Naturwissenschaften (2010) 97:637–644
DOI 10.1007/s00114-010-0679-6

ORIGINAL PAPER

Detrimental effects of carotenoid pigments: the dark side of bright coloration

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Received: 6 November 2009 / Revised: 6 May 2010 / Accepted: 7 May 2010 / Published online: 22 May 2010

Abstract Carotenoid pigments produce yellow, orange, and red integumentary color displays that can serve as reliable signals of health and condition. In many birds and fish, individuals gain competitive or mating advantages by ingesting and utilizing large quantities of carotenoid pigments. Carotenoid pigments serve as antioxidants, performing important functions as free-radical scavengers. The beneficial effects of carotenoid pigments are well documented, but rarely have researchers considered potential detrimental effects of high-level accumulation of carotenoids. We maintained American goldfinches (*Carduelis tristis*) on high- or low-carotenoid diets through molt and tested for damage to the liver and skeletal muscle. High intake of carotenoids had no measurable effect on liver enzymes but caused an increase in creatine kinase, an indicator of skeletal muscle breakdown, and a reduction in vertical flight performance, a measure of skeletal muscle integrity. The detrimental effects of high-level carotenoid accumulation were approximately equivalent to the negative effects of removing carotenoids from the diet. The adverse effects observed in this study have important implications for theories of the function and evolution of colorful plumage.

Keywords *Carduelis tristis* · Honest signaling · Lutein · Muscle breakdown · Sexual selection

Introduction

Carotenoid-based plumage coloration is a condition-dependent sexual ornament (Hill 2002, 2007). Unlike other pigments used to color integumentary structures such as melanins, carotenoids cannot be synthesized by animals and must be ingested as intact macromolecules (McGraw 2006). Because they must be gathered from the environment, carotenoid pigments are potentially a limiting resource for some species with carotenoid-based color displays (Grether et al. 1999; Hill 2002). Expression of the carotenoid coloration of feathers is adversely affected not just by restricted dietary access to carotenoid pigments (Hill 2002) but also by parasitic infection (Brawner et al. 2000; Hill et al. 2004) and poor nutrition (Hill 2002). Thus, encoded in carotenoid pigmentation can be information about pigment access, health state, and nutritional condition.

In some species of birds and fish, females use expression of carotenoid coloration as a criterion in choosing mates (Hill 2006; Houde 1997). By choosing brightly colored males, females often gain direct resource benefits—more food for themselves or their offspring (Hill 2007), and some studies also hint at genetic benefits acquired by choosy females that may also be associated with expression of carotenoid coloration (Hill and Farmer 2005; Lindstrom and Lundstrom 2000).

Carotenoids can function as a protective mechanism against oxidative damage; they eliminate free-radical scavengers by quenching radical chemical species that have oxidative properties such as singlet oxygen and peroxyl

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radicals (Surai 2002). Carotenoids also suppress the production of reactive oxygen species by rat macrophage cells in vitro, suggesting that they have the ability not only to "clean up" oxidative molecules, but also to prevent their formation (Zhao et al. 1998). It has been proposed that there is a tradeoff in animals between the use of carotenoids for color displays and the use of carotenoids as antioxidants so that only individuals in good condition can produce the brightest and most saturated color display (Lozano 1994; von Schantz et al. 1999). This theory assumes that animals benefit by ingesting and utilizing greater quantities of carotenoids. However, while carotenoids have documented antioxidant properties, specifically in mammals, a meta-analysis conducted by Costantini and Møller (2008) indicated that their antioxidant role in birds may be minor at best.

