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Ascorbate improves metabolic abnormalities in *Wrn* mutant mice but not the free radical scavenger catechin

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Abstract

Werner syndrome (*WS*) is a premature aging disorder caused by mutations in a RecQ-like DNA helicase. Mice lacking the helicase domain of the WRN homologue exhibit many phenotypic features of WS. Importantly, mutant $Wrn^{\Delta hel/\Delta hel}$ mice show abnormal increases in visceral fat deposition and fasting blood triglyceride levels followed by insulin resistance and high blood glucose levels. These mice also exhibit increased heart and liver tissue reactive oxygen species concomitantly with oxidative DNA damage, indicating a pro-oxidant status. We treated mice with either ascorbate or catechin hydrate for 9 months. Vitamin C supplementation reduced oxidative stress in liver and heart tissues and reversed hypertriglyceridemia, hyperglycemia, and insulin resistance and reduced fat weight in mutant $Wrn^{\Delta hel/\Delta hel}$ mice. Although the free scavenger catechin hydrate also reduced oxidative DNA damage in heart and liver tissues, it did not reverse any of the metabolic phenotype aspects in treated mutant mice. Finally, vitamin C and catechin hydrate did not affect the metabolic status of wild-type mice. These results indicate that vitamin C supplementation could be beneficial for WS patients.

Keywords

Werner syndrome; metabolic syndrome; vitamin C; catechin; Wrn mutant mice

Aging is defined as a progressive deterioration of physiological functions that impairs the ability of an organism to maintain its homeostasis and consequently increases susceptibility to diseases and death. Much of the recent progress in the understanding of aging has been fueled by the study of human progeroid syndromes.[1] One of the most fascinating of human aging disorders is Werner syndrome (*WS*). WS is a human autosomal recessive disorder characterized by genomic instability and the premature onset of a number of age-related diseases, including osteoporosis, ocular cataracts, graying and loss of hair, diabetes mellitus, arteriosclerosis, and atherosclerosis.[2–5] It is, to our knowledge, the human disease model closest to normal aging. The defective enzyme responsible for WS is a helicase or

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Conflicts of interest
The authors declare no conflicts of interest.

exonuclease involved in DNA repair, replication, transcription, and telomere maintenance.[6–10] We previously generated a mouse model with a deletion in the helicase domain of the murine WRN homologue[11] that recapitulates most of the WS phenotypes, including an abnormal hyaluronic acid excretion, higher reactive oxygen species (*ROS*) levels, and increased genomic instability and cancer incidence.[12,13] In addition, $Wrn^{\Delta hel/\Delta hel}$ mice show hallmarks of a metabolic syndrome that includes premature visceral obesity, hypertriglyceridemia, and insulin-resistant type 2 diabetes, like WS patients.[13] Metabolic syndrome afflicts up to one-half of Western populations and is considered an age-related, proinflammatory lipidic disorder.[14] As WS patients exhibit a pro-oxidative status *in vivo*,[15] it is believed that an antioxidant treatment might alleviate some of the risk factors (including metabolic anomalies) associated with age-related diseases in such patients. The $Wrn^{\Delta hel/\Delta hel}$ mouse provides a unique opportunity to test such compounds. In this study, we first tested the impact of two molecules on visceral obesity, hypertriglyceridemia, insulin-resistant diabetes, and ROS levels in specific tissues known to have antioxidant properties, namely, ascorbate (or vitamin C) and the polyphenolic flavonoid (+)-catechin hydrate [or (+)-cyanol-3; Sigma, St. Louis, MO, USA].

The drinking water of $Wrn^{\Delta hel/\Delta hel}$ mice was supplemented daily with either 0.4% sodium L-ascorbate (w/v) as described before[16] or with 0.2% catechin (w/v) at weaning. The concentration of catechin used was based on the observation that it prevented vascular endothelial dysfunction and reduced vascular ROS production in dyslipi-demic mice expressing the human apolipoprotein B-100 gene.[17] We first examined ROS and DNA damage levels in the liver and heart tissues of ascorbate- and catechin-treated $Wrn^{\Delta hel/\Delta hel}$ mice compared to untreated mutant and wild-type mice. We measured ROS and oxidative DNA damage in five males and five females of each cohort at 9 months of age as described previously.[18] $Wrn^{\Delta hel/\Delta hel}$ animals exhibited higher amounts of ROS and oxidative DNA damage in the liver and heart than wild-type animals (Fig. 1A–D). Both ascorbate and catechin treatments of $Wrn^{\Delta hel/\Delta hel}$ mice reduced these amounts to equal or even inferior values than those observed in the untreated wild-type cohort. These results indicate that both ascorbate and catechin molecules were absorbed by mutant mice and exerted their antioxidant effects in different tissues.

