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Research Article

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# Factors Affecting Vitamin C Absorption Using Rat Everted Gut Sac Model

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## ABSTRACT

Vitamin C (L-ascorbic acid), is water-soluble vitamin that is naturally present. Humans and guinea pig are unable to synthesize ascorbic acid endogenously, due to series of inactivating mutations of the gene encoding gulonolactone oxidase (GULO); this enzyme is responsible for vitamin C synthesis. Ascorbic acid is a potent reducing agent, it has two major functions as an antioxidant and as an enzyme cofactor.

The study aimed to study different physiological factors as pH and temperature that may affect the absorption of ascorbic acid in rat ileum; also the effect of nicotine on ascorbic acid absorption was studied. Everted gut sac model was adopted throughout all the work. Each treated group is repeated for five times.

It is concluded that, ascorbic acid ideal absorption is at pH 7 (normal physiological condition). At acidic and alkaline media ascorbic acid absorption is decreased. The highest absorption level of ascorbic acid was at 37°C (normal physiological condition); while the absorption is decreased when the temperature increase (39°C) or decrease (35°C). Nicotine reduces the absorption of ascorbic acid significantly ($P \leq 0.05$).

***Key words-*** *Rat ileum; Vitamin C; PH; Temperature; Nicotine.*

## INTRODUCTION

Vitamin C is a water-soluble vitamin that is naturally present in some foods, and available as a dietary supplement. Humans, unlike most animals, are unable to synthesize vitamin C endogenously, so it is an essential dietary component.[1] Though most animals are able to synthesize large quantities of vitamin C endogenously, humans lost this capability as a result of a series of inactivating mutations of the gene encoding gulonolactone oxidase (GULO)[2] a key enzyme in the vitamin C biosynthetic pathway.[2,3]

Vitamin C is a potent reducing agent, it readily donates electrons to recipient molecules. Related to this oxidation-reduction (redox) potential, two major functions of vitamin C are as an antioxidant and as an enzyme cofactor.[4,5] Vitamin C is easily oxidized to form dehydroascorbic acid (DHAA), and thus oxidation is readily reversible.[1,6] The presence of glutathione in cells and extracellular fluids helps maintain ascorbate in a reduced state.[7]

Vitamin C is required for the biosynthesis of collagen, L-carnitine, and certain neurotransmitters; it is also involved in protein metabolism.[1,6] Collagen is an essential component of connective tissue, which plays a vital role in wound healing. Vitamin C is also an important physiological antioxidant.[8] Under physiological conditions, it functions as a potent reducing agent that efficiently quenches potentially damaging free radicals produced by normal metabolic respiration of the body.[9]

Vitamin C can protect indispensable molecules in the body, such as proteins, lipids, carbohydrates, and nucleic acids (DNA and RNA), from damage by free radicals and reactive oxygen species (ROS) that are generated during normal metabolism, by active immune cells, and through exposure to toxins and pollutants (e.g., certain chemotherapy drugs and cigarette smoke). Vitamin C also participates in redox recycling of other important antioxidants; for example, vitamin C is known to regenerate vitamin E from its oxidized form.[6,10]

Research suggests that vitamin C is involved in the metabolism of cholesterol to bile acids, which may have implications for blood cholesterol levels and the incidence of gallstones.[11] Vitamin C increases the bioavailability of iron from foods by enhancing intestinal absorption of non-heme iron.[4] Vitamin C plays an important role in immune function.[12]

Plasma vitamin C concentration is tightly controlled by three primary mechanisms: intestinal absorption, tissue transport, and renal reabsorption.[13] While plasma vitamin C concentration reflects recent dietary intake, leukocyte vitamin C is thought to be more closely reflect tissue stores. However, a recent randomized controlled trial (RCT) demonstrated that human skeletal muscle, a major body pool for vitamin C, is highly labile and more responsive to vitamin C intake than neutrophils or mononuclear cells.[14] Thus, leukocyte vitamin C concentration does not accurately reflect skeletal muscle ascorbic acid, and may underestimate muscle tissue vitamin C uptake. However, plasma concentrations of vitamin C ≥50 micromoles/L are sufficient to saturate muscle tissue vitamin C.[15]