Alternatively, Zahavi and Zahavi (1997) suggested that carotenoids might also exert detrimental effects on cell membranes, and Olson and Owens (1998) discuss the possibility that detrimental effects associated with carotenoid accumulation could maintain the honesty of the signal. Indeed, there are reasons to suspect that the large-scale accumulation of carotenoids that occurs during avian molt cycles may exert detrimental effects (Olson and Owens 1998). Carotenoids are prone to one-electron oxidations or reductions making them potential prooxidative molecules such as toxic aldehydes that could be damaging to cellular systems, leading to tissue damage (Hartley and Kennedy 2004; Martin et al. 1999; Russel 1999; Palozza 1998; Palozza et al. 1995). Long-term exposure to high levels of carotenoids is also associated with higher incidences of lung cancer and coronary heart disease in humans (Blomhoff 2001). In addition, because carotenoids are lipid-soluble like vitamin A, they may be absorbed into fat, allowing toxicities to persist for months or even years (Hathcock et al. 1990). If the high levels of circulating carotenoids that are required for bright-feather coloration exert detrimental effects, the coloration based on these potentially prooxidative molecules may illustrate a male's resilience to the detrimental effects imposed by exposure to these compounds. So far, however, studies documenting detrimental effects of carotenoids have been done either in vitro systems or in humans or other mammals. It is still unclear whether these effects translate to birds and other animals that routinely accumulate high concentrations of carotenoids in blood.

In this study, we test for physiological costs associated with the accumulation of high levels of carotenoid pigments during molt, a natural process in bird species with ornamental feather coloration (Hill 1995; Negro et al. 1998). We maintained male American goldfinches (*Carduelis tristis*), a small passerine bird species that accumulates high levels of carotenoids while growing bright yellow feathers each spring, on physiologically high- and low-carotenoid diets through the time of natural molt. We then tested for indicators of impairment of the liver and skeletal muscle, tissues that are known to be reservoirs for carotenoids (i.e., liver) and are highly perfused with blood that would be carrying carotenoids (both liver and skeletal muscle). We predicted that if high levels of circulating carotenoids become prooxidative in body tissues, then we would detect signs of greater tissue damage and a decrease in the functional integrity of those tissues in goldfinches supplemented with high compared to low levels of dietary carotenoids.

Materials and methods

Collection and housing

This experiment was conducted over two consecutive molting seasons during 2004 and 2005. Males were captured in mid-January to mid-February of each year using mist nets and basket traps. The experimental period was chosen so supplementation and measurement protocols would coincide with periods of molt and breeding (Middleton 1993) (Fig. 1). Captured males were kept on the Auburn University campus in flocks (N=20 per cage, total N=80 per year) maintained in adjacent outdoor flight cages sharing similar environmental conditions (2.31×2.54×1.16 m). Birds were provided black oil sunflower and thistle seeds for the duration of the experiment. Both seed types have negligible carotenoid content. Sulfadimethoxine was added to water as an anticoccidial agent as this species is prone to coccidial infection in captive flocks (Brawner et al. 2000; McGraw and Hill 2000), and coccidiosis has been shown to reduce intestinal uptake of carotenoids in passerines (Brawner et al. 2000).

Carotenoid supplementation

American goldfinches initiate pre-alternate molt near the beginning of March, acquire carotenoid pigments, undergo molt for approximately 2 months (March and April), and then begin transition into the breeding stage. Male goldfinches were captured in mid-February and were maintained on only sunflower and nyjer seeds, a diet that contains negligible amounts of carotenoids for 7–14 days. For 60 days (through March and April), the birds were maintained on either a low or high-carotenoid treatment. Lutein and zeaxanthin, the dietary precursor pigments that American goldfinches use to synthesize canary xanthophylls a and b, which are, in turn, deposited as feather colorants, were added to each enclosure's water supply in the form of water-soluble starch gel beadlets (DSM Nutritional Products) in a ratio of 70:30 lutein:zeaxanthin. The low-treatment contained 7 mg of lutein and 3 mg of zeaxanthin per liter of water, and the high treatment contained 700 mg of lutein and 300 mg of zeaxanthin per

