Ascorbic acid or vitamin C is involved in a number of biochemical pathways that are important to exercise metabolism and the health of exercising individuals. This review reports the results of studies investigating the requirement for vitamin C with exercise on the basis of dietary vitamin C intakes, the response to supplementation and alterations in plasma, serum, and leukocyte ascorbic acid concentration following both acute exercise and regular training. The possible physiological significance of changes in ascorbic acid with exercise is also addressed. Exercise generally causes a transient increase in circulating ascorbic acid in the hours following exercise, but a decline below pre-exercise levels occurs in the days after prolonged exercise. These changes could be associated with increased exercise-induced oxidative stress. On the basis of alterations in the concentration of ascorbic acid within the blood, it remains unclear if regular exercise increases the metabolism of vitamin C. However, the similar dietary intakes and responses to supplementation between athletes and nonathletes suggest that regular exercise does not increase the requirement for vitamin C in athletes. Two novel hypotheses are put forward to explain recent findings of attenuated levels of cortisol postexercise following supplementation with high doses of vitamin C.

**Key Words:** ascorbic acid, antioxidant, cortisol, adrenal gland, exercise, oxidative stress, gluconeogenesis

**Introduction**

Ascorbic acid, or vitamin C, is a six-carbon compound similar in structure to glucose. It exists in two active forms: the reduced form known as ascorbic acid and the oxidized form, dehydroascorbic acid. The molecular structure contains two ionizable enolic hydrogen atoms that give the compound its acidic character (Figure 1; 54). Ascorbic acid is considered to be an outstanding antioxidant. Chemically, ascorbic acid exhibits redox characteristics as a reducing agent. Physiologically, ascorbic acid provides electrons for enzymes, for chemical compounds that are oxidants, or other electron acceptors (91). The intermediate free radical formed in
the oxidation of ascorbic acid to dehydroascorbic acid is relatively non-reactive, particularly with oxygen (14). Furthermore, dehydroascorbic acid is reduced by cells back to ascorbate, which can then be reutilized (107).

Ascorbic acid is distributed in varying concentrations throughout the body and is involved in a variety of metabolic reactions relating to exercise, such as the synthesis and activation of neuropeptides, collagen, carnitine, and protection against the harmful effects of reactive oxidant species (79). In light of this involvement, it is conceivable that exercise may enhance the utilization, metabolism, and excretion of ascorbic acid, thereby increasing the dietary requirement for the vitamin. The first evidence of a possible link between vitamin C and exercise came rather unexpectedly from the observation of scurvy symptoms exhibited by explorers on early polar expeditions (78). The diets consumed by these explorers were likely to be seriously lacking in fresh foods containing vitamin C. However, it is also plausible that such a stressful existence could have exacerbated the effects of a vitamin C–deficient diet in such a way that the turnover and requirement for ascorbic acid may have increased. While vitamin C does not appear to improve athletic performance (39), athletes may have increased dietary vitamin C requirements, and supplementation may have important effects on athletes that are more subtle than improvements in aerobic capacity or performance measures (30).

The purpose of this review is to: (a) discuss the role of ascorbic acid in exercise metabolism, (b) summarize the results of studies examining dietary intakes of vitamin C and the responses to supplementation, and (c) address exercise-induced alterations in the distribution of ascorbic acid within the body, with reference to the mechanisms and physiological significance of such changes.

The Distribution, Transport, Function, and Metabolism of Ascorbic Acid

The distribution of ascorbic acid within the human body is outlined in Table 1. Exercise studies have been limited to examining changes in ascorbic acid concentrations within plasma/serum and leukocytes. Saturated plasma ascorbic acid concentrations are in the range of 70 to 80 μmol/L, whereas saturated lymphocyte, monocyte, and neutrophil ascorbic acid concentrations occur at 3.4, 3.2, and 1.3 μmol/L, respectively (64). Leukocytes store ascorbate against very high concentration gradients (43), probably for protection against intracellular reactive oxidants (91). Ascorbic acid and its oxidized form, dehydroascorbic acid, are transported independently. Whereas dehydroascorbic acid is transported into cells by GLUT 1
and GLUT 3, the transport protein for ascorbic acid is yet to be identified. Substrate availability is likely to be critical in determining which molecule (i.e., ascorbic acid or dehydroascorbic acid) is transported at any particular time. Under non-oxidizing conditions, ascorbate is present in greater amounts and is therefore more likely to be transported. In contrast, when oxidants are present, ascorbate is oxidized, resulting in the transient formation of dehydroascorbic acid (91). Now available to surrounding cells, dehydroascorbic acid is preferentially transported for intracellular reduction (91) either by enzymatic reduction (80) or glutathione (109). This recycling of ascorbic acid is likely to be important under conditions of oxidative stress (91).

The redox properties of ascorbic acid (Figure 1) make it an effective reducing agent in several important enzymatic reactions within the body, all of which require that enzyme-bound metal ions are maintained in their reduced state (79). In the adrenal gland ascorbic acid is stored in high concentrations where it is utilized by the enzyme dopamine β-monooxygenase within chromaffin granules to synthesize noradrenaline (Figure 2A; 24). In the pituitary gland and the brain, ascorbic acid is involved with the enzyme peptidyl-glycine α-amidating monooxygenase (72) for the activation of a variety of neuropeptides and hormone releasing-factors (Figure 2D; 79). The vitamin is required by the prolyl- and lysyl-hydroxylase enzymes for the hydroxylation of proline and lysine residues, respectively, in the production of collagen (Figure 2B; 12), and also by the enzymes 6-N-trimethyllysine hydroxylase and γ-butyrobetaine hydroxylase in carnitine synthesis (Figure 2C; 74). This latter role could possibly account for the ascorbic acid content of both cardiac and skeletal muscle (74). In the liver, ascorbic acid is possibly involved in glucose (11), cholesterol, and lipid metabolism (54). A variety of immune functions are enhanced by ascorbic acid (2), which could explain the concentration of the vitamin in the spleen.

<table>
<thead>
<tr>
<th>Organ/fluid</th>
<th>Ascorbic acid concentration (mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pituitary gland</td>
<td>40–50</td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>30–40</td>
</tr>
<tr>
<td>Eye lens</td>
<td>25–31</td>
</tr>
<tr>
<td>Liver</td>
<td>10–16</td>
</tr>
<tr>
<td>Pancreas</td>
<td>10–15</td>
</tr>
<tr>
<td>Spleen</td>
<td>10–15</td>
</tr>
<tr>
<td>Kidney</td>
<td>5–15</td>
</tr>
<tr>
<td>Cardiac muscle</td>
<td>5–15</td>
</tr>
<tr>
<td>Brain</td>
<td>3–15</td>
</tr>
<tr>
<td>Lungs</td>
<td>7</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>3–4</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.4–1</td>
</tr>
</tbody>
</table>
It is currently unclear exactly how ascorbic acid influences pancreatic or renal function. It has been suggested that high doses of vitamin C might influence insulin secretion and blood glucose concentrations (8, 10), possibly through competition between dehydroascorbic acid and glucose for GLUT 1 and GLUT 3 transporters (92). However, this possible regulatory influence seems unlikely to account for the levels of ascorbic acid found within these tissues. It is the antioxidant activity of ascorbic acid which is important in many tissues such as the lungs (68), the eye (23), the cardiovascular system (67), and plasma (37). This antioxidant activity is discussed in more detail in a later section.