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Fruits and vegetables are the best sources of vitamin C.[16] Other sources include red and green peppers, kiwifruit, broccoli, strawberries, brussels sprouts, and cantaloupe.[16,17] Although vitamin C is not naturally present in grains, it is added to some fortified breakfast cereals. Vitamin C content of food may be reduced by prolonged storage and by cooking because vitamin C is water soluble and is destroyed by heat.[17,18]

As a polar compound with a relatively large molecular weight, vitamin C cannot readily cross the cell membrane by simple diffusion. The flux of vitamin C in and out of the cell is controlled by specific mechanisms, including facilitated diffusion and active transport, which are mediated by distinct classes of membrane proteins such as facilitative glucose transporters (GLUT) and sodium vitamin C co-transporters (SVCT), respectively.[1,19]

***Facilitated diffusion through glucose transporters*:** Gradient-driven transport of the oxidized form of vitamin C, dehydroascorbic acid (DHA), is mediated by a class of facilitative GLUT, which has no detectable affinity for the reduced, biologically-active forms such as ascorbic acid and ascorbate.[20] The reduced vitamin C, DHA, can be indirectly imported by three-step mechanism involving:1) extracellular oxidization of ascorbate to DHA; 2) transport of DHA by the GLUT transporter and 3) intracellular reduction of DHA to ascorbate.

Sharing the same transporters as glucose, GLUT-mediated transport of DHA is competitively inhibited by glucose.[20-24] This raises the possibility that changes in serum glucose levels, especially those occurring during disease, may attenuate the bioavailability of vitamin C leading to secondary pathologies due to the depletion of circulating vitamin C. Indeed, this characteristic type of secondary pathology has been observed under hyperglycemic conditions caused by diabetes[21,25] and may be treated, at least partially, by clinical administration of vitamin C. In addition to glucose inhibition, the GLUT transporters are also subject to hormonal control. In the presence of both follicle-stimulating hormone and insulin-like growth factor I, the expression of GLUT 1 is upregulated in granulosa cells.[26] Another dehydroascorbic acid (DHA) transporter, GLUT4, was identified.[20] GLUT4 expression in cells is stimulated by addition of insulin.[27]

***Active transport by sodium vitamin C co-transporters*:** In addition to the facilitated mechanism, vitamin C is also transported by active SVCT, which transport ascorbate directly into the cell. Sodium vitamin C co-transporters ( SVCT) have higher affinity for ascorbate than do GLUT for DHA and thus are considered high-affinity vitamin C transporters.[28] The sodium vitamin C co-transporters ( SVCT) system transports ascorbate at the expense of the sodium electrochemical gradient across the cell membrane and, as such, are classified as secondary active transporters.[26]

***Aim of the work***

The aim of this work is to study the different physiological factors as pH and temperature that may affect the absorption of ascorbic acid in rat ileum; also the effect of nicotine on ascorbic acid absorption was studied. Everted gut sac model was adopted throughout all the work.

## MATERIALS AND METHODS

***Materials***

Sodium chloride, magnesium chloride, potassium chloride were purchased from Carlo Erba, SPA. Calcium chloride 2-hydrat, Sodium hydrogen carbonate, Glucose, were purchased from Riedel-De-Haen AG, Franc. Sodium dihydrogen orthophosphate dehydrate was purchased from Fisher Scientific, UK. Potassium dihydrogen orthophosphate was purchased from BDH Analar, BDH Limited Poole, England. Sodium monohydrogen phosphate was purchased from Park Scientific Limited, Northampton, U.K. Sodium oxalate was purchased from BDH Limited Poole England. Sodium hydroxide GRG (pellets) was purchased from WINLAB, UK. Orthophosphoric acid was purchased from T-Baker Lab chemicals, INDIA. Nicotine was purchased from BDH chemical Ltd Poole England. Vitamin C was purchased from Pharmaceutical Industries Society (PIS), Ben- arous Tunisia.

***Instruments***

Spectrophotometer designed and manufactured in U.K. by Bibby Scientific Ltd. Dunmow Essex CM6 3LB, at wave length 270 nm; quartz cell was used. Shaking water bath from Gesellschaft fur Labortechnik MBH, Germany. Electric balance was Mettler Toledo United States. Ultrasonics Telsonic TPC-15 Frauenfeld, Switzerland.