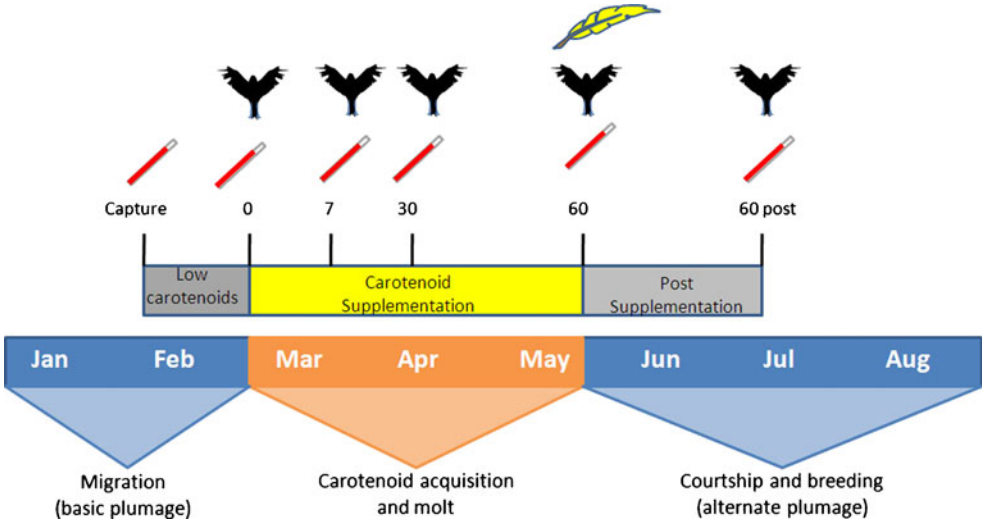

Fig. 1 Experimental timeline: American goldfinches initiate pre-alternate molt near the beginning of March, acquire carotenoid pigments, undergo molt for approximately 2 months (March and April), and then begin to transition into the breeding stage. Male goldfinches were captured in mid-February and were maintained on only sunflower and nyjer seeds, a diet that contains negligible amounts of carotenoids, for 7–14 days. For 60 days (through March and April), the birds were maintained on either a low- or high-carotenoid treatments; after which, treatment ceased and birds were maintained for another 30 days on the sunflower/nyjer seed diet. Blood samples were taken for enzymatic analyses at capture, the day before carotenoid supplementation began, at the start of carotenoid supplementation, at three time points during supplementation, and 60 days after supplementation ceased. Vertical flight challenges were given to a separate subset of birds at similar time points, and feathers were collected for spectral analyses just after supplementation had ceased on day 60 (*capillary tubes* indicate blood sampling time points, *birds in flight* represent vertical flight challenge time points, and the *feather* indicates feather collection)

liter of water. Previous studies indicate that captive American goldfinches ingest approximately 3 ml water per day (McGraw et al. 2005), making daily dosages for this study of 0.03 mg total carotenoids for the low-treatment group and 3 mg total carotenoids for the high-treatment group. The carotenoid supplementation levels for this study were based on levels of carotenoids that are inadequate and just adequate for the brightest and most saturated expression of ornamental coloration in a previous study with American goldfinches (McGraw and Hill 2001; McGraw et al. 2002; Navara and Hill 2003). It is not possible to have a true control for this experiment because a diet lacking all carotenoids is not a situation these birds would ever experience in the wild. The low-treatment group serves as a better comparison in our study because the same dietary carotenoid treatment has been shown to produce circulating plasma carotenoid levels similar to wild, non-molting goldfinches in previous studies (McGraw et al. 2004), and concentrations in non-molting wild goldfinches are well below those in molting wild goldfinches (McGraw and Gregory 2004). Water was changed every other day to minimize carotenoid degradation. After 60 days of carotenoid supplementation, treatment ceased, and birds were maintained for another 60 days on the sunflower/nyjer seed diet.

