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Increased Oxidative Damage in Vitamin C Deficiency is Accompanied by Induction of Ascorbic Acid Recycling Capacity in Young But Not Mature Guinea Pigs

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Ascorbic acid (AA) recycling, i.e. the intracellular regeneration of AA from its oxidized forms semidehydroascorbyl radical and dehydroascorbic acid (DHA), presumably has a key function in maintaining redox homeostasis. Like humans, guinea pigs cannot synthesize AA. In the present paper, the effects of severe AA deficiency on the AA recycling capacity in erythrocytes (RBCs) and liver homogenates were studied in young and mature guinea pigs. Twelve animals of each age category were divided into weight-matched groups of six animals and fed either an AA deficient or sufficient diet. After 5 weeks, they were sacrificed and RBC and liver ascorbate recycling was estimated along with glutathione, tocopherols, AA, SOD, and malondialdehyde (MDA).

For young animals, AA recycling capacity was significantly increased in RBCs from the deficient group as compared to the controls ($p < 0.001$). RBC MDA was not increased by incubation with *t*-butylhydroperoxide (TBH) while the initial MDA level was significantly elevated ($p < 0.001$). In mature animals, neither RBC recycling nor MDA levels depended on AA status. Liver recycling capacity was not affected by age or diet, while liver MDA was significantly higher in young but not in mature deficient animals compared to respective controls ($p < 0.01$). In young animals, incubation with TBH resulted in significant MDA formation in the deficient compared to sufficient animals in both liver and RBCs ($p < 0.05$). RBC glutathione was not significantly changed by age or diet indicating that the observed changes in recycling capacity are enzyme dependent.

The results suggest that young guinea pigs may have a more adaptable antioxidant defense system compared to mature animals while also being more susceptible to oxidative stress.

Keywords: Ascorbic acid; Dehydroascorbic acid; Recycling; Vitamin C deficiency; Oxidative damage; Lipid peroxidation

INTRODUCTION

Aerobic organisms are continuously exposed to oxidative challenges. As a first line of defense, evolution has provided them with an array of antioxidant systems consisting of both high and low molecular weight antioxidants.

Ascorbic acid (AA) is perhaps the most important water-soluble antioxidant in plasma[1] and plays a fundamental role in the antioxidant defense both as a direct radical scavenger and possibly in the regeneration of other antioxidants. While plasma and tissue concentrations of AA have been studied extensively under various physiological conditions and its oxidized metabolite dehydroascorbic acid (DHA) gains more attention, the recycling of AA has not been as thoroughly investigated. AA recycling occurs both via semidehydroascorbyl radical and DHA and reduction can proceed by either enzymatic or chemical means. Recycling of AA plays a key role in its antioxidant function. Thus, in e.g. humans and guinea pigs, that are not capable of synthesizing AA and depend on dietary AA to prevent pathologies like scurvy, this process must be very efficient and of major importance.

Vitamin C deficiency has been studied extensively in guinea pigs for many years because of its relevance to humans. Low levels or absence of AA in the diet for 3–5 weeks have been shown to cause widespread increase in oxidative stress and

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damage,[2–5] while subsequent re-administration of AA results in restored redox homeostasis.[6]

We have previously studied the effect of age on AA levels and recycling capacity in rat hepatocytes and found that antioxidant levels declined with age.[7,8] AA recycling was also impaired with age as demonstrated by the fact that recycling capacity during increased oxidative stress was decreased in old animals compared to young, although no difference was observed at the basal metabolic level.[9,10] However, because rats are capable of synthesizing AA, they constitute an unsatisfactory model when seen from a human perspective.

In particular during vitamin C deficiency, the capacity for recycling of AA is the most important factor in the conservation of the limited AA pool. Consequently, if the possibility of enhancing this process exists, it could play a major role in adaptability of the antioxidant defense during oxidative challenges. In the present paper, the effect of dietary vitamin C intake on AA recycling capacity, antioxidant levels and lipid peroxidation was studied in erythrocytes (RBCs) and livers of young (2 months) and mature (9 months) guinea pigs. The results indicate that the capacity for AA recycling is inducible in young but not mature guinea pigs and that recycling activity proceeds via various enzymatic pathways *in vivo*.