***Methodology***

*Everted gut sac*: Adult male albino rats (300-350g weight) were starved for 24h, killed by cervical dislocation and the entire small intestine quickly excised and flushed for several times with tyrode[29] at room temperature. The intestine was immediately placed in oxygenated air tyrode. 5 cm of ileum was slid onto a glass rod (2.5 mm diameter) and fastened with silk suture; the ileum was then gently everted over the rod and the everted ileum slid into fresh oxygenated tyrode. One end of the ileum was clamped and the whole length of the ileum was filled with 2 ml fresh oxygenated tyrode physiological solution. The ileum was then sealed with a second clamp.

Each sac was then placed in 100 ml volume beaker containing 20 ml of tyrode. Tyrode's solution (20 ml) with 20 mg Vitamin C to have 1 mg/ml vitamin C concentration was prepared; the beakers were shaken in a shaking water bath for homogeneity. At the appropriate times points (5, 15, 30, 60 minutes), sacs were removed, washed four times in tyrode and blotted dry. The sacs were cut open and the fluid content drained into small tubes.[30] After a certain time, 0.1 ml of sac content is added to 9.9 ml of buffer (pH 5.7)[31], followed by measuring the absorption of each vitamin C concentration by spectrophotometer.

*Acidic and alkaline media*: Luminal pH 6.5-7.5 in the distal ileum.[32] Orthophosphoric acid was used to adjust tyrode's pH into 7. Acidic media (pH 6) was prepared by addition Orthophosphoric acid drop by drop to tyrode solution until reach pH 6. Alkaline media (pH8 and pH 9) was prepared by adding 1% NaOH drop by drop till the required pH is obtained. pH meter is used to adjust the required pH.

*Temperature control*: The ideal core temperature is considered to be around 37°C; any temperature above or below this temperature is considered abnormal.[33] Shaking temperature control water bath is used to adjust the required temperature.

*Nicotine*: Nicotine was prepared by mixing 400 mg of nicotine in 100 ml tyrode to prepare 4 mg/ml nicotine solution.

***Evaluation of vitamin C by Spectrophotometer***: Vitamin C concentration was evaluated in sac fluid content using spectrophotometer.[34]

Different concentration of vitamin C (0, 0.25, 0.5 and 1 mg/ml) were prepared for dose-response curve in buffer (PH 5.7). Calibration of spectrophotometer was carried out using 0 mg/ml vitamin C. Absorption of each vitamin C concentration was measured at wave length 270 nm.

## RESULTS

***Effect of different pH on the absorption of vitamin C.***

Vitamin C absorbed was significantly increased by time at all the pHs at $P \leq 0.05$. Absorption of vitamin C was increased significantly at pH 7 compared to pH 6, pH 9 after 5, 15 and 30 minutes. There was insignificant decrease ($P = 0.058$) in vitamin C absorbed at pH 9 compared to pH 7 after 60 minutes (Table 1). After 60 minutes, vitamin C levels absorbed were significantly higher at pH 7 than vitamin C levels absorbed at pH 6. The absorbed vitamin C levels at pH 7 after 5, 15, and 30 minutes did not show any significant difference with that absorbed at pH 8 of the same times ($P > 0.05$); while vitamin C levels absorbed was significantly increased after 60 minute at pH 7 compared to pH 8 after 60 minutes ($P = 0.034$) (Table 1).

It could be concluded that the total amount of vitamin C absorbed after 60 minute at pH 6 was 0.50±0.053mg/ml (100%); out of this vitamin C concentration 22% is absorbed after 5 minutes, while 46% of vitamin C was absorbed after 15 minutes. 72% of the total vitamin C was absorbed after 30 minutes; in the last 30 minutes, 28% of vitamin C was absorbed (Figure 1).

The amount of vitamin C absorbed after 60 minute at pH 7 was 0.86±0.011mg/ml (100%); out of this vitamin C concentration 26.7% is absorbed after 5 minutes, while 51.2% of vitamin C was absorbed after 15 minutes. 75.6% of the total vitamin C was absorbed after 30 minutes; in the last 30 minutes, 24.4% of vitamin C was absorbed (Figure 1).