We next examined visceral fat weight, blood triglyceride, fasting blood glucose, insulin levels, and the homeostatic model assessment of insulin resistance (*HOMA-IR*) index in treated animals. As indicated in Figure 2A, visceral fat weight was increased in $Wrn^{\Delta hel/\Delta hel}$ mice compared to wild-type animals. Ascorbate treatment decreased visceral fat weight of $Wrn^{\Delta hel/\Delta hel}$ mice to wild-type levels. In contrast, catechin had no effect on the accumulation of visceral fat weight in $Wrn^{\Delta hel/\Delta hel}$ mice. Similarly, blood triglyceride levels were increased in $Wrn^{\Delta hel/\Delta hel}$ mice compared to wild-type animals (Fig. 2B). Ascorbate treatment decreased visceral fat weight of $Wrn^{\Delta hel/\Delta hel}$ mice to wild-type levels, but catechin had no effect.

Fasting blood glucose levels were increased in $Wrn^{\Delta hel/\Delta hel}$ mice compared to wild-type animals. Similarly, despite this increase in glucose levels, $Wrn^{\Delta hel/\Delta hel}$ mice also exhibited an increase in insulin levels and HOMA-IR index. Ascorbate decreased the blood glucose levels of $Wrn^{\Delta hel/\Delta hel}$ mice to wild-type levels. The blood insulin levels were higher in

ascorbate-treated *Wrn*$^{\Delta hel/\Delta hel}$ mice than untreated wild-type animals (Fig. 3). Calculation of the insulin resistance index, however, indicated a significant trend toward normalization. In contrast, catechin had no impact on the increased values of fasting blood glucose, insulin, and HOMA-IR index (Fig. 3). Finally, neither ascorbate nor catechin had a significant impact on wild-type fat or glucose metabolism (data not shown).

The above data indicate that ascorbate can reverse the increased ROS and oxidative DNA damage in liver and cardiac tissues as well as the increased fat, blood triglyceride, glucose, and insulin levels observed in *Wrn*$^{\Delta hel/\Delta hel}$ mice. These results suggest that treatment of WS patients with high doses of ascorbate may be beneficial for such individuals. Similar experiments with *Wrn* null mice lacking a functional telomerase will be required. Such double-knockout mice exhibit a more severe premature aging phenotype than *Wrn*$^{\Delta hel/\Delta hel}$ mice.[19] Notably, high doses of ascorbate do not ameliorate the mean life span of wild-type animals.[20] This suggests that ascorbate may have a beneficial effect only on individuals or animals with specific metabolic abnormalities. Other animal models exhibiting genomic instability and premature aging will need to be tested[21] to resolve this question.

Although catechin is as efficient as ascorbate in reducing overall ROS and oxidative DNA damage, it did not reverse the fat and glucose metabolic anomalies observed in *Wrn*$^{\Delta hel/\Delta hel}$ mice. Since the concentration of catechin used did not exacerbate the health effects in these animals when administered for up to 9 months, it is possible that a higher concentration of catechin would be required to have a positive effect on the metabolism of these mice. Alternatively, the antioxidant effect of catechin alone was not sufficient to improve the metabolism of *Wrn*$^{\Delta hel/\Delta hel}$ mice. In this regard, ascorbate is not only an antioxidant but it can also affect the expression of many genes *in vivo* by an unknown mechanism.[20] We will need to test several different antioxidants to determine whether regulating tissue redox status is sufficient to improve the metabolism of *Wrn*$^{\Delta hel/\Delta hel}$ mice. Finally, chemicals known to affect directly metabolic enzymes involved in fat or glucose metabolism will be tested in our mouse model to determine their effects on overall health and longevity.

