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Breaking down, starting up: can a vitamin C–enriched gelatin supplement before exercise increase collagen synthesis?[1]

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Obesity is a major challenge to maintaining health (1) and is associated with the metabolic syndrome, whose components include diabetes, hypertension, and hypercholesterolemia (2, 3). Complications from the metabolic syndrome include microvascular disease, heart disease, kidney disease, blindness, and stroke. These complications result in suffering and decreased life span. Strategies to decrease obesity have enormous possibility to improve health, relieve suffering, and reduce health care costs (4, 5).

When considering therapeutic options for the obese patient, a linchpin in management is exercise (5, 6). To have a better chance for success, exercise is coupled with dietary modifications. Together, these options have the potential to decrease food intake while increasing the utilization of excess stored fat. Unfortunately, a major concern of exercise is injury, especially to soft tissues, including tendon and ligament breakdown and muscle damage (6). Indeed, the occurrence of exercise-related injuries is not limited to obese subjects and is found across the spectrum of people who engage in exercise, from recent initiates to athletes (7, 8). Exercise-related injuries increase suffering and place unexpected additional burdens on many patients, regardless of weight. For the overweight person, an exercise-induced injury may be particularly burdensome. Because an injury often necessitates rest, obesity that exercise was intended to mitigate may conversely worsen until an injury heals. Recommendations after exercise-induced injury have remained similar for years and include analgesia, rest, heat or cold application locally, and physical therapy (7, 9). Physical therapy adjuncts include electrical stimulation and ultrasound, with the more recent addition of acupuncture. Despite these options, standards and guidelines are often lacking, and new therapeutic approaches are decidedly needed.

A major therapeutic advance would be one that increases the speed of musculoskeletal, ligament, and/or tendon repair. Methods considered have shown a key role of animal models and engineering models that used animal tissues. Although these systems certainly can provide clues to hasten healing, challenges remain. Engineered systems have used chicken tendons as source material (10). It is unclear whether avian findings will apply to humans. Rat models have also been used (11). In these models, injuries can be introduced by utilizing appropriate animal protocols to minimize suffering. Either before or after injury, targeted dietary modifications can be made and their effects on recovery assessed. Unfortunately, the animal-to-human limitation remains. Furthermore, although dietary additions may have some effects, doses are impractical or unacceptably high when considered for human use (12).

In this issue of the Journal, Shaw et al. (13) studied whether gelatin supplementation in a vitamin C–containing beverage could increase collagen synthesis. The investigation used merging in vitro and in vivo techniques. One separate human subject was the source of cells that were used to grow all of the engineered ligaments in the study. The ligaments were treated with 10% serum obtained from the study participants. Eight healthy men participated in each of the 3 study arms. For every study arm, there were 9 periods of rope skipping, for 6 min/period, spread over 72 h. Subjects were allowed rest days, and then repeated the study until all 3 treatment arms were completed. The treatment arms, in random order, comprised a gelatin supplement with either 5 or 15 g gelatin or a placebo. All treatments were provided as a dry powder and were dissolved in a beverage containing 48 mg vitamin C. To determine the effects of treatment on engineered ligaments, blood was drawn once before and once 1 h after the ingestion of the treatment. Serum was obtained from these samples and incubated with the ligaments. Blood was drawn at multiple time points for amino acid measurements and assessment of N-terminal peptide of procollagen.

The N-terminal procollagen peptide measurements were new and informative. These measurements are indicative of bone turnover rather than of new collagen synthesis in cartilage and tendons. Nevertheless, these data are provocative, because they suggest that providing more amino acid substrates via the gelatin nutritional intervention increased bone collagen synthesis in vivo after exercise in a dose-dependent fashion. Whether this increased synthesis has clinical benefit should be a subject of future work.

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As a mixture of collagen, gelatin was expected and found to increase blood concentrations of amino acids found in collagen, consistent with both the collagen peptide data and previous work. Engineered ligaments, single-subject derived, were treated with 10% serum from participants. The experimental media for the ligaments contained only minimal amino acids with antibiotics other than the added serum. Increasing gelatin consumption produced more collagen in the engineered ligaments. This finding is expected, given that gelatin increased amino acids that are collagen derived in serum and that are necessary for its synthesis.

Gelatin had no effect on the mechanical properties of the ligaments that were treated with serum. Instead, one-time treatments of placebo and low- and high-dose gelatin all showed an increase in mechanical properties of the engineered ligaments. The comparison was to control ligaments that were treated with serum obtained before the administration of any intervention. Because all of the treatments were administered with a beverage containing vitamin C, it is tempting to conclude that increased mechanical effects in the ligaments were a consequence of the 48 mg vitamin C consumed in the beverage. It is not possible to be sure, however, because the vitamin C measurements in serum or plasma from the subjects before or after the beverage was consumed were not provided. Even so, it is unlikely that vitamin C in the beverage contributed to the findings, for several reasons. On the basis of the bioavailability data, 48 mg vitamin C ingested orally will have minimal effects on plasma concentrations, with a predicted increase of $\leq$10%, unless subjects were deficient at baseline (14, 15). Although this is possible, it is not likely in 8 healthy athletic subjects, at least some of whom would be expected to make their food choices carefully. Such minor changes in vitamin C plasma concentrations would be very unlikely to increase collagen synthesis. A test of whether vitamin C played a role in these experiments would have been to use a beverage with no vitamin C as a control. Attention to vitamin concentrations before and after an experimental manipulation, rather than only to dose, remains key to understanding clinical outcomes (16).

Vitamin C is required for hydroxylation of proline and lysine residues on procollagen (17, 18). In addition, collagen production was increased by vitamin C independently of hydroxylation of amino acids, via increased gene transcription of procollagen (19, 20). It is likely that gene transcription changes induced by vitamin C were due to the inadvertent formation of lipid peroxides (21, 22). Lipid peroxides are oxidants that may form in cell culture systems due to ascorbate and iron, via Fenton reactions (23–25). Whether these reactions have a consequence in vivo is unknown. Nevertheless, it is improbable that these reactions would occur in vivo with a change in plasma vitamin C of $\leq$10%. Rather, multiple hormone signaling accompanies calorie ingestion (26, 27), and it is much more likely that $\geq$1 of these signaling changes in serum mediated the observed effects on mechanical properties of the ligaments rather than a minimal change in vitamin C. A conclusive interpretation of results with the ligaments presented here is limited by representative display of data that were obtained by treating ligaments with serum from only 1 subject, in addition to the use of cells from 1 subject to form ligaments.

This study does not provide definitive answers to improving tendon and ligament strength postexercise in humans. Despite limitations, the data here present a new way forward by merging in vitro and in vivo systems to provide answers to questions about optimizing nutritional conditions for exercise. Studies like these serve as a foundation for the conduct of outcome-based clinical studies. Definitive answers will come from studies in which patients are administered different treatments in blinded fashion and in which an in vivo consequence, or lack thereof, is measured.

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