The amount of vitamin C absorbed after 60 minute at PH8 was 0.65±0.070 mg/ml (100%); out of this vitamin C concentration 33.8% is absorbed after 5 minutes, while 55.4% of vitamin C was absorbed after 15 minutes. 80% of the total vitamin C was absorbed after 30 minutes; in the last 30 minutes, 20% of vitamin C was absorbed (Figure 1).

The amount of vitamin C absorbed after 60 minute at PH9 was 0.68±0.094 mg/ml (100); out of this vitamin C concentration 17.6% is absorbed after 5 minutes, while 44.1% of vitamin C was absorbed after 15 minutes. 66.2% of the total vitamin C was absorbed after 30 minutes; in the last 30 minutes, 33.8% of vitamin C was absorbed (Figure 1).

***Effect of different temperature on the absorption of vitamin C.***

Absorption of vitamin C significantly increased by time at temperature 35 and 37°C. The concentration of vitamin C absorbed at 39°C increased insignificantly after 15 minutes compared to 5 minutes; while the absorption was significantly increased after 30 and 60 minutes compared to the amount of vitamin C absorbed after 5 minutes. There was no changes in the levels of vitamin C absorbed after 15 compared to the levels after 30 minutes at 39°C; while the levels of vitamin C absorbed after 60 minutes at 39°C were significantly higher compared to the levels after 30 minutes of the same temperature ($P = 0.038$).

After 5 minutes, the levels of vitamin C absorbed were significantly higher at 37°C compared to 35 and 39°C. This relationship was repeated, significantly, after 15, 30 and 60 minutes (Table 2).

It could be conclude that the amount of vitamin C absorbed after 60 minute at temp. 35 was 0.35±0.015 mg/ml (100%); out of this vitamin C concentration 17.1% is absorbed after 5 minutes, while 42.9% of vitamin C was absorbed after 15 minutes. 68.6% of the total vitamin C was absorbed after 30 minutes; in the last 30 minutes, 31.4% of vitamin C was absorbed (Figure 2).

The amount of vitamin C absorbed after 60 minute at temp. 37 was 0.86±0.011mg/ml (100%); out of this vitamin C concentration 26.7% is absorbed after 5 minutes, while 51.2% of vitamin C was absorbed after 15 minutes. 75.6% of the total vitamin C was absorbed after 30 minutes; in the last 30 minutes, 24.4% of vitamin C was absorbed (Figure 2).

The amount of vitamin C absorbed after 60 minute at temp. 39 was 0.34±0.073mg/ml (100); out of this vitamin C concentration 14.7% is absorbed after 5 minutes, while 32.4% of vitamin C was absorbed after 15 minutes. 61.8% of the total vitamin C was absorbed after 30 minutes; in the last 30 minutes, 38.2% of vitamin C was absorbed (Figure 2).

***Effect of nicotine on vitamin C absorption***

In normal physiological conditions, the levels of vitamin C absorbed increased significantly; the increase in vitamin C absorbed was time dependent. Nicotine treated condition, vitamin C absorbed increased significantly by time, except between 15 and 30 minutes in which it did not show any difference (Table 3).

It could be conclude that the amount of vitamin C absorbed after 60 minute at normal was 0.86±0.011mg/ml (100%); out of this vitamin C concentration 26.7% is absorbed after 5 minutes, while 51.2% of vitamin C was absorbed after 15 minutes. 75.6% of the total vitamin C was absorbed after 30 minutes; in the last 30 minutes, 24.4% of vitamin C was absorbed (Figure 3).

The amount of vitamin C absorbed after 60 minute at nicotine was 0.33±0.042mg/ml (100); out of this vitamin C concentration 9.1 % is absorbed after 5 minutes, while 36.4% of vitamin C was absorbed after 15 minutes. 60.6% of the total vitamin C was absorbed after 30 minutes; in the last 30 minutes, 39.4% of vitamin C was absorbed (Figure 3).

