioxidant protection have not been determined but are much larger than the quantities presently recommended.

VI-I-5 Synergistic effect of antioxidants

Citrus and citrus juices contain a large array of different antioxidant compounds including vitamin C, flavonoids, carotenes and limonoids. Among these, vitamin C and flavonoids are present in the largest amounts. Studies have shown that antioxidants can have a synergistic effect and are much more powerful when acting together than separately. This has been shown in two different areas.

VI-I-5-a Antioxidant activity of juices and fruits as compares with individual antioxidants

Reports have shown that whole citrus juices are more effective antioxidants than each component ingested separately. Orange juice is more effective than flavonoids which in turn are more effective than vitamin C. Since citrus contain various types of antioxidants, they can be expected to provide a significant protection against the different kind of oxidative species. Such synergistic effect may exist between vitamin C and some flavonoids. Bioflavonoids and vitamin C appear to work synergistically to maintain blood capillary health and prevent capillary fragility. For example, there is some *in vitro* evidence that ascorbic acid acts synergistically with the flavonoid quercetin to protect cutaneous tissue cells against oxidative damage induced by glutathione deficiency.

VI-I-5-b Synergistic antioxidant activity of citrus juice taken with other foods

This co-antioxidant activity has been shown to exist between compounds originating from various types of food. This was originally discovered with vitamin C which was shown to be much more effective in preventing fat oxidation (one of the main causes of CVD) when acting in conjunction with vitamin E (tocopherol). A recent study has shown a 250 fold increase in antioxidant potential of almond skins taken with vitamin C as compared with each taken separately. Such synergistic action could be promoted with meals containing citrus products and other foods
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VI-II Citrus antioxidants and the restoration of the oxidative balance

As mentioned previously, it has been estimated that up to 2% of the consumed energy may be diverted toward the production of oxidants. This occurs in a few specific physiological steps when energy is produced and used. The consumption of citrus during these oxidative "bursts" should help neutralize some of the toxic free radicals. Furthermore many inflammatory steps generate oxidants. Some of the areas which are the most promising in reestablishing the "oxidative balance"will be reviewed.

VI-II-1 Neutralizing the postprandial oxidative burst

The first of these steps is associated with the process of breaking up food into its elemental components (sugars, amino acids, fatty acids), after a meal. It has been called the postprandial oxidative burst. Citrus juices and fruits ingested with or after a meal may help neutralize the excess of oxidants produced during that time.

VI-II-2 Neutralizing the exercise oxidative burst

During intensive exercise, the demand for energy rises quickly and our glucose reserves (glycogen) are used and if necessary fat is burned. To remain healthy it is important to be able to provide antioxidants when energy generating oxidation increases. Citrus juices and fruits rich in antioxidants may help neutralize some of the oxidative species generated during exercise. At the same time, other citrus components could also be beneficial. Exercise is certainly a time where providing some sugar would be useful in providing an extra boost of energy. Potassium in the juices could also replenish minerals lost during strenuous efforts. Citrus juices could be promoted as exercise drinks providing energy, minerals, and antioxidants.

VI-II-3 Slowing the aging process

Many believe that oxidants are one of the main causes of aging. We enter a spiral where more oxidants are produced as the body become less able to neutralize them. Providing extra antioxidants with citrus may help slow the aging process although this would require extensive research, since the reasons for the age related deterioration of the oxidative balances are unclear.

VI-II-4 Improving brain handling of oxidants

The brain is one of the main targets of the aging process. The brain constantly needs large amounts of energy, but it is protected from the rest of the body by the blood brain barrier, which prevents many molecules from crossing from the rest of the body. The condition of the brain is even more delicate since central nervous system (CNS) neurons cannot regenerate and die if deprived of oxygen and glucose. Accumulation of oxidants may be one of the main causes of brain inflammation leading to many cerebral ailments such as Alzheimer's and Parkinson's diseases**.** Providing more antioxidants may prevent or slowdown some of these problems and there are reports that several citrus antioxidant flavonoids particularly tangeretin and nobeletin and some hesperedin metabolites can cross the blood brain barrier.

VI-II-5 Limiting psychologically induced inflammation (stress, depression)

Psychological stress and depression have been shown to be associated with a rise in the inflammation of the central nervous system (CNS) and the production of inflammation markers. Citrus compounds may play a role in limiting psychological diseases. This effect could lead to the recommendation that citrus should be ingested during periods of heavy stress. This is however somewhat speculative at the present.

VI-II-6 Preventing blood lipid oxidation (LDL and plaque)

Lipid oxidation is a major component of the inflammation process particularly in the area of cardiovascular diseases.
The formation of plaque in the blood vessels is promoted by the oxidation of LDL which are deposited in the arteries and leads to atherosclerosis (artery wall hardening), and blood vessel narrowing preventing blood flow and generating blood clots. Many CVD diseases can result from these problems including stroke, high blood pressure, infarctus and embolism. A significant number of studies have already shown that many compounds present in citrus such as vitamin C, tangeretin and nobeletin can slow blood lipid oxidation and could play a role in limiting CVDs by preventing lipid oxidation and the subsequent plaque formation.

