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Inhibition of Platelet Activation by Lachrymatory Factor Synthase (LFS)-Silenced (Tearless) Onion Juice

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Supporting Information

ABSTRACT: Onion and garlic are renowned for their roles as functional foods. The health benefits of garlic are attributed to di-2-propenyl thiosulfinate (allicin), a sulfur compound found in disrupted garlic but not found in disrupted onion. Recently, onions have been grown with repressed lachrymatory factor synthase (LFS) activity, which causes these onions to produce increased amounts of di-1-propenyl thiosulfinate, an isomer of allicin. This investigation into the key health attributes of LFS-silenced (tearless) onions demonstrates that they have some attributes more similar to garlic and that this is likely due to the production of novel thiosulfinate or metabolites. The key finding was that collagen-induced in vitro platelet aggregation was significantly reduced by tearless onion extract over normal onion extract. Thiosulfinate or derived compounds were shown not to be responsible for the observed changes in the inflammatory response of AGS (stomach adenocarcinoma) cells to tumor necrosis factor alpha (TNFα) when pretreated with model onion juices. A preliminary rat feeding trial indicated that the tearless onions may also play a key role in reducing weight gain.

KEYWORDS: *Allium cepa, thiosulfinate, tearless onion, LFS-suppressed onion, platelet aggregation, IL-8*

INTRODUCTION

Plants from the genus *Allium*, especially onion and garlic, have long been cultivated for their health effects and flavors.[1] These are due to the unique sulfur chemistry of members of this genus, which store large amounts of alkylated cysteine sulfoxides and specialized enzymes that break these down when tissues are damaged. In onions, *Allium cepa* L., the main alkylated cysteine sulfoxide is 1-propenylcysteine sulfoxide (isoalliin) (**1**, Figure 1), which is the substrate for a series of

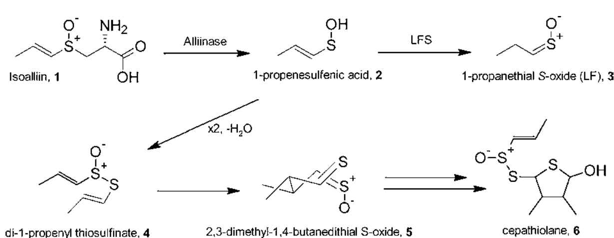

Figure 1. Major sulfur metabolism in cut onions.

rapid reactions initiated upon onion cutting. The enzyme alliinase (EC 4.4.1.4) catalyzes the conversion of **1** to (*E*)-1-propenesulfenic acid (**2**), which is then rearranged to the volatile and highly reactive lachrymatory factor (LF) (*Z*)-propanethial S-oxide (**3**) by the closely associated LF synthase (LFS) (Figure 1).[2] In garlic, the main alkylated cysteine sulfoxide is 2-propenylcysteine sulfoxide (alliin). This is also acted upon by alliinase but converted to 2-propenesulfenic acid, which condenses to di-2-propenyl thiosulfinate (allicin).

Recently, a Japanese–New Zealand collaboration produced genetically modified onions with the LFS silenced, producing tearless onions.[3] The reduced production of LF (**3**, Figure 1) was demonstrated, causing the onion to be termed "tearless". In the LFS-silenced onions di-1-propenyl thiosulfinate (**4**), an isomer of allicin, was expected to be formed.[3] Compound **4** and isomeric forms have been synthesized and shown to be thermally unstable.[4] Recent analysis of LFS-silenced onion has shown compound **4** is rearranged to a cepathiolane (**6**) via **5** by further reaction with 1-propenesulfenic acid (**2**).[5]

The health attributes of alliums are well documented[6,7] and include references to fructans as a soluble fiber for gut health,[8] flavanoids and quercetins as powerful antioxidants,[9,10] and γ-glutamyl transpeptides for bone health,[11] anticancer attributes,[12] and cardiovascular health.[13] Allium sulfur compounds have been shown to block tumor necrosis factor alpha (TNFα)-induced inflammatory pathways by inhibiting nuclear factor kappa B (NF-κB) activation.[14] Cardiovascular health research has focused on the ability of various allium-derived thiosulfinates to inhibit human platelet aggregation,[15] and research has shown garlic-derived sulfur compounds to be approximately 14 times better at reducing platelet aggregation than onion-derived compounds.[16] This was attributed in garlic to the presence of allicin. Initial in vitro investigations of synthesized cepathiolane compounds demonstrated inhibition

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of cyclooxygenase-1 (COX-1) that catalyzes the formation of prostaglandin G2 from arachidonic acid, a crucial step in platelet aggregation.[5] This indicated that the sulfur compounds in tearless onions may produce a response more akin to that of garlic. The study also linked the cepathiolane to antidiabetic activity through their ability to inhibit α-glucosidase activity, an enzyme important in the digestion of carbohydrates.[5]

The first objective of this work was to determine whether onion extracts from tearless and normal onions had differing human platelet aggregation activities, as suggested by the recent model studies.[5] The second objective was to see if the tearing and tearless onion-derived sulfur compounds differed in their ability to suppress the inflammation response by inhibiting NF-κB, thus preventing production of IL-8 anti-inflammatory response. Third, we undertook a preliminary study using the laboratory rat to model in vivo effects of tearing and tearless onion juice. These data in conjunction with other recent findings are discussed.