**Table 1:** Effect of different pH on vitamin C absorption through isolated rat ileum

| Different pH | 5min | 15min | 30min | 60min |
|---|---|---|---|---|
| PH6 | 0.11±0.020 * | 0.23±0.020 *,a | 0.36±0.030 *,a,b | 0.50±0.053 *,a,b,c |
| PH7 | 0.23±0.046 | 0.44±0.020 a | 0.65±0.007 a,b | 0.86±0.011 a,b,c |
| PH8 | 0.22±0.060 | 0.36±0.044 | 0.52±0.102 a | 0.65±0.070 *,a,b |
| PH9 | 0.12±0.020 * | 0.30±0.049 *,a | 0.45±0.043 *,a | 0.68±0.094 a,b,c |

* Significantly different from the normal (pH 7) at $P \leq 0.05$; Vitamin C concentration (mg/ml); a, Significantly different from 5 minutes of the same pH at $P \leq 0.05$; b, significantly different from absorbed vitamin C levels after 15 minutes; c, significantly different from absorbed vitamin C levels after 30 minutes.

**Table 2:** Effect of different temperature on the absorption of vitamin C through isolated rat ileum

| VIT. C Absorption (mg/ml) | 5min | 15min | 30min | 60min |
|---|---|---|---|---|
| Temp.35 | 0.06±0.003 * | 0.15±0.003 *,a | 0.24±0.005 *a,b | 0.35±0.015 *a,b,c |
| Temp.37 | 0.23±0.046 | 0.44±0.020 a | 0.65±0.007 a,b, | 0.86±0.011 a,b,c |
| Temp.39 | 0.05±0.015 * | 0.11±0.014 * | 0.21±0.001 *a | 0.34±0.073 *a,b,c |

* Significantly different from the normal (37 C) per each time at $P \leq 0.05$; a, significantly different from absorbed vitamin C levels after 5 minutes; b, significantly different from absorbed vitamin C levels after 15 minutes; c, significantly different from absorbed vitamin C levels after 30 minute.

**Table 3:** Effect of nicotine on the absorption of vitamin C through isolated rat ileum

| VIT. C Absorption (mg/ml) | 5min | 15min | 30min | 60min |
|---|---|---|---|---|
| Normal | 0.23±0.046 | 0.44±0.020 a | 0.65±0.007 a,b | 0.86±0.011 a,b,c |
| Nicotine | 0.03±0.012 * | 0.12±0.032 *,a | 0.20±0.026 *,a | 0.33±0.042 *,a,b,c |

* Significantly different from the normal per each time at $P \leq 0.05$; a, significantly different from absorbed vitamin C levels after 5 minutes; b, significantly different from absorbed vitamin C levels after 15 minutes; c, significantly different from absorbed vitamin C levels after 30 minute.