MATERIALS AND METHODS

Chemicals

All chemicals were of the highest quality available. Specifically, AA, DHA, glutathione (GSH), danzyl chloride, α- and γ-tocopherol, *t*-butylhydroperoxide (TBH) were from Fluka (Milwaukee, IL). *meta*-Phosphoric acid (MPA) and disodium ethylenediamine tetraacetic acid (EDTA) were from Merck (Darmstadt, Germany). Phosphate buffered saline (PBS) was purchased from Biochrome KG (Berlin, Germany) while superoxide dismutase (SOD) was from Sigma (St. Louis, MO).

Animals and Diets

Male outbred Dunkin Hartley guinea pigs (Charles River Laboratories, Wilmington, MA) aged two (young) or 9 months (mature) were acclimatized for 1 week at the university research animal facility before the diet regimen was initiated. Each age group of animals was randomized into two weight-matched groups ($n = 6$). All animals were fed pathogen free standard diets (Altromin International, Lage, Germany). The diets were essentially identical except for vitamin C content. Control animals were placed on a normal guinea pig diet (Diet #3010, 1036 mg vitamin C/kg), while deficient animals were supplied with a diet low in vitamin C (Diet #2010, 36 mg vitamin C/kg). The dietary regimen was continued for 30–34 days. Animals were checked daily by educated staff at the animal facility and weighed twice a week. One 9 months control animal died during the course of the study and was not replaced.

At the end of the feeding regimen, animals were anaesthetized using pentobarbital (55 mg/ kg i.p.). One thousand units of heparin (200 μl) was injected into the heart and after 2 min, a 2 ml blood sample was obtained by cardiac puncture carefully avoiding hemolysis and processed immediately as described below. Meanwhile, while still anaesthetized, the animal was sacrificed by cutting the portal vein and the liver was removed.

Sample Handling

The blood sample was immediately centrifuged (2000*g*, 5 min, 4°C). One 100 μl plasma aliquot was acidified with an equal volume of 10% MPA containing 2 mM EDTA, briefly vortex mixed, centrifuged (16,000*g*, 2 min, 4°C), and the supernatant frozen at −80°C for ascorbic and uric acid analysis. The remaining plasma aliquots were stored neat at −80°C.

RBCs were washed three times with five volumes of PBS followed by centrifugation (2000*g*, 5 min, 4°C). After the final wash, the hematocrit was adjusted to 25% with PBS (or water for SOD analysis) and aliquots were frozen at −80°C or used immediately for recycling experiments as described below.

The liver was weighed and immediately homogenized in ice-cold 1.15% potassium chloride (9 ml/g tissue) by using a Potter–Elvehjem (600 rpm, 3 min). One liver homogenate aliquot was prepared for AA analysis as above and another one was used immediately for recycling experiments. The remaining aliquots were stored at −80°C until analysis.

Analyses

AA and uric acid in MPA stabilized samples were analyzed by HPLC with coulometric detection as described previously.[11,38] Tocopherols were analyzed by HPLC with amperometric detection as described by Sattler *et al*.[12] Total glutathione was measured after derivatization with danzyl chloride by HPLC with fluorescence detection.[13] SOD activity was quantified by the pyrogallol method.[14]

Malondialdehyde (MDA) was used as an index of lipid peroxidation and was assessed by thiobarbituric acid derivatization of followed by quantification by HPLC with fluorescence detection as described elsewhere.[15] As a measurement of

TABLE I Effect of diet and age on various antioxidant concentrations in plasma, liver and erythrocytes in young and mature guinea pigs fed a diet with either normal (1036 mg/kg, control) or low (36 mg/kg, deficient) vitamin C for 5 weeks