In light of these exercise-related metabolic requirements for ascorbic acid, it is plausible that periods of regular intense exercise, musculoskeletal growth, and repair may promote the need for vitamin C in the diets of athletes. However, owing to obvious limitations inherent to the biopsy of internal tissue in humans, any direct assessment of the actual utilization of ascorbic acid in these reactions as a result of either acute or chronic exercise is difficult. Given that ascorbic acid is an antioxidant in plasma (37), changes in the plasma content of ascorbate may provide indirect evidence of its utilization in neutralizing reactive oxidant species; nevertheless, no studies of exercise-induced oxidative stress have reported changes in the circulating levels of ascorbic acid derivatives, such as dehydroascorbic acid, which would offer some indication of the metabolism of ascorbic acid itself. Two studies compared the urinary output of ascorbic acid between sedentary individuals and athletes and found no difference (32, 89). Hence, although there is in vitro evidence that ascorbic acid is likely utilized in several exercise-related metabolic pathways, measurements of ascorbic acid concentrations in blood and urine do not reflect its consumption within these reactions.

An alternative approach to determining the physiological requirement for ascorbic acid has been to compare the dietary intake of vitamin C between athletes
and nonathletes, in addition to assessing the response of athletes to vitamin C supplementation. If athletes exhibit low plasma/serum ascorbic acid concentrations pre-supplementation, then it may be anticipated that these values would increase upon the addition of extra vitamin C within the diet.

**Vitamin C Status**

The vitamin C status of individuals has been assessed using several different methods. Dietary intakes of ascorbic acid have been used for the general assessment and prediction of vitamin C status. The correlation between plasma/serum ascorbic acid concentration and dietary ascorbic acid intake is only modest \((r = 0.32–0.55; 89)\). One group failed to find any relationship (44). The normal ranges for plasma and leukocyte ascorbic acid concentrations are listed in Table 2. There has been some debate as to which component of whole blood provides the most reliable index of vitamin C status and whether whole blood does in fact reflect the true tissue stores of the vitamin (18, 29, 42, 53, 65). In two studies, the responses to supplementation with vitamin C were similar between plasma and leukocytes (9, 55). Furthermore, plasma and leukocyte ascorbic acid concentrations are correlated with each other (15). In view of the inherent difficulties associated with measuring the ascorbic acid content of internal organs in humans, a combination of measurement of dietary intakes, plasma/serum, and leukocyte ascorbic acid concentrations appears to be the best available indication of vitamin C status.

**Vitamin C Intakes of Exercising and Non-exercising Individuals**

The recommended daily allowance for vitamin C varies among countries. In Great Britain and Canada, it is 30 mg, in New Zealand and Australia it is 40 mg, in the United States it is 60 mg, whereas in Germany it is 75 mg (108). Vitamin C consumption is related to total energy intake (17). Some investigators have assessed the dietary vitamin C intake of athletes on the basis of the recommended daily allowance (25, 75, 96, 106). With the exception of a small number of athletes in two studies (25, 96), the majority of data on vitamin C intakes in athletes have indicated that most athletes are receiving adequate amounts of vitamin C in their diets. Mean

<table>
<thead>
<tr>
<th>Body pool (mg)</th>
<th>Plasma µmol/L (mg/dL)</th>
<th>Mixed leucocytes µg/10⁶ cells (nmol/10⁸ cells)</th>
<th>Mononuclear leucocytes µg/10⁶ cells (nmol/10⁸ cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>500–1500 (10–22 mg/kg)</td>
<td>23–84 (0.4–1.5)</td>
<td>114–301 (20–53)</td>
<td>142–250 (25–44)</td>
</tr>
</tbody>
</table>
intakes in athletes are reported to range from 90 to 140 mg per day. Intakes were even higher in those athletes taking vitamin C supplements, which in one study was found to be the most commonly used supplement among a large number of athletes (34). Others have compared the intakes of exercising individuals with those of sedentary controls and have generally observed higher intakes within the athletic population (33, 34, 44, 81, 89).

Several studies have examined vitamin C intakes in athletes from a variety of sports (25, 44, 89, 96). The results from a 24-h dietary recall suggested wide variation among sports, with some indication of seasonal variation, but no consistent trends emerged (96). Low intakes have been observed in male gymnasts and wrestlers (96), and also in female athletes (25). In contrast to these findings, the results from another study indicated very little difference in 1- or 7-day vitamin C intakes among athletes from different sports, including wrestlers (96). Lower intakes in some athletes may reflect dietary interventions directed at weight control in these athletes, as it has been demonstrated that vitamin C intakes are related to total energy intakes (34). Therefore, the amount of vitamin C in the diets of most athletes appears to be sufficient, based on the recommended daily allowance. Another approach to measuring vitamin C status has been to examine the response of plasma or serum ascorbic acid concentrations to supplementation with varying doses of vitamin C.

**Supplementation Studies**

Athletes have been given vitamin C supplements ranging from 85 to 1500 mg vitamin C, for periods of 1 day up to 8 months (5, 44, 58, 69, 76, 81, 83, 98, 106). The effects of supplementation on both resting and exercise-induced changes in ascorbic acid concentration have been investigated (Table 3). There was little or no effect of supplementation on plasma or serum ascorbic acid concentrations in several studies (48, 58, 95, 98), whereas others have demonstrated increases in the concentration of ascorbic acid in blood following supplementation (5, 44, 69, 90, 106). However, there are several issues to consider when interpreting these results.

The dose of vitamin C in the supplement, the regular dietary intakes of vitamin C, and the concentration of ascorbic acid within plasma/serum and leukocytes prior to supplementation would all appear to be important determinants of the response to supplementation. Plasma ascorbic acid concentrations in one study plateaued at vitamin C intakes of 250 mg and above per day, whereas the ascorbic acid content of neutrophil, monocytes, and lymphocytes plateaued at lower intakes of 200 mg per day (64). In another study, the optimal intake for absorption capacity was reported to be 3 mg vitamin C per kilogram of body weight per day (89). The athletes in many of the studies in Table 3 were receiving ≥ 200 mg of vitamin C per day in total. Therefore, plasma/serum levels were likely close to saturation. Himmelstein et al. (48) have reported that the athletes in their study had higher total dietary intakes of vitamin C than sedentary individuals and consequently showed a smaller response to supplementation. Similarly, Thompson et al. (101) observed a modest but significant increase in plasma ascorbic acid concentration but no change in the ascorbic acid of lymphocytes following 2 weeks of supplementation with 400 mg vitamin C per day. The mean (±SD) dietary intake of vitamin C prior to supplementation was 147 (±70) mg per day, and this level of intake was offered in explanation for the lack of change in lymphocyte ascorbic acid content with supplementation.
Table 3  Mean (±SD) Changes in Ascorbic Acid Concentration Following Dietary Supplementation Including Vitamin C