Feather collection and saturation analysis

Feather coloration was measured to confirm that supplemented carotenoids were being deposited into feathers during the molt period and to compare feather color between birds in the two treatment groups. Feathers were collected the day after carotenoid supplementation ended (day 60, Fig. 1), and feather coloration was measured using a reflectance spectrophotometer following standard techniques as in Shawkey et al. (2006). Reflectance measurements were taken with an Ocean Optics S2000 spectrometer (range 250–880 nm: Dunedin, FL, USA) using a bi-furcated micron fiber optic probe at a 90° angle 5 mm from the feather surface. This 2-mm measurement area was illuminated with both UV (deuterium bulb) and a visible (tungsten–halogen bulb) light source. Reflectance data were generated relative to a white standard (Labsphere, Inc.). Color variables were calculated from spectral reflectance data between 320 and 700 nm. We used yellow chroma (percentages of total light reflected between 575–600 nm) in all comparisons because chroma is a function of carotenoid concentration in yellow feathers (Saks et al. 2003) and concentrating carotenoids in feathers is what most affects coloration in American goldfinches (Andersson and Prager 2006).

Measurement of creatine kinase and aspartate amino transferase

Blood samples were taken from subsets of birds from the high- and low-carotenoid supplemented groups in 2004 and 2005. In 2004, we sampled birds at capture, the day before supplementation began (after 7–14 days on only sunflower

and nyjer seeds and before carotenoid supplementation), and at 30 and 60 days on carotenoid supplementation. In 2005, we sampled a new set of birds more frequently and continued the sampling longer. In the second year of the study, we sampled birds at capture, the day before supplementation began (after 7–14 days on the same diet as 2004), and then at 7, 30, and 60 days on carotenoid supplementation, and 60 days after carotenoid supplementation was removed. Birds were caught with butterfly nets from outdoor aviary enclosures and placed in brown paper bags until samples were collected. All birds were bled within 1 h of capture. Venipuncture was performed by nicking the brachial (wing) vein and collecting the blood using heparanized microhematocrit tubes. The blood samples were refrigerated until centrifuged. Samples were centrifuged at 14,000 rpm for 6 min, and plasma was collected and stored at −20°C until assayed for liver and muscle enzymes. The same birds were never sampled twice for these measures.

To test liver function, aspartate amino transferase (AST), an enzymatic indicator of hepatocellular damage in avian species (Jaensch et al. 2000), was measured in the 2004 experiment. In addition, creatine kinase (CK) was measured as an indicator of systemic muscle degradation (Harr 2002; Jaensch et al. 2000) in both the 2004 and 2005 experiments. All plasma enzyme analyses were performed using Roche automated clinical chemistry analyzers by Auburn University School of Veterinary Medicine Clinical Pathology Laboratory. In 2004, plasma samples were analyzed for AST and CK within 30–90 days of freezing. In 2005, plasma samples were analyzed for CK 30–60 days after freezing. AST frozen at −20°C has been shown to be stable up to 3 months (Lawson et al. 1982), and CK has been documented to be stable up to 50–90 days (Jakubowski et al. 1998; Lawson et al. 1982).

Muscle performance

To test for functional skeletal muscle degradation, an assessment of pectoral muscle performance was used in the 2005 experiment. The pectoral muscle mass comprises the majority of skeletal muscle in songbirds (Hartman 1961) and produces the most aerodynamic power during the downstroke (Askew and Marsh 2002). Muscle performance was tested by measuring the height that individuals could fly vertically (similar to Veasey et al. 1998) because vertical ascent flight has been shown to be the most challenging flight type in some bird species (Dial and Biewener 1993). The vertical flight performance test was conducted by placing a single bird in a rectangular Plexiglas® chamber measuring 122 cm (h)×20 cm (w)× 20 cm (d) (Fig. 2). Width and depth of the chamber dimensions were chosen to be about 2.5 cm smaller than