Measurement	Young (2 months)		Mature (9 months)	
	Controls ($n = 6$)	Deficient ($n = 6$)	Controls ($n = 5$)	Deficient ($n = 6$)
Plasma				
Ascorbic acid (μM)$^{\#\#\#}$	123.4 ± 10.9	0.3 ± 0.3***	75.0 ± 15.2§	1.2 ± 0.7***
Uric acid (μM)$^{\#}$	44.7 ± 6.2	70.6 ± 14.0	28.8 ± 2.0	75.6 ± 22.0
α-Tocopherol (μM)	7.5 ± 1.1	10.0 ± 1.5	9.4 ± 2.0	10.0 ± 1.9
γ-Tocopherol (μM)	0.50 ± 0.05	0.38 ± 0.05	0.49 ± 0.09	0.41 ± 0.09
Liver				
Ascorbic acid (nmol/mg tissue)$^{\#\#\#}$	1.55 ± 0.11	0.02 ± 0.01***	1.20 ± 0.09§	0.04 ± 0.02***
Glutathione (nmol/mg tissue)$^{\#\#\#}$	3.42 ± 0.11	2.39 ± 0.20**	3.11 ± 0.11	2.61 ± 0.27**
α-Tocopherol (nmol/g tissue)$^{\#\#\#}$	29.6 ± 2.2	11.7 ± 1.4***	27.2 ± 4.0	20.1 ± 2.0
γ-Tocopherol (nmol/g tissue)$^{\#\#\#,\dagger\dagger\dagger}$	1.38 ± 0.10	0.30 ± 0.05***	2.78 ± 0.25§§§	1.94 ± 0.17*
SOD (mg/g tissue)$^{\#\#\#}$	0.98 ± 0.10	0.81 ± 0.11*	0.94 ± 0.03	0.72 ± 0.17*
Erythrocytes				
Glutathione (mM in RBC volume)	0.94 ± 0.10	0.94 ± 0.19	0.98 ± 0.04	0.83 ± 0.20
SOD (μg/ml RBC volume)	91.2 ± 35.9	91.9 ± 12.2	102.6 ± 16.5	90.1 ± 17.9

Data are presented as means ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to control, $^{\S}p < 0.05$, $^{\S\S\S}p < 0.001$ compared to young control by 1-way breakdown ANOVA. Two-way ANOVA using age and diet as factors showed a significant effect of diet, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, or age, $^{\dagger\dagger\dagger}p < 0.001$.

oxidizability, lipid peroxidation was also assessed in samples of liver and RBCs that had been subjected to a 30 min incubation with 2 mM TBH at 37°C.

AA recycling capacity was estimated by measuring DHA reduction as described previously with some modifications.[10] Each experiment (1000 μl) consisted of 80 μl liver homogenate (typically about 25 mg protein/ml) or 25% hematocrit RBCs (typically about 90 mg protein/ml), PBS, and 6 mM DHA and was assayed in the presence or absence of 0.2 mM NADPH. Immediately after addition of DHA, the sample was mixed by inversion and a 100 μl aliquot of sample was added to an equal volume of freshly prepared 10% MPA containing 2 mM EDTA, vortex-mixed, centrifuged (16,000*g*, 2 min, 4°C), and frozen to −80°C until analysis. The DHA solution was prepared in 5 mM sodium acetate buffer pH 4.0 immediately before use and kept on ice. Meanwhile, the remainder of the sample was incubated for 10 min in an incubator set at 37°C with gentle rocking (8 inversions/min), after which a second 100 μl aliquot was quenched with MPA and treated as above. The samples were typically stored less than 1 week before AA analysis as described.[11] The capacity for AA recycling was calculated by subtraction of the baseline sample from the 10 min sample and given as per minute rate. The baseline concentration of AA was usually about 20–60% of the 10 min sample. The susceptibility of the recycling process to oxidative insults was tested by performing equivalent experiments with samples that had been subjected to a 30 min incubation with 2 mM TBH at 37°C.

Protein concentrations were measured by using the Bradford method.[16]

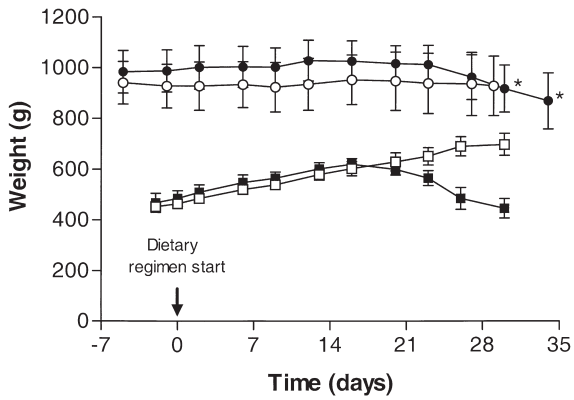

FIGURE 1 Animal weights during the depletion period. Young controls (open squares); young deficient (closed squares); mature controls (open circles); mature deficient (closed circles). Data are shown as mean ± SD. *n*-Values are as indicated in Table I except where an asterisk indicates that only three animals were left.