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Supplement</th>
<th>Supplementation period</th>
<th>Dietary intake (mg)</th>
<th>Blood compartment</th>
<th>Pre-supplement</th>
<th>Post-supplement</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Runners (n = 30)</td>
<td>85-mg placebo</td>
<td>3 months</td>
<td>108.7 (± 56.6)</td>
<td>Whole blood</td>
<td>ND</td>
<td>76.0 (± 17.0) *</td>
<td>(106)</td>
</tr>
<tr>
<td>Athletes (n = 55)</td>
<td>200 mg</td>
<td>1 month</td>
<td>94.9 (± 17.5)</td>
<td>Serum</td>
<td>73.3 (± 28.9)</td>
<td>101.0 (± 22.2) †</td>
<td>(44)</td>
</tr>
<tr>
<td>Inactive (n = 20)</td>
<td>87.8 (± 50.6)</td>
<td>ND</td>
<td>ND</td>
<td>Plasma</td>
<td>ND</td>
<td>55.7 (± 22.6) a</td>
<td>(98)</td>
</tr>
<tr>
<td>Athletes (n = 86)</td>
<td>550-mg placebo</td>
<td>7–8 months</td>
<td>ND</td>
<td>Plasma</td>
<td>ND</td>
<td>56.2 (± 18.1)</td>
<td></td>
</tr>
<tr>
<td>Inactive (n = 8)</td>
<td>400-mg placebo</td>
<td>3 weeks</td>
<td>ND</td>
<td>Serum</td>
<td>76.3 (± 34.8)</td>
<td>112.5 (± 36.3) *</td>
<td>(69)</td>
</tr>
<tr>
<td>Basketballers (n = 16)</td>
<td>250-mg placebo</td>
<td>1 month</td>
<td>201 (± 205) mg 320 (± 257) mg</td>
<td>Serum</td>
<td>49.1 (± 22.3)</td>
<td>47.0 (± 45.0)</td>
<td>(95)</td>
</tr>
<tr>
<td>Runners (n = 10)</td>
<td>500-mg placebo</td>
<td>2 weeks</td>
<td>ND</td>
<td>Plasma</td>
<td>40.0 (± 21.4 )</td>
<td>66.0 (± 29.5) *</td>
<td>(84)</td>
</tr>
<tr>
<td>Runners (n = 16)</td>
<td>200-mg placebo</td>
<td>4.5 weeks</td>
<td>ND</td>
<td>Serum</td>
<td>50.0 (± 53.0)</td>
<td>69.8 (± 80.0) *</td>
<td>(90)</td>
</tr>
<tr>
<td>Runners (n = 41)</td>
<td>1000-mg placebo</td>
<td>2 months</td>
<td>442 (± 457) mg</td>
<td>Plasma</td>
<td>78 (± 13)</td>
<td>80 (± 10)</td>
<td>(48)</td>
</tr>
<tr>
<td>Runners (n = 30)</td>
<td>1000-mg placebo</td>
<td>2 months</td>
<td>378 (± 518) mg</td>
<td>Plasma</td>
<td>83 (± 10)</td>
<td>80 (± 10)</td>
<td></td>
</tr>
<tr>
<td>Inactive (n = 29)</td>
<td>1000-mg placebo</td>
<td>2 months</td>
<td>312 (± 360) mg</td>
<td>Plasma</td>
<td>62 (± 22)</td>
<td>72 (± 16)</td>
<td></td>
</tr>
<tr>
<td>Inactive (n = 35)</td>
<td>1000-mg placebo</td>
<td>2 months</td>
<td>227 (± 226) mg</td>
<td>Plasma</td>
<td>51 (± 19)</td>
<td>52 (± 14)</td>
<td></td>
</tr>
</tbody>
</table>

Note. ND = no data available. *Significantly different compared to placebo (p < .01); †significant increase (p < .05); ‡significant increase (p = .005). aPlacebo group used as comparison for effect of supplementation; bathletes from a variety of sports.
Pre-supplementation levels of plasma/serum ascorbic also seem to affect the response to supplementation. In one study (106), the athletes were seemingly randomly assigned to either a supplement or placebo group with no attempt to match the groups for pre-supplementation whole blood ascorbic acid concentrations. Considering also that these pre-supplementation values were not reported, the true efficacy of supplementation in this study is difficult to evaluate. Among the studies listed in Table 3, serum ascorbic acid concentration increased by 40–50% in those individuals with serum levels in the middle of the normal range (44, 69, 90). A much more dramatic increase was reported among individuals with plasma ascorbic acid concentrations below the normal range; administration of an acute 500-mg dose of vitamin C increased plasma ascorbic acid significantly from 26.3 (±18.2) to 117 (±28.3) µmol/L (5). This latter result may indicate that athletes with low circulating levels of ascorbic acid are more likely to exhibit a greater response to supplementation. Alternatively, it has been suggested that there may be an initial transient increase in plasma ascorbic acid followed by a decrease during prolonged supplementation (98). However, data from Levine et al. (64) indicate that with a 60-mg dose of vitamin C, plasma levels of ascorbic acid were saturated after 3 weeks and remained stable thereafter. Hence, although no data are available on the saturation of cellular stores of ascorbic acid, based on the plasma response, a minimum period of 3 weeks of vitamin C supplementation may be necessary for the saturation of cellular stores of ascorbic acid.

In several of the studies listed in Table 3, the participants were given a combination of vitamins and minerals, which may have influenced the absorption of ascorbic acid and, consequently, the response to supplementation (98). The assessment of the effectiveness of supplementation is also likely to be influenced by the type of vitamin C supplement and the timing of supplementation and blood sampling. In the study by Levine et al. (64), intravenous injection of vitamin C predictably caused a very rapid increase in plasma ascorbic acid concentration when compared to oral ingestion of the same dose. Furthermore, the appearance of ascorbic acid in plasma occurred at a two-fold faster rate following the oral ingestion of 1250 mg compared to 250 mg vitamin C. Plasma ascorbic acid concentrations also returned to baseline twice as rapidly following the higher dose. Following the 200-mg dose, plasma ascorbic acid remained elevated up to 10 hours after ingestion (64). In contrast, Alessio et al. (1) found no difference in the effects of supplementation with 1000 mg for either 1 day or 2 weeks on postexercise changes in thiobarbituric acid reactive substances or oxygen radical absorbance capacity. These factors should be borne in mind when evaluating the alterations in blood measures following supplementation.

Several groups have attempted to address the issue of ascorbic acid requirements of athletes and have reported conflicting findings. One group observed that athletes had intakes of vitamin C that were, on average, around 40% higher than the controls (34). Together with the finding that plasma ascorbic acid concentrations were in fact similar between the athletes and controls, this difference in intakes would suggest that either athletes do have increased requirements for ascorbic acid, and/or they retain more. In contrast, in the study by Guilland et al. (44), although athletes demonstrated a marked increase in serum ascorbic acid concentration after supplementation, this increase was of similar magnitude to that of sedentary controls.
The dietary intakes were also similar between the two groups. The fact that the athletes did not exhibit a greater response to supplementation is suggestive that regular exercise does not actually increase the requirement for ascorbic acid. Nevertheless, it is reasonable to contend that through its utilization in metabolic pathways such as carnitine, noradrenaline, and collagen synthesis and antioxidant reactions, athletes may require more vitamin C than sedentary individuals. At similar dietary intakes, athletes may use a greater proportion of the vitamin C consumed in their diet than nonathletes. Nonathletes may not need all of the vitamin C they consume and may excrete the excess. In such a way, athletes would actually have increased requirements. However, two studies of the ascorbic acid content in urine revealed no difference between athletes and sedentary controls (32, 89).