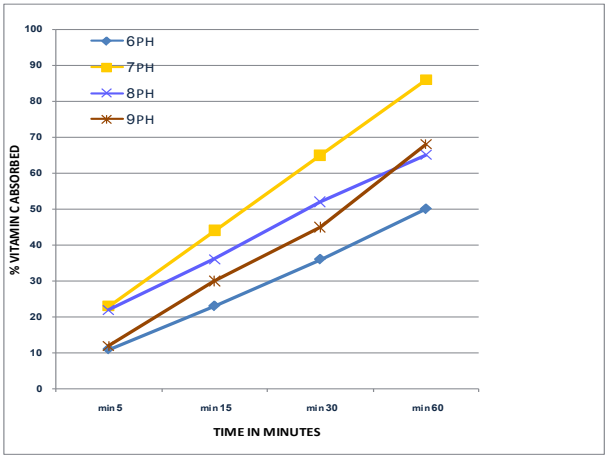


**Figure 1**: Effect of different pH on vitamin C absorption

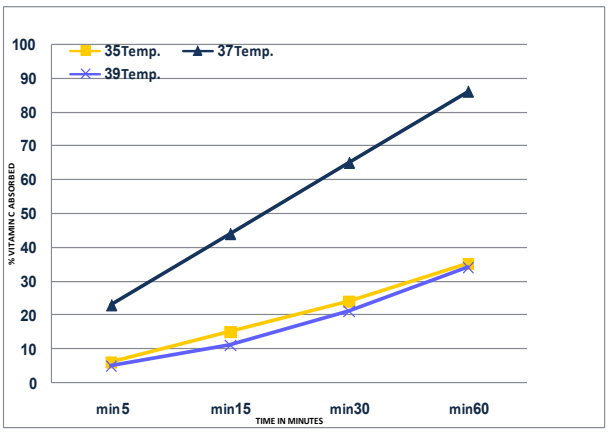


**Figure 2**: Effect of different temperature on the absorption of vitamin C

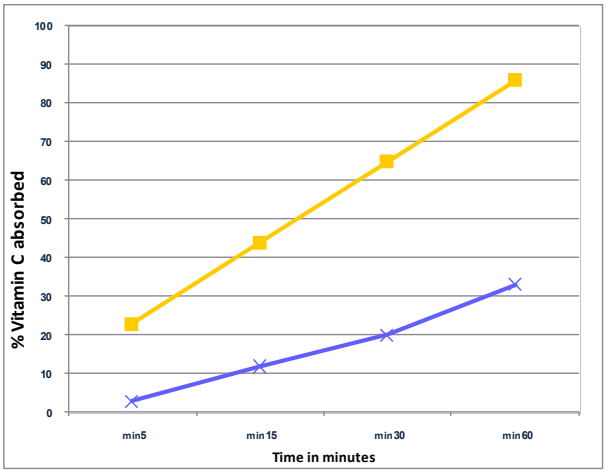


**Figure 3**: Effect of nicotine on vitamin C absorption

## DISCUSSION

***Effect of acidic pH on vitamin C absorption***

Luminal pH in the proximal small bowel ranges from 5.5 to 7.0 and gradually rises to 6.5-7.5 in the distal ileum.[32] Ascorbate was taken up along the entire length of the small intestine with a threefold higher initial uptake rate in distal than proximal segments; whereas dehydroascorbic acid (DHAA) uptake was higher in jejunal segments.[35]

The relatively low affinity of DHAA transport compared with ascorbate transport indicates that most vitamin C is absorbed as ascorbate. Both the inward- and the outward-directed, pH gradients inhibited ascorbate uptake but were ineffective on DHAA uptake.[35]

At low pH solutions, $H^+$ concentrations are high. The lower the pH, the more $H^+$ ions present; this will lead to degradation of ascorbic acid into dehydroascorbic acid. $H^+$ ions oxidizes vitamin C molecule by grabbing an electron from it. Vitamin C molecule is oxidized and becomes dehydroascorbic acid ; therefore the levels of vitamin C will be decreased in acidic media, if other factors of oxidation (such as temperature, light intensity, time) are kept constant.[36]

Vitamin C is more powerful antioxidant at low pH; meaning that vitamin C degrades (or is oxidized) more quickly at low pH medium. An increased acidity (lower pH), vitamin C is oxidized rapidly by the abundant $H^+$ ions.[37]

***Effect of alkaline pH on vitamin C absorption***

The weak base is absorbed at a faster rate from the intestine (pH 7.50 - 8), this is because the basic substances can not be ionized in basic medium. So the uncharged substances can be passed easily due to its lipid solubility. Similarly, weak acid is absorbed at a faster rate from stomach (pH 1.4 - 2).[38]

The intestinal absorption of acidic drugs is decreased several fold and the absorption of basic drugs increased several fold when the pH of the intestinal contents is raised from 4 to 8. This supports the hypothesis that the intestinal mucosa preferentially allows the absorption of the unionized form of a drug.[39]

Ascorbic acid is easily oxidized in alkaline pH and high temperature.[40] There is a sufficient association between absorption of drugs and the lipid: water partition coefficients of their unionized moieties to justify the continued assumption that the gastrointestinal cell membranes are essentially lipoidal. Highly lipid-soluble drugs are in general rapidly absorbed while decidedly lipid-insoluble drugs are in general poorly absorbed.[39]

Ascorbic acid absorption was decrease in alkaline media; this decrease could be explained that ascorbic acid being weak acid will present in ionized form in alkaline media in ionized state as this will decrease absorption.

***Effect of nicotine on vitamin C absorption***

Nicotine is a natural ingredient acting as a botanical insecticide in tobacco leaves. It is the principal tobacco alkaloid, occurring to the extent of about 1.5% by weight in commercial cigarette tobacco and comprising about 95% of the total alkaloid content. Oral snuff and pipe tobacco contain concentrations of nicotine similar to cigarette tobacco, whereas cigar and chewing tobacco have only about half the nicotine concentration of cigarette tobacco.[41] An average tobacco rod contains 10-14 mg of nicotine[42], and on average about 1-1.5 mg of nicotine is absorbed systemically during smoking.[43]