Statistics

Effects of diet and age were tested by using 2-way ANOVA. After tests for homogeneity of variances (Levene's test), individual comparisons were done by using 1-way breakdown ANOVA followed by Fisher's least significant difference (LSD) test. Analysis of oxidant stress susceptibility was done by using paired *t*-test. *n*-Values are as shown in Table I and data are presented as means ± SEM unless otherwise indicated. A two-tailed *p*-value less than 0.05 was considered statistically significant.

RESULTS

Animal Weights

Figure 1 shows animal weights throughout the dietary regimen. For young animals, continuous growth was observed for the control animals for the

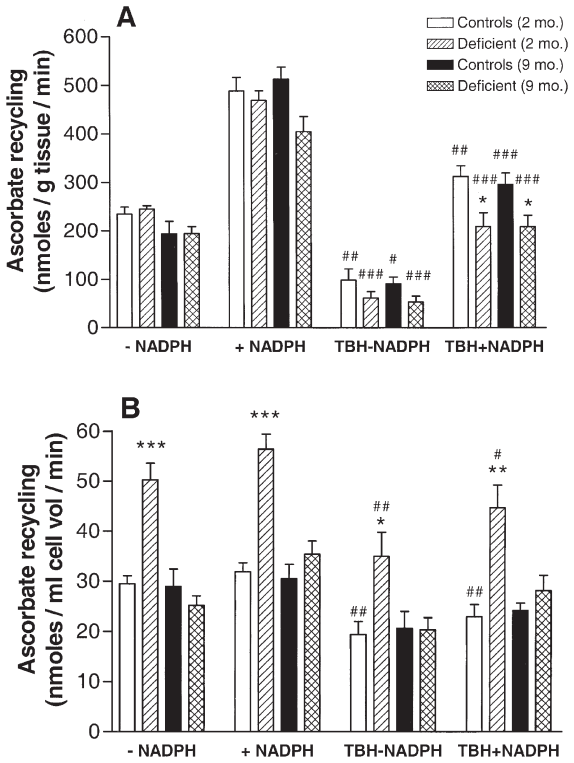

FIGURE 2 Ascorbic acid recycling in liver (A) and erythrocytes (B). Bar assignment is as depicted in panel A. Samples were incubated with or without 0.2 mM NADPH and/or 2 mM *t*-butyl hydroperoxide (TBH) for a total of four treatments. Data are shown as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to respective controls; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared to equivalent sample without TBH treatment.

entire period while deficient animals started loosing weight after about 2 weeks. Mature animals showed no significant weight gain or loss during the first 3 weeks after which the deficient animals began to loose weight.

Low Molecular Weight Antioxidants

Table I shows antioxidant concentrations in plasma, liver and RBCs. Plasma and liver concentrations of AA confirmed the expected AA status of the animals. Severe depletion of AA to less than 3 percent of that of the respective controls were observed among the animals on vitamin C deficient diet ($p < 0.001$ in all cases). Plasma uric acid showed a significant effect of diet ($p < 0.05$) with marked increases among both young (58%) and mature (163%) deficient animals over that of respective controls. Plasma tocopherol concentrations were not significantly affected by age or diet in plasma. In contrast, liver α-tocopherol showed a significant effect of diet ($p < 0.001$). Specifically, a 60% decrease was observed in young deficient animals compared to control ($p < 0.001$), while a 26% decrease among the mature animals was not statistically significant. γ-Tocopherol was affected significantly by both diet ($p < 0.001$) and age ($p < 0.001$). A 78% decrease was observed among young deficient animals compared to controls ($p < 0.001$) while a 30% decrease was found in mature animals ($p < 0.05$). The γ-tocopherol level in mature controls was 2-fold that of young. Total liver glutathione showed a significantly effect of diet ($p < 0.001$) with decreases of 30% for young ($p < 0.01$) and 16% for mature ($p < 0.01$) deficient animals compared to controls. In RBCs, no effect of either diet or age was observed.

Superoxide Dismutase

SOD activity was measured in liver and RBC (Table I). In liver, a significant effect of diet was observed ($p < 0.001$). Both young and mature deficient animals showed about 20% decreases compared to controls ($p < 0.05$ in both cases). In RBC, no significant effects of age or diet were found.