In conclusion, the majority of existing data from dietary and supplementation studies do not support the concept that athletes have increased requirements for vitamin C for the following reasons. First, the dietary intake of athletes and sedentary controls is similar, as is the response to supplementation with vitamin C. Second, there does not appear to be a strong association between dietary intakes of vitamin C and the concentration of ascorbic acid within the blood. Last, the excretion of ascorbic acid in urine—which may be taken as an assessment of the utilization of vitamin C within the body—does not differ between athletes and nonathletes.

### Changes in Plasma, Serum, and Lymphocyte Ascorbic Acid Concentrations Following Acute Exercise

Transient alterations in the concentration of ascorbic acid in plasma and lymphocytes have been reported in plasma and lymphocytes following acute exercise. These changes are summarized in Table 4. Some studies have observed a rise of variable magnitude in circulating ascorbic acid levels following exercise (28, 41, 42, 69, 84, 90), some have found no change (40, 66, 76, 82, 83, 101, 104), while others have reported a reduction (16). Meydani et al. (70) observed that after 45 min of downhill running, when the increase in plasma ascorbic acid was corrected for changes in plasma volume, no significant change was seen. Several studies have also demonstrated significant increases in lymphocyte ascorbic acid concentration (41, 42, 100, 101).

It is worthy of note that in several of the studies listed in Table 4, plasma ascorbic acid concentrations in the days following exercise were actually below baseline (42, 66, 76, 83, 90). In the study by Gleeson et al. (42) post-race haemodilution accounted for the reduced plasma ascorbic acid concentration at days 2 and 3; however, it could not account for the significant decrement seen after 24 hours. This decrement could possibly reflect alterations in the antioxidant demands of tissues in the days following exercise. In the study by Liu et al. (66), commensurate with reduced plasma ascorbic acid concentrations 4 days postexercise, there was also a significant diminution of trapping antioxidant capacity of plasma and a significant increase in the susceptibility of low-density lipoprotein (LDL) to oxidation. Muscle damage resulting from strenuous exercise such as a marathon is likely to cause local inflammatory reactions (97), and phagocytes involved with tissue damage are a source of reactive oxidants (47). Although ascorbic acid is a water-soluble antioxidant...
Table 4 Mean (±SD) Changes in Ascorbic Acid Concentration Immediately Post-exercise

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Activity</th>
<th>Blood compartment</th>
<th>Pre-exercise</th>
<th>Post-exercise</th>
<th>Corrected for HC</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untrained (n = 24)</td>
<td>60 min box stepping</td>
<td>Serum</td>
<td>53.3 (± 12.4)</td>
<td>59.6 (± 21.2)</td>
<td>√</td>
<td>(69)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>112.5 (± 20.9)</td>
<td>140.4 (± 20.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>99.9 (± 46.4)</td>
<td>110.4 (± 56.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untrained (n = 8)</td>
<td>35 min uphill (+5%) walking at 60% VO₂max</td>
<td>Plasma</td>
<td>21.0 (± 2.27)</td>
<td>19.3 (± 2.27)</td>
<td>√</td>
<td>(16)</td>
</tr>
<tr>
<td></td>
<td>35 min downhill (–20%) running at 60% VO₂max</td>
<td></td>
<td>18.2 (± 2.95)</td>
<td>10.8 (± 2.27)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclists (n = 11)</td>
<td>90 min at 65% VO₂max</td>
<td>Plasma</td>
<td>41.1 (± 25.2)</td>
<td>48.1 (± 29.5)</td>
<td>√</td>
<td>(104)</td>
</tr>
<tr>
<td>Triathletes (n = 39)</td>
<td>Ironman triathlon</td>
<td>Plasma</td>
<td>96.0 (± 26.7)</td>
<td>95.4 (± 23.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>98.9 (± 35.8)</td>
<td>94.8 (± 35.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Runners (n = 8)</td>
<td>Marathon</td>
<td>Plasma</td>
<td>54.7 (± 26.6)</td>
<td>79.0 (± 34.7)</td>
<td>√</td>
<td>(41)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lymphocytes</td>
<td>17.4 (± 3.96)</td>
<td>21.3 (± 7.35)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Runners (n = 9)</td>
<td>Half-marathon</td>
<td>Plasma</td>
<td>52.7 (± 12.3)</td>
<td>67.0 (± 15.9)</td>
<td>√</td>
<td>(42)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lymphocytes</td>
<td>15.6 (± 1.80)</td>
<td>19.9 (± 2.70)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Runners (n = 7)</td>
<td>Half-marathon</td>
<td>Plasma</td>
<td>38.4 (± 15.3)</td>
<td>51.5 (± 26.2)</td>
<td>√</td>
<td>(28)</td>
</tr>
</tbody>
</table>

(continued)
<table>
<thead>
<tr>
<th>Subjects</th>
<th>Activity</th>
<th>Blood compartment</th>
<th>Pre-exercise</th>
<th>Post-exercise</th>
<th>Corrected for HC</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Runners (n = 16)</td>
<td>Marathon</td>
<td>Serum</td>
<td>41.9</td>
<td>56.8</td>
<td>√</td>
<td>(90)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>72.8</td>
<td>105.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Runners (n = 11)</td>
<td>Marathon</td>
<td>Plasma</td>
<td>86.0</td>
<td>103</td>
<td></td>
<td>(66)</td>
</tr>
<tr>
<td>Runners (n = 29)</td>
<td>Ultramarathon</td>
<td>Serum</td>
<td>82.9</td>
<td>134</td>
<td>X</td>
<td>(76)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>128</td>
<td>150</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>153</td>
<td>161</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Runners (n = 20)</td>
<td>90 min running at 75% VO2max</td>
<td>Plasma</td>
<td>35.0</td>
<td>42.0</td>
<td>ND</td>
<td>(84)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>66</td>
<td>95</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Runners (n = 29)</td>
<td>Ultramarathon</td>
<td>Serum</td>
<td>82</td>
<td>125</td>
<td></td>
<td>(82)</td>
</tr>
<tr>
<td></td>
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<td>145</td>
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<td></td>
<td>150</td>
<td>146</td>
<td></td>
<td></td>
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<tr>
<td>Runners (n = 16)</td>
<td>Ultramarathon</td>
<td>Serum</td>
<td>118</td>
<td>116</td>
<td>√</td>
<td>(83)</td>
</tr>
<tr>
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<td>85.8</td>
<td>107</td>
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<td></td>
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<tr>
<td>Runners (n = 28)</td>
<td>Ultramarathon</td>
<td>Plasma</td>
<td>69.8</td>
<td>183</td>
<td></td>
<td>(77)</td>
</tr>
<tr>
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<td></td>
<td></td>
<td>39.7</td>
<td>73.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note.** ND = no available data, HC = haemoconcentration. *Significant (p < .05); †significant (p < .01); ‡significant (p < .001); §significant (p < .05) versus placebo. *Concentration expressed as μmol · g⁻¹ of protein; †vitamin C–supplemented group; ‡vitamin E–supplemented group; §placebo group.
and is therefore not directly involved in the protection of LDL against oxidation, it
does neutralize free radicals that can lead to oxidation of LDL (36) through mecha-
nisms explained below. Hence, the decline in plasma ascorbic acid content observed
in the days following the marathon (66) may be attributed to the consumption of
ascorbic acid in oxidative reactions contributing to LDL oxidation. Alternatively, it
could also reflect the involvement of ascorbic acid in maintaining α-tocopherol in a
reduced state (57)—the plasma concentration of which was unchanged 4 days after
the marathon (66). Further research is warranted to confirm these concepts.