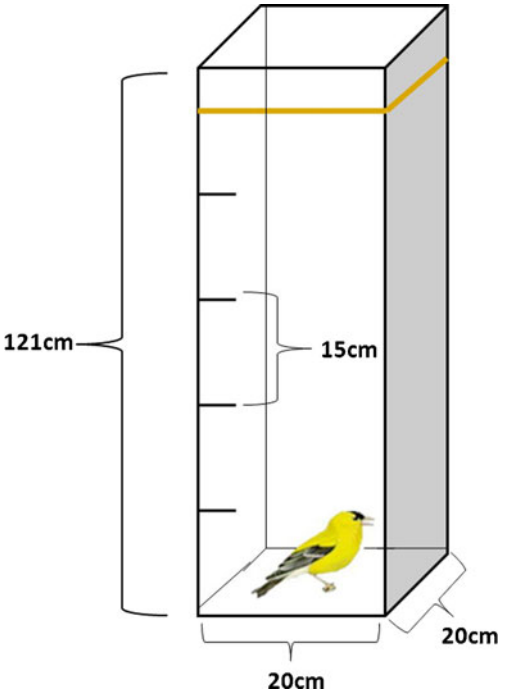

Fig. 2 Diagram of the chamber used to measure vertical flight performance

the average wingspan of American goldfinches (22.86 cm) (Sibley 2000), so individuals were forced to fly vertically inside the chamber. Birds were given 30 s to become familiar with their surroundings and were then stimulated by a research technician approaching the chamber. Subsequently, the first two attempted flight heights were recorded, and the mean was used in statistical analyses. Vertical flight challenges were recorded for subsets of birds from different carotenoid treatments prior to carotenoid supplementation, after 7, 30, and 60 days on carotenoid supplementation and 60 days following carotenoid removal.

Statistical analyses

All measures were initially tested for normality. Because plasma enzyme values as well as the vertical flight performance were non-normally distributed, these values were log transformed. Log transformation was successful in normalizing the AST values. Initial differences in AST values for all birds (prior to division into treatment groups) between the capture date and 2 weeks in captivity were analyzed using a one-way ANOVA on log-transformed values. The effects of carotenoid treatment group on log-transformed AST levels at all sample dates were analyzed using a two-way ANOVA. Log transformation did not successfully normalize CK values nor the performance measures, so non-parametric statistics was used for these analyses. Mann–Whitney *U* tests indicated that CK values did not differ significantly between the 2004 and 2005

experiments and, thus, observations from those years were combined. A Kruskal–Wallis test was used to determine the effect of sample period on enzymatic measures for each carotenoid treatment and a post hoc non-parametric multiple comparison test was used to determine which sample periods differed significantly from one another (Sokal and Rohlf 1995). The effect of treatment on vertical flight performance was analyzed using a Mann–Whitney *U* test within sample period and between sample periods. When multiple comparison were done using Mann–Whitney *U* test, the significant alpha levels were recalculated using a Bonferroni correction. Finally, yellow chroma was compared between the treatment groups using an unpaired *t* test.

Results

After carotenoid supplementation, birds on the high-carotenoid treatment were significantly more colorful—yellow chroma was significantly greater in feathers collected from high-carotenoid birds ($p<0.0001$; $N=20$; Fig. 3a). These results are similar to those shown in a previous study using the same dietary treatments (Navara and Hill 2003) and suggested that birds did, in fact, accumulate high levels of carotenoids.

After several weeks in captivity on diets containing few carotenoid pigments (i.e., prior to the start of carotenoid supplementation experiment), both AST and CK levels were significantly elevated compared to levels at the time of capture (AST, $F_{1,20}=5.20$, $p=0.033$; CK, Z value$=-3.16$, $p=0.0003$; Fig. 3b). Once supplementation began (i.e., at the 7- and 30-day sample), AST and CK levels significantly decreased in both high- and low-carotenoid groups when compared to levels found in the captive non-supplemented sample (AST, $F_{3,77}=4.82$, $p=0.004$; CK, H corrected for ties$=36.28$, $p<0.001$; Fig. 3b). In fact, the AST levels at this time point did not differ significantly from those at capture ($p=0.85$), and CK levels at this point were even significantly lower than those at capture (tied p value$=0.019$; Fig. 3b).