Ascorbic Acid Recycling

Figure 2 shows the results of AA recycling experiments in liver and RBC, respectively. In the liver, no significant effects of age or diet were observed at the basal metabolic level (Fig. 2). Addition of 0.2 mM NADPH to the incubation mixture resulted in markedly increased recycling capacity in all groups (91–166%; $p < 0.001$ in all cases). Conversely, incubation in the presence of 2 mM TBH for 30 min resulted in decreased recycling capacity in all groups ($p < 0.05$), but more so in deficient animals than in controls ($p < 0.05$). Addition of 0.2 mM NADPH to the latter incubations increased the recycling capacity throughout (2 to 3-fold; $p < 0.001$ in all cases) while the significant effect of diet persisted ($p < 0.01$).

The equivalent AA recycling experiments with washed RBCs disclosed a very different pattern (Fig. 2B). In young animals, a highly significant effect of AA deficiency was observed as demonstrated by a 70% increase in AA recycling capacity compared to controls ($p < 0.001$). This significant difference persisted in all RBC experiments while no effect of diet was observed among the mature animals. In contrast to the liver experiments, addition of 0.2 mM NADPH to the incubation mixtures did not result in an increased recycling capacity. Moreover, while incubation in the presence of 2 mM TBH resulted in decreased recycling capacity among young animals ($p < 0.05$ in all experiments), no significant effect of the treatment was observed among either deficient or sufficient mature animals.

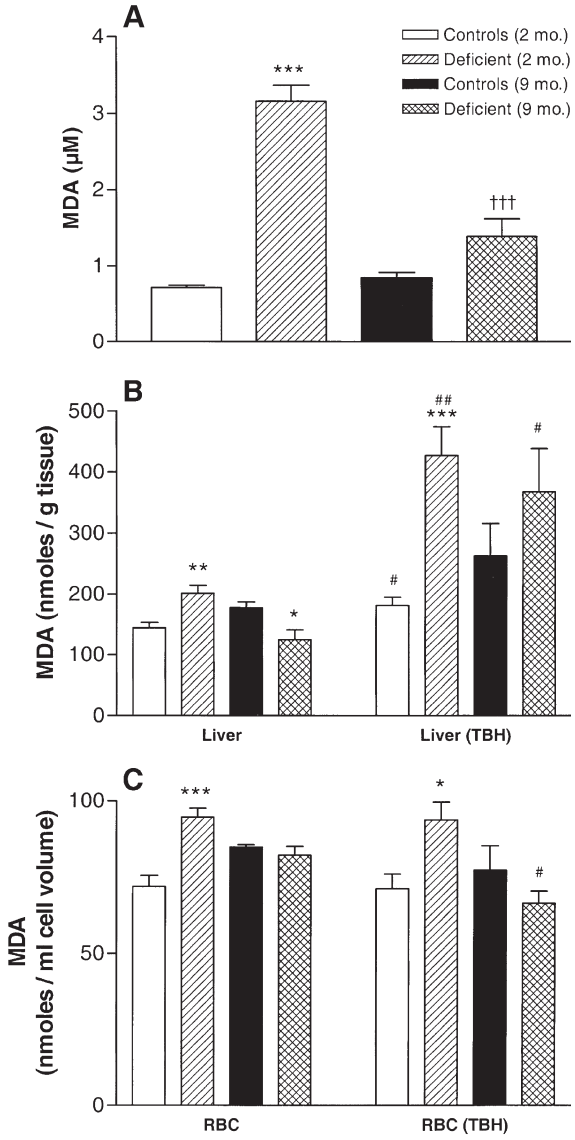

FIGURE 3 Lipid hydroperoxides in plasma (A), liver (B) and erythrocytes (C) as measured by MDA. Bar assignment is as depicted in panel A. Liver and erythrocyte samples were incubated with or without 2 mM *t*-butyl hydroperoxide (TBH). Data are shown as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to respective controls; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared to equivalent sample without TBH treatment. $^{\dagger\dagger\dagger}p < 0.001$ compared to young deficient animals.

Lipid Peroxidation

As an index of lipid peroxidation, i.e. oxidative damage, MDA was quantified in plasma, liver and RBC. Furthermore for liver and RBCs, the susceptibility to oxidative stress was assessed by a 30 min incubation in the presence of 2 mM TBH. The results are shown in Fig. 3.

Plasma MDA was significantly affected by diet ($p < 0.001$, Fig. 3A). Specifically, increases of 440% for young ($p < 0.001$) and 65% for mature deficient animals ($p = 0.06$) were observed compared to respective controls. Moreover, young deficient animals had significantly more MDA compared to the corresponding mature animals ($p < 0.001$).