The reasons for differences with regard to exercise-induced changes between
these studies are not immediately clear, but some suggestions may be offered.
Differences in assay techniques to measure plasma ascorbic acid concentrations
may have contributed to the variability of findings between studies. Alternatively,
the validity of measuring ascorbic acid in the plasma or serum compared to leuko-
cyte concentrations has been questioned (70, 88).

Differences in the extent of oxidative stress caused by exercise could also
have been a factor. Many of the studies in Table 4 involved prolonged endurance
exercise, which is likely to cause an increase in oxidative stress (28, 40, 66, 90), and
it has been suggested that oxidative stress is a stimulus for the release of ascorbic
acid from the adrenal gland (82). During exercise, it has been proposed that a pro-
oxidant-antioxidant equilibrium is in operation, and that the intensity of exercise is
the critical factor in determining alterations in this equilibrium (110). It is possible
that differences in the degree of oxidative stress induced by exercise among the
studies might have caused some variation in the amount of ascorbic acid released. If
this concept is in fact true, then it follows logically that pre-exercise plasma/serum
ascorbic acid concentrations would also influence the extent of exercise-induced
oxidative stress and consequently, the magnitude of ascorbic acid release during
exercise in response to any oxidative challenge. Among the studies in Table 4, there
is partial support for this idea. When pre-exercise plasma/serum ascorbic acid con-
centrations were in the middle of the range (i.e., 40–60 μmol/L), there was an
increase in plasma/serum ascorbic acid (28, 41, 42, 69, 77, 90). In contrast,
when pre-exercise plasma/serum ascorbic acid concentrations were at the high end
of the range (i.e., ≥80 μmol/L), less ascorbic acid was released (40, 66, 69). This was
particularly apparent in those studies involving supplementation (76, 82, 83).

There is also tentative evidence for an exercise mode effect on the magnitude
of changes in plasma ascorbic acid concentration. Among cyclists and triathletes the
increase was modest (40, 104), whereas among runners plasma ascorbic acid levels
appear to be elevated to a greater extent (41, 42, 66, 76, 82, 83, 90). Considering that
there are no existing data with respect to the possible effect(s) of exercise mode on
the degree of oxidative stress, the differences above cannot be reconciled on the
basis of differences in exercise-induced oxidative stress. Differences in tissue dam-
age resulting from each form of exercise also seem an unlikely influence, in light of
the recent findings that supplementation with vitamin C did not influence inflamma-
tory reactions to exercise-induced muscle damage (77, 84). Therefore, it remains to
be determined what exercise factors (i.e., intensity, duration, mode) affect changes
in plasma ascorbic acid during exercise. Future exercise studies could investigate
possible differences between different forms of exercise with respect to alterations
in antioxidant capacity and oxidative stress.
Chronic Effects of Exercise on Resting Plasma, Serum, and Leukocyte Ascorbic Acid Concentrations

Despite evidence from comparisons of exercising and non-exercising individuals indicating similar dietary intakes of vitamin C and similar responses to supplementation, the limited existing data on the effect of training or chronic exercise on circulating ascorbic acid concentrations are unclear. A marginal deficiency in plasma or serum ascorbic acid concentration has been reported in only a small number of athletes (34, 90, 98), although the deficiency criteria varied among these studies. Contrasting results have been obtained from cross-sectional comparisons of plasma ascorbic acid concentrations between athletes and sedentary controls. In their study of a variety of athletes, Fogelholm et al. (34) reported similar levels. The percentage of both controls and athletes classified as ascorbic acid deficient (<22.7 µmol/L) was similar (0–1%). There was also no effect of weight or energy expenditure. Brites et al. (13) found higher plasma ascorbic acid levels in soccer players compared to sedentary controls. Eight weeks of endurance training did not alter the serum ascorbic acid concentration of athletes (32), yet the levels of ascorbic acid were still higher than those of sedentary controls. In contrast, 3 months of training in marathon runners caused a decline in all circulating antioxidants, except serum ascorbic acid, which increased from a mean (±SEM) serum concentration of 107 (±5) to 180 (±22) µmol/L with training (7).

The concentration of ascorbic acid within plasma may be a less reliable index of vitamin C status than that within leukocytes. The relationship between regular physical training and circulating ascorbic acid concentrations was investigated in 6 sedentary subjects and 12 runners (88). The runners were divided into two groups of 6 based on training status. Whereas the plasma concentrations were not related to training status, the lymphocyte concentrations in the highly trained runners were significantly higher compared to the controls. In cyclists, measurements of leukocyte ascorbic acid concentration were made in the 3rd year of a 4-yr training cycle and immediately prior to the Olympics (31). A significant reduction in the ascorbic acid content of both lymphocytes and neutrophils was observed over the course of the study. Compared to the pre-Olympic values, after the Olympics the mean (±SD) ascorbic acid concentration within lymphocytes had declined from 326 (±86) to 24 (±2) nmol per 10^8 cells, whereas neutrophil ascorbic acid concentration fell from 94 (±22) to 69 (±17) nmol per 10^8 cells. The decline in the ascorbic acid content of both these cell types could be due to the consumption of ascorbic acid in neutralizing reactive oxidants (3), particularly if the athletes in this study suffered any infections (52).

For several reasons, few definitive conclusions can be drawn regarding the effects of regular exercise on vitamin C status in athletes, as indicated by the levels of ascorbic acid in serum, plasma, and leukocytes. First, the magnitude and direction of changes in circulating ascorbic acid concentrations among the studies reported above are variable. Second, in the studies reported above, it was not indicated if any attempt was made to control the effect of exercise in the days prior to blood sampling on circulating ascorbic acid concentrations, which fluctuate in the period following exercise (42, 66, 76, 83, 90). Last, it is unclear whether comparisons can reasonably be made for changes in ascorbic acid within different blood compartments.
Ascorbic Acid and Cortisol

In spite of available data from several studies, the precise relationship between changes in ascorbic acid and cortisol with exercise is yet to be elucidated. It was originally suggested that ascorbic acid and cortisol are released simultaneously as part of the general stress response to exercise (42). More recently, it has been reported that when rats (a species capable of synthesizing ascorbic acid endogenously) performed one bout of intense exercise, there was a significant increase in plasma ascorbic acid concentration 2 hrs postexercise that also corresponded to a significant decline in the ascorbic acid content of the adrenal glands. These authors suggested that the responses were indicative of a stress response (102). Unfortunately, no data were available on changes in cortisol. Others have proffered that ascorbic acid and cortisol release during exercise are linked through oxidative stress and inflammatory reactions (82). The evidence for and against these concepts is discussed below.