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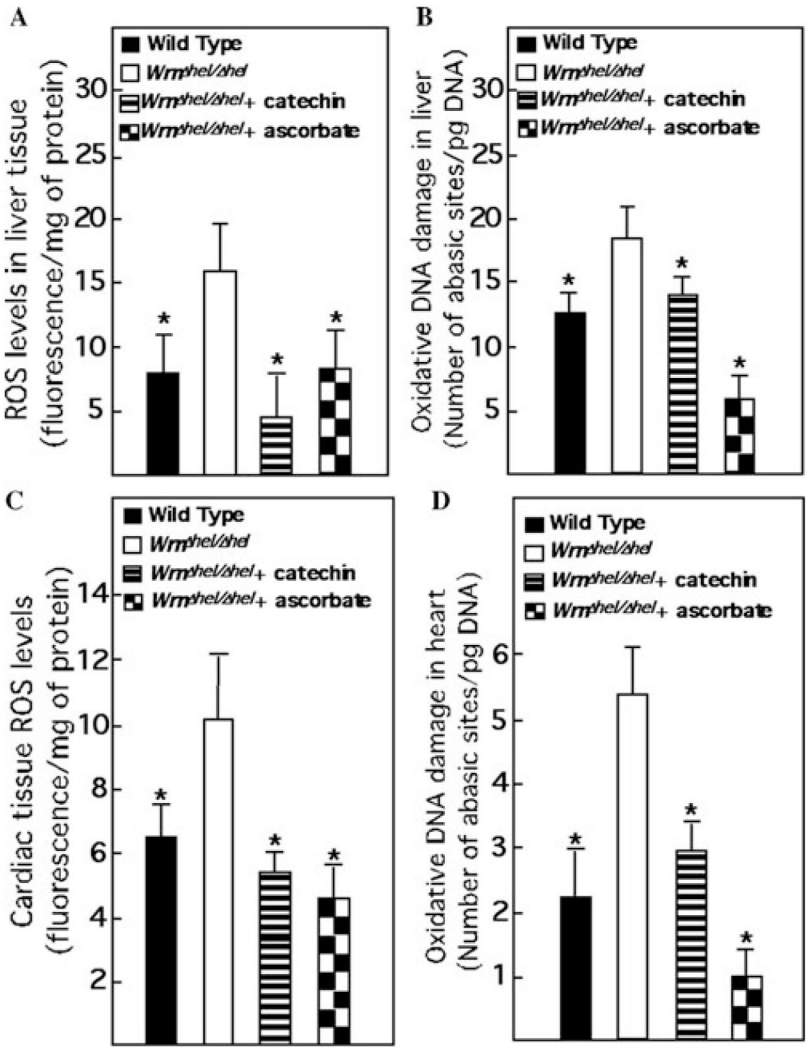

Figure 1.

Impact of ascorbate and catechin on levels of ROS and oxidative DNA damage in liver and heart of $Wrn^{\Delta hel/\Delta hel}$ mice. (A) Liver tissue ROS levels. Tissues were excised and homogenized in RIPA buffer. The homogenate was incubated with the dye 2′,7′-dichlorofluorescein for 1 h at 37°C. This dye is highly fluorescent upon oxidation. (B) Oxidative DNA damage levels in liver. Oxidative DNA damage was measured with the oxidative DNA damage kit from Kamiya Biomedical Co. (Seattle, WA). (C) Heart ROS levels. (D) Oxidative DNA damage levels in heart. Ascorbate and catechin were added in drinking water (0.4% and 0.2%, w/v, respectively) of $Wrn^{\Delta hel/\Delta hel}$ mice at weaning. *, $P < 0.05$ by unpaired *t*-test compared to $Wrn^{\Delta hel/\Delta hel}$ animals. Bars in the histograms represent the standard errors of the means. Nine-month-old males ($n = 5$) and females ($n = 5$) of each cohort were used for all measurements.