While immediate effects of carotenoid accumulation appeared positive (i.e., a decrease in degradative enzymes), we also observed a potentially negative effect of high levels of circulating carotenoids that occurred over a longer time period. Birds in both the high- and low-carotenoid treatment groups exhibited significantly elevated CK levels after 60 days of supplementation (H corrected for ties$=34.22$, $p<0.0001$, Fig. 3b). Sixty days after removal from the carotenoid-supplemented diet, CK levels in birds from the high-carotenoid treatment group was significantly higher than that observed in the low-carotenoid group ($Z=-2.663$, $p=0.008$; Fig. 3b) whose CK values remained virtually unchanged from those observed late in supplementation (60 day sample). We did not find significant

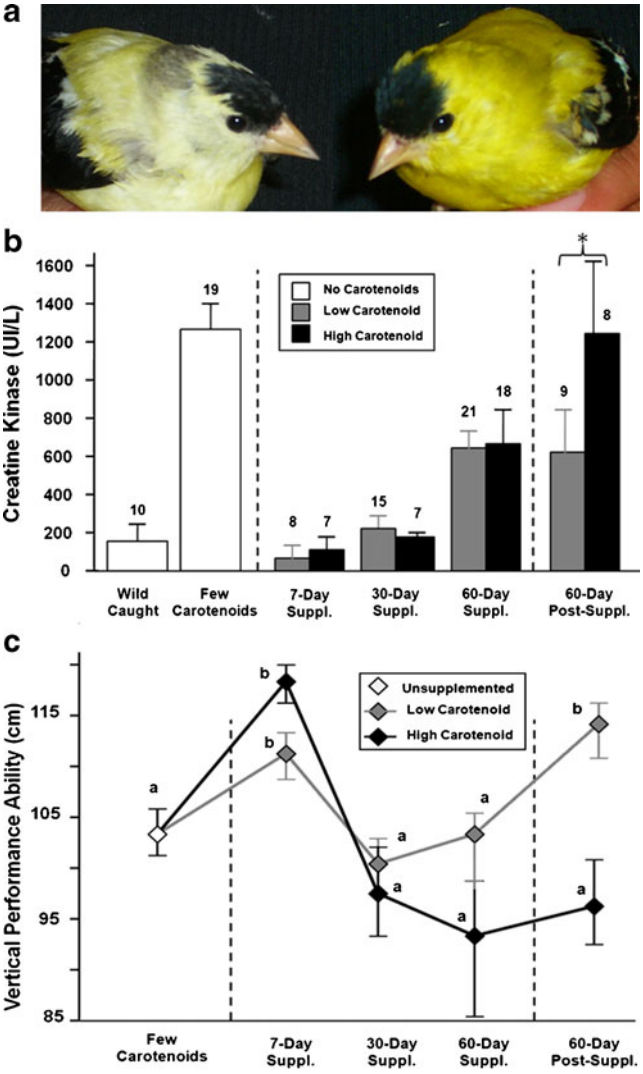

Fig. 3 Effects of high- and low-carotenoid supplementation on American goldfinches. **a** Carotenoid-based yellow feather coloration in male American goldfinches resulting from low- (*left*) and high- (*right*) carotenoid supplementation during molt. **b** Mean (±SE) plasma creatine kinase (CK) assessed in wild-caught birds, after 10–30 days in captivity on a diet containing few carotenoids and at four experimental time points during and after carotenoid supplementation. Significant differences are indicated by an *asterisk* and sample sizes are included above the bars. **c** Mean (±SE) vertical flight performance assessed at similar experimental time points to those describe in **b**. Significant differences are represented by different *lowercase letters*. Sample sizes for the vertical flight performance measures are as follows. Few carotenoids, $N=37$, day 7—low$=16$, high$=20$, day 30—low$=17$, high$=19$, day 60—low$=14$, high$=19$, day 60 post—low$=12$, high$=9$

effects of high-level carotenoid accumulation on liver function measured as plasma AST concentrations in either treatment group in the course of the experiment ($F_{2,1,73}=1.08$, $p=0.36$).