The biosynthesis of cortisol in the adrenal gland begins with the conversion of cholesterol to pregnenolone (see Figure 2). This is the rate-limiting step and is the major site of action for ACTH. This step involves two hydroxylation reactions, followed by the side-chain cleavage of cholesterol. The process requires molecular oxygen and a pair of electrons and is regulated by a single enzyme, CYP11A1. The electrons are donated by NADPH first to adrenodoxin reductase, a flavoprotein, then to adrenodoxin, an iron-sulphur protein, and lastly to CYP11A1. Pregnenolone is then transported out of the mitochondria before further synthesis of steroids occurs. Both adrenodoxin reductase and adrenodoxin are also involved in the action of CYP11B1 (11-β-hydroxylase) (4).

It was originally suggested that ascorbic acid must first be released from the adrenal gland in order to permit the synthesis of cortisol, as ascorbic acid was believed to impede electron transport required for the side-chain cleavage of cholesterol (61). In theory, this concept could explain several experimental observations. First, it may be responsible for the apparent depletion of ascorbic acid within the adrenal gland following ACTH treatment or conditions of stress (27, 51). Second, in young chickens it has been shown that vitamin C administered in doses of 1000 mg per kg of body weight ameliorated the immunosuppressive effects of exogenous cortisol and high environmental temperatures, in addition to reducing the mortality rates associated with such stress (99). Last, this concept may also account for the observation of Gleeson et al. (42) that following exercise, increases in plasma ascorbic acid correlated with a rise in plasma cortisol concentration \( r = 0.89, p < .01 \). They interpreted this finding as possible evidence that ascorbic acid and cortisol release from the adrenal gland could be coupled as part of the stress response to exercise (42).

There is evidence against this coupling, however. Kipp and Rivers (60) demonstrated that the injection of ACTH in guinea pigs caused an increase in plasma cortisol concentrations, in addition to a significant reduction in the ascorbate content within the adrenal gland. Moreover, ACTH significantly enhanced the uptake of radio-labeled ascorbic acid into the adrenal gland. Nevertheless, despite the release of cortisol, there was no change in the concentration of ascorbic acid within plasma or any other tissues (60). Although Kipp and Rivers did not measure dehydroascorbic acid, the oxidation of ascorbic acid to dehydroascorbic acid during the synthesis of cortisol (62) could account for its apparent depletion within the adrenal
gland upon ACTH stimulation. Freeman (35) has reported that 1 hour after the injection of ACTH, relative to chickens not supplemented with vitamin C, there was a greater increase in plasma corticosteroid concentrations in those chickens receiving additional vitamin C in their diet.

In another study by the same group, the vitamin C content of diets fed to guinea pigs was manipulated, injections of ACTH were administered to the animals, and the resultant alterations in adrenal ascorbic acid and plasma cortisol concentrations were measured (63). Although dietary vitamin C strongly influenced the concentration of ascorbic acid in the adrenal glands, it was without effect on basal or ACTH-stimulated changes in plasma cortisol. Furthermore, among the animals treated with ACTH, only a weak association was found between adrenal ascorbic acid and plasma cortisol concentrations. Last, similarly to earlier findings (60), ACTH treatment did not influence plasma ascorbic acid concentrations. Taken together these data indicate that the absolute concentration of ascorbic acid within the adrenal gland is not critical for the synthesis of cortisol (63). While ascorbic acid is required for the synthesis of cortisol, ascorbic acid does not appear to be released simultaneously with cortisol as a stress response.

In 1962, Jenkins (56) demonstrated a role for ascorbic acid in the synthesis of cortisol, specifically in the terminal step of the conversion of 11-deoxycortisol to cortisol (Figure 3). This finding was supported more recently by Moser (71) who
cultured porcine adrenal cells for one week with and without 50 µmol/L ascorbic acid and stimulated the cells on days 2, 3, and 4 with ACTH. While the release of cortisol on day 2 was not dependent on ascorbic acid, on days 3 and 4, the amount of cortisol produced was significantly higher in those cells cultured with ascorbic acid. In contrast, there was a marked decline in cortisol release after repetitive stimulation in the cells cultured without ascorbic acid. Hence, ascorbic seems to be a critical factor in the synthesis of cortisol. It has since been shown that ascorbic acid exerts its influence in this process by acting as an antioxidant in this process, which stands to reason considering the high concentration of ascorbic acid within the adrenal gland (49). The transfer of electrons between NADPH, adrenodoxin reductase, adrenodoxin and CYP11A1, produces superoxide radicals (46), which may impair the activity of CYP11B1 (11-β-hydroxylase). Hornsby et al. (50) demonstrated that when primary bovine adrenocortical cells were cultured in a serum-free medium, the addition of cortisol as a pseudosubstrate caused a decline in 11-β-hydroxylase activity. However, in the presence of 50 µmol/L cortisol, the addition of 5 mmol/L ascorbic acid almost completely prevented this loss of 11-β-hydroxylase activity. This effect was synergistic with low oxygen in the culture medium. Hence, rather than stimulating steroidogenesis per se, ascorbic acid appears to act by protecting cytochromes, such as CYP11B1.

Several other groups have subsequently investigated the possible relationship between the release of ascorbic acid and cortisol during exercise by supplementing athletes with 500–1500 mg vitamin C in the week before, and on the day of, an ultramarathon (76, 82, 83). In each of these studies, vitamin C caused a significant increase in pre-race plasma/serum ascorbic acid concentrations and significantly attenuated the post-exercise serum cortisol responses, relative to the placebo group. In contrast to the work of Gleeson et al. (42), modest negative correlations have been reported between changes in plasma/serum ascorbic acid and cortisol postexercise (76, 77, 83). These findings are supported by other studies not involving athletes. Supplementation with 1000 mg vitamin C for 16 weeks caused a significant reduction in resting serum cortisol concentrations in aged women with coronary heart disease, but not in healthy age-matched controls (22). Patients who were given 1000 mg vitamin C while under anesthetic demonstrated a transient suppression of blood ACTH and cortisol concentrations (73).

In accounting for these findings, it has been suggested that ascorbic acid is utilized during exercise to counter oxidative stress, while cortisol is released to counter inflammatory reactions resulting from exercise-induced muscle damage (82). Oxidative stress has been implicated in chronic inflammation. The findings of De la Fuente et al. (22) outlined above, are supportive of a link between oxidative stress and inflammation. Under some conditions and in certain cell lines, a shift in the glutathione redox state can activate NFκB (26). It is unknown whether there is a signal transduction pathway directly linking alterations in the glutathione redox state with the release of cortisol, or if increases in ascorbic acid and cortisol with exercise are incidental. Certainly, it is tempting to support the concept that oxidative stress is a stimulus for the release of cortisol during exercise, particularly on the basis that elevated plasma ascorbic acid concentrations—which presumably signaled adequate antioxidant defense capacity—attenuated the cortisol response (76, 82, 83). Nevertheless, it must be questioned whether there is in fact a link between oxidative stress reactions and the inflammatory response to exercise-induced muscle damage (77, 84).
Despite the attenuation of cortisol release following vitamin C supplementation, this effect may actually have promoted the inflammatory response (see Figure 4), as indicated by significantly higher levels of acute phase reactants such as C-reactive protein and creatine kinase (83), and significantly reduced concentrations of anti-inflammatory cytokines such as interleukin-1 receptor antagonist and interleukin-10 (76, 82). Another group demonstrated that supplementation with a smaller dose of vitamin C (400 mg) for 2 weeks prior to exercise did not alter the cortisol response, yet the postexercise plasma concentration of the anti-inflammatory cytokine interleukin-6 was significantly reduced (101). Therefore, it seems unlikely that exercise-induced oxidative stress causes the release of cortisol from the adrenal gland, at least not in order to counter inflammation. However, the effects of vitamin C could depend on the supplementation dosage.