Liver lipid peroxidation (Fig. 3B) was also significantly increased in deficient young animals compared to controls ($p < 0.01$), while a decrease was observed among mature animals ($p < 0.05$). Incubation in the presence of TBH resulted in increased formation of MDA in young animals ($p < 0.05$) but significantly more so in deficient animals compared to controls ($p < 0.001$). Among mature animals, only deficient animals were significantly affected by the TBH treatment ($p < 0.05$).

As for liver, RBC MDA was significantly increased in deficient young animals as compared to controls ($p < 0.001$, Fig. 3C). No difference was observed among mature animals. Incubation in the presence of 2 mM TBH did not result in increased MDA formation. A decrease was even observed among deficient mature animals. In contrast, the MDA level of young deficient animals remained significantly elevated ($p < 0.05$).

DISCUSSION

AA recycling plays a vital role in the preservation of the AA pool. Particularly in guinea pigs, which are unable to supply AA by other than dietary sources, the characteristics of this activity are of major importance in understanding the response to vitamin C deficiency and oxidative insults. In the present study, the effects of age and severe vitamin C deficiency on AA recycling and various variables of oxidative stress and damage were investigated in young and mature guinea pigs.

For many years, the recycling of AA in animals was believed to occur exclusively non-enzymatically. Considerable evidence exists for the chemical reduction of DHA by GSH *in vitro*[17,18] and convincing *in vivo* evidence linking GSH to reduction of DHA was provided by Meister and coworkers in a series of studies.[19–23] In plants, DHA reductases have been known for decades and are well characterized.[24–26] More recently, studies with purified enzyme preparations have shown that mammalian thioltransferases such as glutaredoxin and protein disulfide isomerase possess substantial GSH-dependent DHA reductase activity,[27,28] while thioredoxin reductase[29] and 3α-hydroxysteroid dehydrogenase[30] have demonstrated NADPH-dependent DHA reductase activity. However, critics argue that the physiological relevance of enzymes that only enhance the chemical reduction 2 to 5-fold is questionable.[31] The fact remains that the significance of these enzymes in AA recycling, most of which have multiple known functions and broad substrate specificity, has not been sufficiently demonstrated *in vivo*.

In the present study, AA recycling capacity was estimated in liver and RBCs under various conditions. Incubations were made both in the presence and absence of NADPH as a measurement of the involvement of NADPH-dependent DHA reductases in the process. As mentioned above, GSH is capable of reducing DHA non-enzymatically, and therefore analogous experiments adding exogenous GSH were not carried out. In parallel samples, the susceptibility of the DHA reduction activity to oxidative stress was assessed following preincubation with TBH, an oxidant known to induce lipid peroxidation and deplete GSH.[32] In addition to providing evidence for the enzymatic reduction of AA *in vivo*, the results reveal a fundamental difference between liver and RBCs regarding the capacity, regulation and molecular basis of the process (Fig. 2).

In the liver, no differences were observed between the groups at the basal metabolic level. This is in agreement with our previous findings in isolated hepatocytes from young and old rats.[9,10] Addition of NADPH to the incubation mixture significantly increased the recycling capacity in liver by about 100% suggesting the presence of NADPH-dependent DHA reductases. Although NADPH is also required for the recycling of glutathione, no advantage of NADPH addition was observed among the more challenged deficient animals compared to controls. This further substantiates the involvement of NADPH-dependent DHA reductases in the liver AA recycling process.

As for liver, no difference was observed between RBCs of the control groups at the basal metabolic level. However, no significant effect of NADPH was observed for RBCs, indicating the presence of only GSH-dependent DHA reductase activity. The latter results are in agreement with those of May *et al.* who suggested that ascorbate regeneration from DHA in human RBCs is largely GSH-dependent.[33] Thus, it appears that the enzymes involved in AA recycling in the liver are different from those in RBC. Moreover, the recycling capacity of the liver was found to be about 10-fold higher than that of RBC based on DHA reduction per mg protein (data not shown). This, however, does not suggest that RBC recycling capacity is unimportant. In agreement with estimates made by May and coworkers,[34] estimates based on the data obtained in the present study shows that the total amount of AA present in blood can be recycled by the RBCs alone approximately once every 3 min. Consequently, the recycling capacity of the RBCs may constitute a substantial antioxidant reserve *in vivo*.[34]

No effect of diet was observed on liver recycling capacity at the basal metabolic level (Fig. 2). Conversely, among young animals, a surprising 70% increase in AA recycling capacity was found in RBCs ($p < 0.001$). This effect of diet was not present among the mature animals. The increase in RBC recycling capacity among young deficient animals was not accompanied by an increased glutathione concentration (Table I). Consequently, it is concluded that the observed increase in recycling is due to increased DHA reductase activity or increased substrate availability.