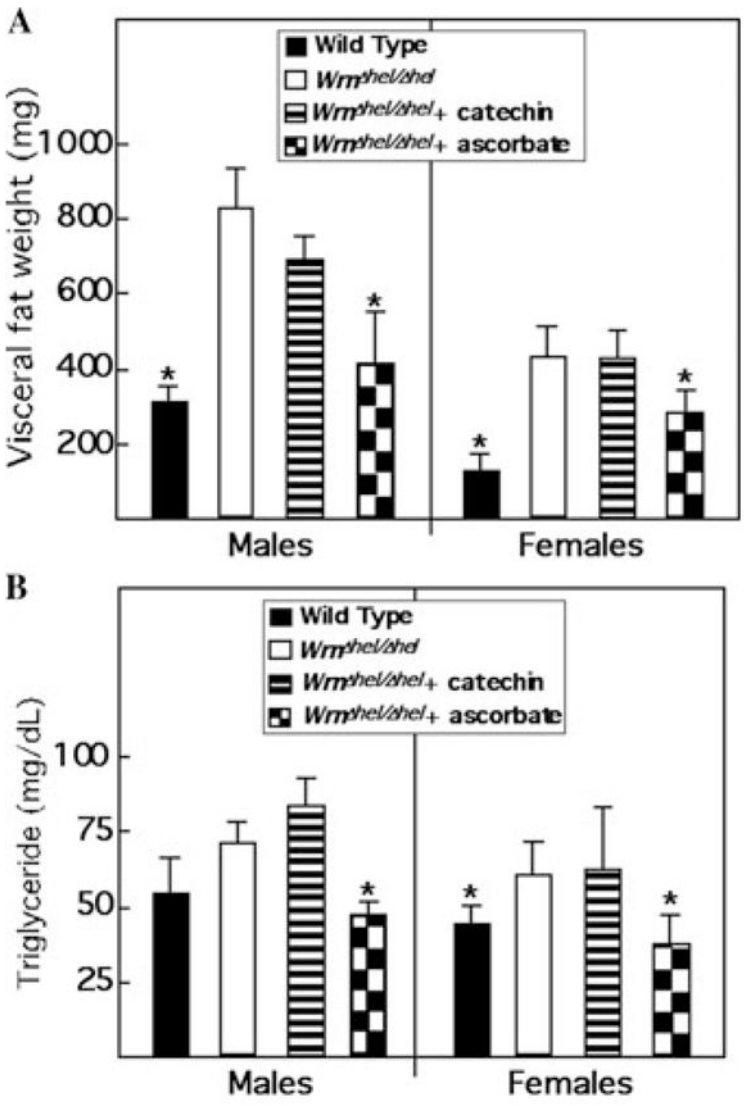

Figure 2.

Impacts of ascorbate and catechin on visceral fat weight and blood triglyceride levels in $Wrn^{\Delta hel/\Delta hel}$ mice. (A) Weight of retroperitoneal (visceral) white adipose tissue in males and females at 9 months of age. (B) Fasting triglyceride levels in serum of untreated wild-type and treated and untreated $Wrn^{\Delta hel/\Delta hel}$ mice at 6 months of age. Five mice of each genotype were evaluated. *, $P < 0.05$ by unpaired *t*-test, compared to $Wrn^{\Delta hel/\Delta hel}$ animals. $Wrn^{\Delta hel/\Delta hel}$ mice were treated with either ascorbate or catechin as described for Figure 1.

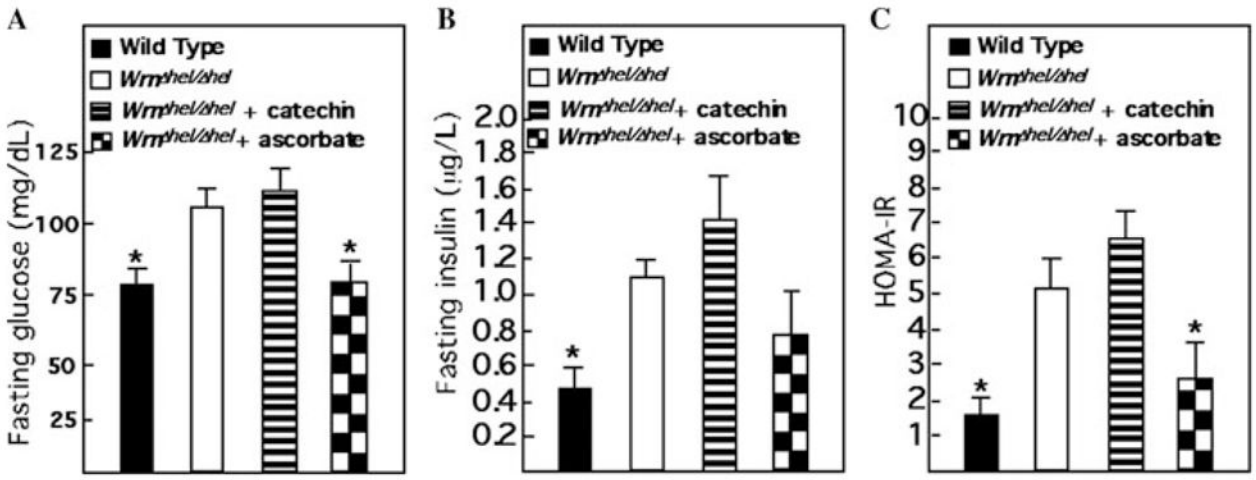

Figure 3.

Impact of ascorbate and catechin on fasting blood glucose, insulin, and HOMA-IR index in $Wrn^{\Delta hel/\Delta hel}$ mice. (A) Fasting blood glucose levels. (B) Fasting insulin levels. (C) Index for the HOMA-IR for fasting mice. *, $P < 0.05$ by unpaired *t*-test compared to $Wrn^{\Delta hel/\Delta hel}$ animals. Bars in the histograms represent the standard errors of the means. Nine-month-old males ($n = 5$) and females ($n = 5$) of each cohort were used for all measurements. $Wrn^{\Delta hel/\Delta hel}$ mice were treated with either ascorbate or catechin as described for Figure 1.