Two novel, yet largely speculative hypotheses are presented below to account for the observation of attenuated postexercise cortisol levels following vitamin C supplementation (76, 82, 83). High doses of vitamin C may inhibit the synthesis of cortisol, possibly through pro-oxidant effects. Under certain conditions in vitro, ascorbic acid may work as a pro-oxidant rather than as an antioxidant by reducing transition metal ions (reaction 1), which in turn drives the Fenton reaction (reactions 2 and 3), potentially resulting in oxidative stress (45).

\[
\begin{align*}
AH^- + Fe^{3+} + A\Sigma^- + Fe^{2+} + H^+ & \quad (\text{reaction } 1) \\
H_2O_2 + Fe^{2+} + H\Sigma + Fe^{3+} + OH^- & \quad (\text{reaction } 2) \\
LOOH + Fe^{2+} + LO\Sigma + Fe^{3+} + H^+ + OH^- & \quad (\text{reaction } 3)
\end{align*}
\]

There is also strong evidence against a pro-oxidant effect of ascorbic acid, however (6). A series of in vitro trials were performed in which iron was added to plasma in amounts exceeding the latent iron-binding capacity. While this caused appreciable oxidation of ascorbic acid and an increase in bleomycin detectable iron, which is potentially catalytic for free radical reactions, ascorbic acid maintained its antioxidant activity, and there was no detectable alteration in the concentration of F₂-isoprostane and protein carbonyls as markers of oxidative stress (6).

In spite of this evidence, data from a more recent study do lend support to the concept that ascorbic acid can act as a pro-oxidant under some conditions (21). Individuals were given either a placebo, or 12.5 mg vitamin C and 10 mg of the

Figure 4 — Proposed relationship between exercise-induced oxidative stress and inflammatory responses to muscle damage.
antioxidant N-acetylcysteine per kg body weight for 7 days prior to a brief, intense session of eccentric resistance exercise. The plasma concentration of serum bleomycin detectable iron was elevated in both groups in the days after exercise but was significantly higher in the supplement group. Supplementation also significantly increased plasma total antioxidant status. Importantly, there was evidence of oxidative stress. In the supplemented group, the plasma concentrations of 8-isoprostaglandin F$_2$$\alpha$ tended to be higher ($p = .07$), whereas lipid hydroperoxide levels were significantly higher ($p < .001$). Although no correlations were reported, it is tempting to speculate that the combination of increases in serum-free iron, plasma antioxidant capacity, and lipid peroxidation markers were indicative of pro-oxidant effects of ascorbic acid during exercise. Unfortunately, plasma ascorbic acid concentrations were not measured, as this may have provided further insight into the mechanisms in operation. The interaction between Fe$^{3+}$ and ascorbic acid may depend on the ratio of ascorbic acid to dehydroascorbic acid (87). It is also unknown if there was any effect of the N-acetylcysteine on plasma ascorbic acid concentration (21).

Of interest to the current discussion is whether interactions between ascorbic acid and iron could also influence the synthesis of cortisol in the adrenal gland during exercise. There is evidence suggesting that oxidative conditions impair the activity of the enzyme responsible for cortisol synthesis, 11-$\beta$-hydroxylase (50). The production of superoxide anions by polymorphonuclear cells during exercise (47) could possibly induce the release of catalytic iron from ferritin (86) and/or myoglobin (85). The combined effects of elevated concentrations of serum-free iron and ascorbic acid in the circulation during exercise could promote the formation of reactive oxidants (93), leading in turn to impaired activity of 11-$\beta$-hydroxylase and less synthesis of cortisol, which could possibly explain the recent findings of Peters et al. (82, 83). In evidence against this novel hypothesis, Nieman et al. (77) recently reported that vitamin C supplementation had no effect on plasma F$_2$-isoprostane and lipid hydroperoxide concentrations following an ultramarathon race, yet postexercise serum cortisol correlated negatively with plasma ascorbic acid ($r = 0.50, p = .0006$).

The second hypothesis offered presently is related to a possible role for ascorbic acid in glucose metabolism during exercise. Although ascorbic acid within the adrenal gland may not influence ACTH activity and cortisol synthesis directly (63), high concentrations of ascorbic acid in other tissues such as the liver could possibly have a regulatory effect on the release of cortisol. The evidence for this concept comes from a study by Kodama et al. (62) who investigated the effect of infusing a glucose-electrolyte solution containing 200 mmol/L ascorbic acid over 1.5 h into a male volunteer. The concentration of ascorbic acid in plasma rose above 1000 $\mu$mol/L within 0.5 h, and began to decrease thereafter, reaching baseline 3 h later. Interestingly, the decline in plasma ascorbic acid corresponded to a rapid and dramatic increase in plasma cortisol and ACTH concentrations. Kodama et al. proposed that the liver acts as a “vitamin C/cortisol absorber” for two reasons: First, the liver contains high concentrations of ascorbic acid (49) and, second, both cortisol (59) and possibly even ascorbic acid are involved in the regulation of carbohydrate metabolism within the liver (11).

Using human erythrocytes as a model of peripheral tissues, Braun et al. (11) demonstrated that the oxidized form of ascorbic acid, dehydroascorbic acid, is converted to glucose-6-phosphate through the pentose phosphate pathway and/or
gluconeogenesis. They proposed that through glycolysis, glucose-6-phosphate is metabolized to form lactate, which is then transported to the liver to be converted back to glucose-6-phosphate and eventually, glucose (11). Interestingly, it has also been demonstrated that glucose can inhibit the cellular uptake of dehydroascorbic acid (105), and that high concentrations of plasma ascorbic acid inhibit the glucose-induced secretion of insulin from the pancreas (8). These factors could influence the role of ascorbic acid in gluconeogenesis described above. Recently, cortisol was also shown to enhance gluconeogenesis in humans. A high dose cortisol infusion was administered to fasted individuals, and this caused a significant 10% increase in gluconeogenesis as a proportion of hepatic glucose production (59). Therefore, it may be hypothesized that high plasma concentrations of ascorbic acid following supplementation with large doses of vitamin C could possibly compete with cortisol by maintaining blood glucose concentrations via the stimulation of gluconeogenesis during prolonged exercise. There is support for this concept in the study by Peters et al. (82) because although supplementation with 1500 mg vitamin C attenuated the cortisol response to exercise relative to those athletes taking 500 mg vitamin C or a placebo, the postexercise alterations in plasma glucose concentrations were similar between the three groups. These are novel concepts that warrant further investigation.