Guinea pig RBCs have a life span of about 2 months. RBCs of an adult mammal lack a nucleus, endoplasmic reticulum, mitochondria, and ribosomes. Thus, induction of enzymatic activity is not possible in the mature erythrocyte. Reticulocytes may have this ability but amounts only to a small fraction of the blood cells. Thus, an increased amount of DHA reductases must originate from an altered RBC production in the stem cells of the bone marrow. During the rapid growth of the young animals, the RBC population turnover is much higher compared to that of mature animals. In fact, over the course of the feeding regimen, the majority of the RBCs have been replaced in the young animals. Consequently, the data indicate that changing the recycling capacity of the RBCs may constitute an important part of the available response to AA deficiency in young guinea pigs but the data also suggest that mature animals do not have this ability or that the response is significantly delayed. The latter may be due to the relatively slower change in RBC population.

Incubating liver homogenates and RBCs in the presence of 2 mM TBH tested the susceptibility of the recycling activity to oxidative stress. In the liver, marked decreases in activity were observed for all groups but more so for deficient animals than controls ($p < 0.05$). In RBCs, only young animals showed significant decreases in recycling activity while mature animals were unaffected. This could indicate that the NADPH-dependent DHA reductase activity is more susceptible to oxidative stress compared to the GSH-dependent counter part.

The various antioxidant measurements shown in Table I verify the effect of the AA deficiency resulting from the dietary regimen. Specifically, liver glutathione and α- and γ-tocopherol were significantly decreased in deficient animals ($p < 0.001$). The latter is interesting as controversy remains regarding the possible sparing-effect of AA on α-tocopherol. The present data suggest that both α- and γ-tocopherol are spared by AA *in vivo* as demonstrated *in vitro* for α-tocopherol by Doba *et al.*[35] The data are in agreement with Tanaka *et al.* who found similar results in AA deficient ODS rats.[36]

Table I also reveals interesting age dependencies. In the majority of rodent studies on aging, young animals have been compared to animals well into the degenerative phase of aging under the assumption that the young animals that have not yet reached maturity can serve as a representative control group.

This approach has rightfully been criticized.[37] The results of the present data also suggest that this assumption is unwarranted, since differences between young and mature animals were observed both in antioxidant concentrations and susceptibility to oxidative stress. Consequently, more age groups are necessary to cover an entire lifespan and to separate the process of maturation from that of aging.

MDA were markedly elevated in deficient animals but more so in young compared to mature animals ($p < 0.001$, Fig. 3A). In liver and RBCs, deficient young animals showed increased MDA, while no effect or even decreased MDA were observed in mature animals (Fig. 3B,C). A similar pattern was observed when liver and RBCs were incubated with 2 mM TBH. Generally, significantly elevated MDA levels were observed in deficient young animals compared to controls while the changes among the mature animals were more moderate or absent (Fig. 3). Moreover, the changes in liver were generally larger than those observed in RBCs indicating that RBCs are better protected against lipid peroxidation.

In conclusion, the data presented in this paper suggest that in guinea pigs, DHA reduction is catalyzed by both glutathione- and NADPH-dependent DHA reductases in the liver, while only a glutathione dependent activity is present in RBCs. AA deficiency results in significantly increased AA recycling capacity in RBCs of young but not mature animals. The enzymatic origin of this increase is substantiated by the fact that glutathione levels in the same samples were unchanged. These findings suggest that young guinea pigs have a more responsive antioxidant defense compared to mature animals. However, parallel measurements of oxidative damage also demonstrate that young animals are more susceptible to oxidative insults compared to mature animals and that the ability to increase AA recycling apparently does not efficiently prevent the resulting oxidative damage. The differences between young and mature animals should be taken into account when designing experiments to study the effects of oxidative stress in aging.

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