VI-III Citrus ability to decrease inflammation markers

Inflammation has been linked to an extremely broad spectrum of diseases, and the monitoring of inflammation markers is rapidly becoming the method of choice in determining the progression and the intensity of many inflammatory diseases and particularly CVDs. Screening for markers will very soon be as widely used as measuring cholesterol levels is now. This is an extremely important area that has reached the medical community and is now spreading to the general public. Patients are now concerned with their LDL and HDL levels and in the next few years will be asking physicians to follow inflammation markers and particularly the CRP. Unfortunately, so far only one study linking citrus juices and these markers have been found. Showing citrus compounds can decrease inflammation markers would be of great importance in establishing and proving the importance of citrus in promoting health. Inflammation is an area of medical research that is growing rapidly and is already used for diagnostics. At present many other inflammation markers are being studied. It seems likely that in the next few years the use of markers will become more sophisticated and several diseases may be linked to specific markers.
High blood level of the amino acid homocysteine has also been implicated with CVD . Rather than being a result of inflammation like most markers, homocysteine is a cause of inflammation. Studies have shown that homocysteine is cytotoxic and can damage many cells and organs from the cardiovascular system to the kidneys. It seems likely that homocyteinemia has several origins, one is a genetic disorder and another one may be due to a lack of absorption of folic acid, vitamin B6 and B12 from food. In most cases folic acid can limit problems related to the accumulation of homocysteine and orange juice is a good source of folic acid.

VI-IV Citrus and other inflammation induced diseases

VI-IV -1 Diabetes:

The ingestion of high amounts of fructose, from drinks rich in high fructose corn syrup, is very detrimental and promotes insulin resistance and some CVDs. However, studies have shown that if fructose represents less than 10% of the total carbohydrates, it improves liver glucose management even in the presence of insulin resistance. The presence of small amounts of fructose in meals help regulate glucose uptake and glycogen synthesis. Citrus contain glucose and fructose in identical amounts and if ingested during meals rich in starch, that is broken down into glucose, they should help maintain the proper glucose metabolism**.** The question of citrus juices and sugars is of major importance as it is linked to the glycemic index controversy.

VI-IV-2 Blood Pressure:

Citrus antioxidants act on blood pressure by preventing lipid oxidation and atherosclerosis but citrus juices with its high potassium content may favor the ability of the cellular Na/K pumps to remove excess sodium since these cellular pumps expel three sodium ions from cells for two potassium ions they take in. This is another area where citrus juices could play a role in lowering blood pressure.

The hormone angiotensin II which increases blood pressure can also generate inflammation compounds and oxidative species. An effect of citrus compounds in decreasing inflammation in the kidney could be possible.

VI-IV-3 Cancer:

Both citrus antioxidants and folic acid can prevent DNA damage and may play a role in preventing mutation induced cancers. There are many reports of the anti-carcinogenic potential of flavonoids and limonoids. This research is done mainly with in vitro culture of cancer cells and the extrapolation of the results for human benefit is difficult to explain. Of interest are recent reports on the possible beneficial effect of grapefruit juice on tobacco induced cancers. The level of the liver enzyme CYP1A2 is very high in smokers. This enzyme transforms some tobacco compounds into carcinogens. Two new studies have shown the ability of grapefruit juice to decrease the liver level of CYP1A2 in smokers. It has been suggested that this decrease in CYP1A2 could have a positive effect on tobacco induced cancer by decreasing the formation of carcinogens. It may also be possible to study if grapefruit juice could decrease CYP1A2 in people living in areas with high air pollution. .A role of folic acid in protecting DNA could be important as it may minimize DNA errors that start cancers.

VI-IV-4 Immunity:

Citrus and particularly vitamin C can boost the production of T cells and raise the immunity level of the body. Citrus have been shown to help against the flu, but the data are not always extremely

convincing. This is one of the best perceived beneficial effects of citrus at least to the general public. Of particular interest is a recent study that has shown that orange juice may prevent rheumatoid inflammation

VI-V- Some vitamin potentials

VI-V-1 Vitamin C:

One of the most well known properties of vitamin C is its beneficial effect on collagen formation. As we age, wrinkles are largely due to the decrease of the quality of the skin collagen. This property could be used to promote the prevention of skin degradation with age.

VI-V-2 Folic acid:

Folic acid has been shown be able to prevent up to 75% of neural tube defects although the role of folic acid in CNS fetal development is not clear. It has been suggested that this was due to the ability of folic acid to boost DNA synthesis. Indeed, there seems to be proofs that folate promotes the formation of nucleic acid precursors for DNA and RNA and prevents the split of DNA strands. That could account for the beneficial effects of folic acid during periods of active cellular multiplication such as fetal development and spermatogenesis.

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