In summary, ascorbic acid is necessary for the synthesis of cortisol within the adrenal gland. Increases in the plasma concentration of ascorbic acid during exercise likely occur to counter exercise-induced oxidative stress, but do not appear to cause the release of cortisol, either as a general stress response or to counter inflammatory reactions. It is possible, yet unproven, that the synthesis of cortisol during exercise may be influenced by interactions between ascorbic acid and free iron and/or that ascorbic acid may have a role in glucose metabolism during prolonged exercise.

**Ascorbic Acid and Exercise-Induced Oxidative Stress**

If ascorbic acid is released during exercise in response to oxidative stress (82), then what exactly is the stimulus for its release? A comprehensive review of free radicals in their regulation of physiological functions was published recently (26). The concentration of reactive oxygen species depends on the balance between their rate of production and their rate of clearance by various antioxidant compounds and enzymes. Cells or tissues are in a stable state if the rates of oxidant formation and antioxidant scavenging capacity are constant and in balance. The intracellular thiol/disulfide redox state is the key determinant of this redox homeostasis. An alteration of this redox state, which may occur either by an increase in the concentration of reactive oxidant species or a decline in the activity of one or more antioxidant systems, induces redox-sensitive signal cascades that lead to enhanced expression of antioxidant enzymes, elevated concentrations of nonenzymatic antioxidants or an increase in the cysteine transport system. This latter response may in turn facilitate an increase in intracellular glutathione in certain cell types (26). Within the context of exercise, the thiol/disulphide ratio may represent the concept of the pro-oxidant-antioxidant equilibrium, as discussed by Žembron-Lacny and Szyszka (110).

If the production of reactive oxidants during exercise disturbs the intracellular redox state, what role does ascorbic acid play in restoring the balance? Ascorbic acid represents the first line of antioxidant defense in human plasma, and also effectively
protects low-density lipoprotein against oxidative stress reactions (37, 38). During exercise, ascorbic acid likely exerts its antioxidant activity both directly and also in concert with alpha-tocopherol and glutathione (Figure 5). The hydrogen atoms produced in the oxidation of ascorbic acid first to ascorbyl radical and then to dehydroascorbate (see Figure 1), react with a lipid peroxyl radical (LOO•) to form the non-radical product, LOOH. Trapping of LOO• prevents this radical from attacking polyunsaturated fatty acid side chains (L’H) and other lipoproteins in plasma, and thereby halts the chain reaction of lipid peroxidation (reaction 4) (36).

\[
\text{LOO}^\cdot + \text{L’H} \rightarrow \text{LOOH} + \text{L’}^\cdot \quad (\text{reaction 4})
\]

Dehydroascorbic acid is reduced back to ascorbic acid by glutathione (109) and enzymatic reduction (80). Ascorbic acid may assist alpha-tocopherol in trapping aqueous radicals. Alpha-tocopherol also traps peroxyl radicals before they propagate radical chain reactions leading to lipid peroxidation (36). By donating a hydrogen atom, ascorbic acid may reduce the alpha-tocopherol radical produced in this trapping reaction back to alpha-tocopherol (57) (Figure 5).

In view of this role for ascorbic acid, it has been suggested that oxidative stress induced by regular exercise could increase vitamin C requirements (13, 31, 95). However, the evidence for this postulate is conflicting. Some studies have noted changes in biomarkers of oxidative stress without any change in circulating levels of ascorbic acid (28, 40, 90), some have demonstrated increases in ascorbic acid without a change in indices of oxidative stress (7, 77, 95, 104), and others have reported commensurate increases in both ascorbic acid and oxidative stress indicators (58, 101). While an increase in indices of oxidative stress following exercise may not necessarily correspond to an elevation of ascorbic acid levels per se, there is some evidence supporting more generalized antioxidant responses to oxidative stress during exercise. Following a half-marathon, plasma malondialdehyde increased after exercise, in conjunction with a dramatic increase in the total antioxidant capacity of blood (19, 20). The majority of data have indicated that the vitamin C supplementation does not completely prevent, but attenuates, exercise-induced oxidative stress as indicated by increases in the levels of serum diene conjugation (103),
thiobarbituric acid–reactive substances (90), malondialdehyde and exhaled pentane (5, 58, 101), the oxidation of low-density lipoproteins (5, 94), and electron spin resonance (5). Another group (1) demonstrated that supplementation with 1000 mg vitamin C attenuated the increase in thiobarbituric acid–reactive substances following submaximal exercise, but there was no effect on oxygen radical absorbance capacity.

The results from two other groups contrast with the findings above. Relative to a placebo group, supplementation with 1000 mg vitamin C pre-exercise was without effect on plasma malondialdehyde concentrations after 90 min of high-intensity shuttle running (100). This suggests that supplementation was ineffective because endogenous antioxidant defenses were adequate prior to supplementation. However, in apparent contradiction to this suggestion, they also contended that antioxidant defenses had been overwhelmed as indicated by increased levels of malondialdehyde after exercise. One month of supplementation with 1000 mg vitamin C was also without effect on serum ascorbic acid concentrations, yet it reduced resting levels of plasma lipoperoxide. The placebo group displayed a decline in serum ascorbic acid concentration but no change in plasma lipoperoxide (95). It was not stated whether the effects of recent exercise had been controlled for at the time of sampling, as this factor could have influenced the results.

The disparity among these studies could be due to the fact that ascorbic acid is a water-soluble antioxidant, and indices of oxidative stress are based on lipid peroxidation. Ascorbic acid is effective in preventing the initiation of lipid peroxidation. Aside from the chain-propagating reaction (reaction 4), lipid peroxyl radicals may also be converted to a cyclic peroxide, which then degrades to other breakdown products, such as malondialdehyde (36). Ascorbic acid is not effective in preventing the formation of these products, and this could explain some of the discrepancies between changes in plasma/serum ascorbic acid concentration and markers of oxidative stress in the studies above.

Conclusions

Ascorbic acid is known to be involved in a number of metabolic pathways that are important to exercise. Nevertheless, reports of alterations in the concentration of ascorbic acid within plasma/serum or urine following vitamin C supplementation and exercise do not support the concept that athletes have a greater requirement for vitamin C in their diets. During acute exercise, it is commonly contended that the major role of ascorbic acid is in counteracting oxidative stress. However, whether ascorbic acid is oxidized to dehydroascorbic acid in these reactions during exercise has not been investigated to date. Future exercise studies should investigate measuring the concentration of ascorbic acid derivatives, such as dehydroascorbic acid following exercise. It is conceivable that ascorbic acid is efficiently recycled by exercise, thereby obviating the need for supplementation beyond regular dietary intakes. Two novel hypotheses have been put forward in this review. First, in addition to causing lipid peroxidation, the products of prooxidant reactions that potentially follow supplementation with high doses of vitamin C may have more wide-ranging effects on other aspects of physiological function. Second, although it has been questioned whether cortisol is a factor in stimulating gluconeogenesis, there is recent evidence that lends tentative support to the idea that both cortisol and ascorbic acid have a regulatory influence on gluconeogenesis. These concepts await further
investigation before they can be applied to the control of physiological systems during exercise.

References