During the same periods in which we measured an increase in CK (indicative of increased breakdown of skeletal muscle) in the high-carotenoid group, we also

saw a decline in vertical flight performance for birds in this same group. Initially (i.e., after only 7 days of carotenoid supplementation), birds in both the high- and low-carotenoid treatment groups showed a significantly elevated vertical flight performance compared to their previous performance on the non-supplemented diet (high, $Z=-4.175$, $p<0.0001$, low, $Z=-2.570$, $p=0.0006$; Fig. 3c). After 30 days of supplementation, vertical flight performance of birds in both treatment groups significantly decreased from that observed 7 days after supplementation (high, $Z=-4.826$, $p<0.001$, low, $Z=-2.641$, $p=0.007$; Fig. 3c). The flight performance measures did not differ significantly between the two treatment groups until 60 days after carotenoid supplementation was removed, when the performance of birds in the low-carotenoid treatment group significantly improved ($Z=-4.82$, $p=0.001$), while the performance of the high-carotenoid birds remained low. At this point, low-carotenoid birds were able to fly significantly higher compared to high-carotenoid birds ($Z=-2.629$, $p=0.0086$; Fig. 3c).

Discussion

Carotenoids present a double-edged sword to molting American goldfinches. Males need carotenoid pigments to color their feathers, and they may benefit from antioxidant properties of carotenoids, but maintaining high levels of circulating carotenoid pigments can cause tissue damage. These statements are supported by the changing responses to carotenoids shown by male American goldfinches through our experiment. Our results indicate that ingestion of both moderate and high levels of carotenoids had short-term physiological benefits. Measures of enzymatic degradation after 1 week of limited access to carotenoids increased significantly compared to capture levels. Once the birds were returned to diets containing carotenoid supplements, these enzymatic indicators significantly declined, returning to levels observed at the time of capture. Perhaps carotenoids provided protection from oxidative stress. However, we also found that long-term ingestion of high levels of carotenoids resulted in skeletal muscle tissue deterioration and a decrease in flight performance.

Increasing intake of carotenoid pigments for ornamental coloration may have initial beneficial effects as some carotenoids have been shown to reduce oxidative stress in vertebrates (Surai 2002). The initial drop in levels of both AST and CK that we observed in goldfinches following the onset of carotenoid supplementation provides evidence for beneficial effects associated with carotenoid ingestion, as these measures serve as indicators of liver and skeletal muscle breakdown, respectively. Despite these indications of a potential initial benefit, however, accumulation of carotenoid pigments at the levels needed for brilliant pigmentation of thousands of contour feathers in male goldfinches, appears to exceed levels of pigment concentration that are beneficial and to exert a stress on organ systems in the long term. Costantini and Møller (2008) suggest that while carotenoids serve as potent antioxidants in mammals, the reliance on their antioxidant function by birds is likely minimal.

Instead, we found that levels of carotenoid accumulation naturally experienced by molting goldfinches exerted detrimental effects. We found two independent lines of evidence for deterioration of skeletal muscle under high-carotenoid supplementation. First, after 60 days of carotenoid supplementation, CK levels in both the low- and high-carotenoid treatment groups rose significantly, and after supplementation had ceased, blood levels of CK were significantly elevated in the high-carotenoid treatment group when compared to the low-carotenoid treatment group. Concentrations of creatine kinase in blood have previously been used as an indicator of muscle damage in migratory birds (Guglielmo et al. 2001), but the interpretation of how creatine kinase concentrations can influence muscle function and thus flight is often difficult. In horses, significant muscle damage is accompanied by an approximate 100-fold increase in creatine kinase concentrations (Volfinger et al. 1994). However, in humans, even a threefold increase is enough to indicate significant muscle damage or disease (O'Connell et al. 2002). Here, goldfinch CK and AST levels were within similar ranges to those found in other avian species measured (Bollinger et al. 1989; Mitchell and Sandercock 1995; Lanzarot et al. 2005). Birds in the high-treatment group showed an approximate sixfold increase in CK levels over time, but the relationship between the range of CK values and the magnitude of functional muscle damage is clearly species specific. For that reason, it was necessary to characterize muscle function in addition to CK concentrations in response to carotenoid supplementation. Vertical flight performance, a measure of pectoral muscle efficiency, decreased markedly in both treatment groups after only 7 days on carotenoid supplementation. By 60 days after supplementation had ceased, birds in the high-carotenoid treatment group showed a significantly impaired vertical flight performance compared to those in the low-carotenoid treatment group. These data suggest that high levels of dietary carotenoids may stimulate skeletal muscle breakdown and related performance challenges in the long term. Additionally, the observed detrimental effects occurred at the time of year when these birds would typically initiate breeding behaviors. Exposure to high levels of carotenoid accumulation during pre-alternate molt may impair flight performance in the subsequent breeding period for those birds that cannot deal with the detrimental effects carotenoids, and this negative effect of carotenoids may present a formerly unconsidered cost associated with carotenoid display.