National Health and Nutrition Examination Survey (NHANES II) data confirms that cigarette smoking is associated with decreased serum vitamin C levels. Dietary vitamin C intakes were lower in smokers than non-smokers a finding which might potentially explain this inverse correlation). The lowered serum vitamin C levels in smokers could be due to either impaired vitamin C absorption or increased turnover.[44]

Pelletier[45,46] suggested the presence of impaired bioavailability but normal turnover of vitamin C in smokers. Vitamin C kinetics were measured using radio-labeled ascorbic acid. It demonstrated an increase in turnover in smokers but only small differences in absorption when compared to non-smokers.[47] Others have reported that smoking acutely increases urinary excretion of vitamin C[48], also suggesting an accelerated metabolism in smokers.[44]

The significance of the increased risk of hypovitaminosis C produced by the interaction between smoking and serum vitamin C levels is not currently known, a specific association of smoking with vitamin C intake is not well recognized. Vitamin C intake was lowest in individuals smoking 20 cigarettes or more daily, and that smokers who have stopped for greater than one year have intakes similar to individuals who have never smoked, suggest a specific aversive effect of smoking on vitamin C rich foods which may be reversible when smoking is discontinued.[44]

Absorption of nicotine across biological membranes depends on pH. Nicotine is a weak base with a pKa of 8.0. In its ionized state, such as in acidic environments, nicotine does not rapidly cross membranes. The PH of smoke from flue-cured tobaccos, found in most cigarettes, is acidic (pH 5.5-6.0); at this pH, nicotine is primarily ionized.[41] As a consequence, there is little buccal absorption of nicotine from flue-cured tobacco smoke, even when it is held in the mouth.[49] Smoke from air-cured tobaccos, the predominant tobacco used in pipes, cigars, and some European cigarettes, is more alkaline (pH 6.5 or higher) and, considerable nicotine is unionized.[41] Smoke from these products is well absorbed through the mouth.[50] The alkaline pH facilitates absorption of nicotine through mucous membranes.[51]

It has been proposed that the pH of cigarette smoke manufactory is higher; therefore a larger portion of nicotine would be in unionized form; this will facilitate rapid pulmonary absorption.[52]

When tobacco smoke reaches the small airways and alveoli of the lung, nicotine is rapidly absorbed. Blood concentrations of nicotine rise quickly during a smoke and peak at the completion of smoking. The rapid absorption of nicotine from cigarette smoke through the lungs, presumably because of the huge surface area of the alveoli and small airways, and dissolution of nicotine in the fluid of pH 7.4 in the human lung facilitate transfer across membranes.[41] After a puff, high levels of nicotine reach the brain in 10-20s, faster than with intravenous administration, producing rapid behavioral reinforcement.[53] Nicotine meets all criteria of a highly addictive drug. While it is the case that initial tobacco use often escalates to compulsive use accompanied by tolerance and physical dependence. The rapidity of rise in nicotine levels permits the smoker to titrate the level of nicotine and related effects during smoking, and makes smoking the most reinforcing and dependence-producing form of nicotine administration.[54]

It appears that nicotine *N*-oxide (nicotine metabolite) is not further metabolized to any significant extent, except by reduction back to nicotine in the intestines, which may lead to recycling nicotine in the body.[41]

In acidic urine, nicotine is mostly ionized and tubular reabsorption is minimized; renal clearance may be as high as 600 ml $min^{-1}$ (urinary pH 4.4), depending on urinary flow rate.[55] In alkaline urine, a larger fraction of nicotine is unionized, allowing net tubular reabsorption with a renal clearance as low as 17 ml $min^{-1}$ (urine pH 7.0).[41]

Ascorbate oxidase is present in young and growing tissues of tobacco. Ascorbate oxidase is a multi-copper enzyme that catalyzes the oxidation of ascorbic acid to dehydroascorbic acid.[56]

It is suggested that the absorption of vitamin C (weak acid) in ileum will be decreased in smokers due to presence of nicotine (weak base)[41]; where vitamin C will be ionized and less absorbed. In addition, in smokers, ascorbate oxidase enzyme, which is present in tobacco will catalyze (oxidation) of ascorbic acid to dehydroascorbic acid.[56]

***Effect of high temperature on vitamin C absorption***

Vitamin C is very easily decomposed by increases in temperature, ascorbic acid will be oxidized to become L-dehydroascorbic acid. This is hard to prevent in foodstuff processing of which contains vitamin C, such as vegetables and fruits.[57]

It is readily oxidized, particularly in the presence of copper and iron but not of aluminum. This vitamin is also rapidly destroyed by alkalis but is fairly stable in weak acid solutions. Steam cooking destroys very little amount of ascorbic acid.[58]

The oxidation of vitamin C occurs very quickly in a base environment at high temperatures. Light and heat damage vitamins B and C in fruits and vegetables.[57]

Ascorbate oxidase, which is present in the mitochondria[59], has been proposed to be the major enzyme responsible for enzymatic degradation of ascorbic acid.[40] Ascorbate oxidase is a copper-containing enzyme that oxidizes ascorbic acid to DHA in the presence of molecular oxygen.[60]

Higher temperatures increase the oxidation of reduced ascorbate, due to intensified ascorbate peroxidase (APX) and ascorbate oxidase (AO) activities and a depression of the enzyme dehydroascorbate reductase (DHAR).[61]

Ascorbic acid is easily oxidized, especially in aqueous solutions, and greatly favored by the presence of oxygen, heavy metal ions, especially $Cu^2$, Ag, and $Fe^3$, and by alkaline PH and high temperature.[40]

A very important reaction in regard to vitamin C degradation leading to DHAA that does not have biological activity as vitamin C.[62] The excess amount of heat can destroy Vitamin C completely.[63] High temperature effects on vitamin C content of fruits; blanching in hot water can cause an appreciable loss in vitamin C that is thermally labile. Ascorbic acid oxidase needs to be inactivated; this prevents enzyme-catalyzed reaction during processing. Heat and water reduce vitamin C content, cooking reduce the vitamin C content in fruit juice because vitamin C content is sensitive to heat, water, and air.[58] Vitamin C is first leached out of the fruit into the water, and then degraded by the heat.[63,64]

At high temperature, in the presence of sunlight and oxygen in air, vitamin C will be oxidized.[65] For high retention of vitamin C while cooking, it is recommended that the vegetables are cooked in low heat and small amounts of water for short periods to minimize the loss of vitamin C. Heating time has significant effect on the vitamin C content of all the vegetables, as the heating time increases, the percentage loss of vitamin C increases too. Vitamin C is easily destroyed by excessive heat and water, as well as exposure to air. For retention of vitamin C in cooked foods, it is recommended that foods containing vitamin C be cooked as fast as possible with less heat and small amount of water.[63]

Oxidation can occur in the presence of catalysts, oxidase enzymes, or as a result of heat during processing. Loss of vitamin C is enhanced by extended storage, higher temperatures, low relative humidity, physical damage, and chilling injury.[40]

Dehydroascorbic acid (DHAA) can be reduced to ascorbic acid (AA) by reducing agents and also can be irreversibly oxidized to form diketogulonic acid, which has no vitamin C activity.[66] In general the extent of loss in ascorbic acid (AA) content in response to elevated temperatures was greater in vegetables than in acidic fruits, such as citrus, because ascorbic acid (AA) is more stable under acidic condition.[67,68] Citrus juices in unopened bottles and cans contain high AA content and a low level of DHA, whereas those juices exposed to air and stored at warm temperatures contain higher levels of DHA and diketogulonic acid.[40]

Cooking in high temperature is also a cause for the destruction of vitamin C therefore food containing vitamin C should be cooked on low temperature.[63] Areas with cool nights produce fruits with higher vitamin C levels. Hot tropical areas produce fruit with lower levels of vitamin C.[68]

***Effect of low temperature on vitamin C absorption***

It was found that storage of orange juice at low temperature destroys vitamin C to some extent, and sterilization may destroy it completely.[69] This agree with our finding that ascorbic acid is decreased with lower temperature (□ 37°C).

## CONCLUSION

The results show that, the ascorbic acid ideal absorption is at pH

7 (normal physiological condition). At acidic or alkaline media ascorbic acid absorption is decreased. The highest absorption level of ascorbic acid is at 7°C (normal physiological condition); while the absorption is decreased when the temperature increases or decreases. Nicotine reduces the absorption of ascorbic acid significantly.

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