The question remains, however, why it took 60 days for the detrimental effects of high-level carotenoid accumulation to present and why these effects occurred after carotenoid supplementation had ceased. We suggest that carotenoids provided during the molt period were primarily deposited into feathers, as was evident from the variation in feather saturation between the two treatment groups. In fact, the feathers taken from birds in the high-carotenoid treatment group did not exceed the highest chroma observed in wild goldfinches, suggesting that these birds were likely using a majority of the available carotenoids towards creating feathers with high-pigment concentration. Upon ingestion, carotenoid pigments are absorbed unchanged from the intestine into the lymphatics, where they are either sent whole into circulation or shuttled to the liver for metabolism. Pigments and metabolites are mobilized in the blood as components of the very-low-density lipoprotein particles and are accumulated in the adipose tissue due to their lipophilic nature (Blomhoff 2001; Schmitz et al. 1991). Perhaps the long-term effects seen after feather development was completed are due to the slow, long-term release of these lipophilic carotenoids from the fat stores, as is often the case with lipid-soluble vitamins (Hathcock et al. 1990).

Despite the effects of high-level carotenoid accumulation on skeletal muscle breakdown and function, liver function appeared unaffected by the treatment. The reasons for the different responses by liver and skeletal muscle are unknown. Perhaps, as in newly hatched chickens, the performance of antioxidant systems are higher in the liver relative to other tissues (Gaal et al. 1995), allowing for greater exposure to pro-oxidants without detriment to organ integrity.

In male American goldfinches, expression of yellow feather coloration is positively related to female mate preferences (MacDougall and Montgomerie 2003); male goldfinches that ingest and mobilize large quantities of lutein and zeaxanthin achieve brighter and more saturated yellow coloration (McGraw et al. 2005) and are then better able to attract females. Indeed, the degree of carotenoid supplementation in our study directly affected plumage saturation, as males in the high-carotenoid treatment group had plumage that was significantly more saturated when compared with males in the low-carotenoid treatment group. The yellow chroma measured in birds of both treatments fell within the range of plumage saturation found in wild-caught goldfinches during molt by McGraw and Gregory (2004), suggesting that the birds in this study were, in fact, accumulating carotenoid concentrations similar to levels that molting goldfinches accumulate naturally.

Carotenoid-based color displays are proposed to be honest signals because of the challenges of concentrating pigments in feathers (Hill 2002). Parasites, poor nutrition, and inability to ingest sufficient carotenoid pigments have all been shown to diminish expression of carotenoid-based coloration (Hill 2002). Another proposed cost of carotenoid-based coloration is that carotenoid pigments used as colorants are not available to function as free-radical scavengers (Lozano 1994; von Schantz et al. 1999). This latter hypothesis assumes that carotenoid pigments are limiting such that there are tradeoffs between body maintenance and ornament display. Here, we provide evidence that not only are carotenoids not limiting for use as antioxidants when they are accumulated in high concentrations as feather pigments, but that the very high levels of carotenoids needed to color feathers poses a challenge to biological systems. The costs of high levels of carotenoid accumulation will have to be considered in studies of carotenoid-based color signals.

Acknowledgements Birds were collected and handled according to federal (# 21661), state (#97181), and IACUC (2005-0825) permits. We thank Erin Carroll, Tyler Hicks, and Maranda B. Wood-Holley for assistance with collecting and husbandry and Bob Montgomerie for use of his software for calculating yellow chroma. Work was funded by NSF grants IBN0235778 and DEB0218313 to G.E.H.

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