■ MATERIALS AND METHODS

Plant Material. Transgenic green fluorescent protein (GFP) positive, LFS-silenced dehydration onion line D2[3] was crossed with its original nontransgenic dehydration parent and in a second cross to a brown intermediate day-length Pukekohe Longkeeper (PLK) type. The progeny from these crosses produced GFP-positive and GFP-negative individuals as observed by fluorescence microscopy, indicating that LFS-silenced and LFS-active progeny had been produced. The cross with the parent onion produced white dehydration type onions, whereas the cross with the PLK onion produced visually brown fresh market type onions. The visually brown type onions were grown to maturity and selfed to produce a population of brown and white onions that segregated for GFP-positive and -negative offspring, again indicating inheritance, or not, of the LFS-silencing phenotype. This was supported by DESI-MS analysis of sample material that confirmed the reduced LF and increased thiosulfinate phenotype of these plants.[17] Material was grown in a Biotron growth chamber (Lincoln University) with temperature and daylight conditions changed every 3 months to reflect external conditions to produce mature bulbs. Mature bulbs, stored for up to 4 months, were used in bioactivity assays.

Nontransgenic PLK onion bulbs and Printanor garlic cloves were used as source material for the production of model onion and garlic juice.

Preparation of Model Onion and Garlic Extracts. Alliinase was prepared from fresh garlic cloves following the procedures previously described.[2] Recombinant LFS (rLFS) and purified isoalliin were supplied by Housefoods Corp., Chiba, Japan. Inactivated onion juice and garlic juice was prepared by microwaving 250 g of cut fresh bulb material under plastic wrap for 6 min at 600 W. This was mixed 1:1 with water and homogenized. Juice was extracted by filtration, and isoalliin or alliin concentrations were determined as previously described.[3] Model tearless onion juice and model garlic juice were prepared by mixing inactivated onion juice or inactivated garlic juice with alliinase; model onion juice was prepared by mixing inactivated onion juice with alliinase and rLFS. A model system utilizing purified isoalliin mixed with alliinase, ± rLFS, was also used to specifically investigate the reaction with the isoalliin compound. In all cases initial starting alliin and isoalliin concentrations were normalized to 1 μg/μL.

Addition of alliinase ± rLFS was set as reaction time t = 0. Model extracts were incubated at room temperature and analyzed at various time points ranging from 30 s to 120 min after the addition of alliinase ± rLFS.

Preparation of Transgenic Onion Extracts. Due to the logistics of assay testing and the need to capture very early time points in the reaction mix of onion extracts, onion material was ground in the presence of liquid nitrogen and stored at −80 °C. Transgenic onion bulbs were sectioned; one portion was frozen in liquid N_2 and ground to a fine powder, and another portion was microwaved for isoalliin determination (as described earlier). For platelet aggregation assays, onion powder was mixed with water to give 1 μg/μL of estimated isoalliin. Samples were centrifuged at 14000 rpm for 1 min and then passed through a 0.4 μm filter.

The addition of water to onion powder was set as reaction time t = 0. Samples were incubated at room temperature and analyzed at various time points.

Human Platelet Preparation. Whole blood was taken from healthy volunteers, males and females between the ages of 25 and 45 years of age, under informed consent. Volunteers were well-hydrated, without any medication or supplements, and had abstained from consuming alliums for at least 48 h prior to blood sampling.

Platelet extraction was carried out essentially as described by Allison et al.,[18] with some modifications. Whole blood was mixed with trisodium citrate (3.8 wt %/vol) at a ratio of 9:1, whole blood/trisodium citrate, and centrifuged at 200g for 15 min. Centrifugation was carried out using a swinging bucket rotor (Eppendorf 5810R) at room temperature with no braking. Platelet-rich plasma (PRP) was transferred to a fresh tube; platelet-poor plasma (PPP) was extracted following a further centrifugation of the red cell fraction at 1500g for 20 min.

Platelet counts were estimated by staining a small aliquot of PRP with malachite green and counting on a hemocytometer. When needed, PRP was diluted using PPP to approximately 250 × 10^6 platelets/mL. Samples with low platelet counts were excluded. Platelets were used within a 3 h period from the time of blood taking.

Human Platelet Aggregation. Platelet aggregation was measured using a four-channel Aggram aggregometer (Helena Laboratories) following the Canterbury District Health Laboratories (CDHB, Christchurch, New Zealand) in-house protocol.

PRP (225 μL) was gently mixed with 5 or 10 μL (5 or 10 μg estimated alliin or isoalliin) of model onion or garlic mixtures (after incubation at room temperature for 30 s and 5, 30, or 120 min) or 5 or 10 μL (5 or 10 μg estimated isoalliin) of transgenic onion extract (after incubation at room temperature for 5, 15, 30, 60, 90, or 120 min). As a control, 10 μL of phosphate buffer saline (PBS, pH 7.4) was used. Then 225 μL was transferred to a cuvette and incubated at 37 °C for 2 min. Aggregation was activated by the addition of 25 μL of ADP (80 μM, final 8 μM) or 25 μL of collagen (20 μg/mL, final 2 μg/mL). Aggregation was followed for up to 5 min at 37 °C, and aggregation was measured as a percentage of aggregation and compared to that of the PBS control, which was repeated at least three times.

Maximum aggregation was measured for all assays. When the maximum aggregation of the PBS control sample was found to be <75%, results from these individuals were excluded as it was generally found that volunteers did not meet all stipulated conditions such as eliminating allium from the diet. Affects on aggregation by the model onion and garlic juice and transgenic onions were calculated as a percentage of the average maximum aggregation for the PBS control.

IL-8 Anti-inflammatory Assay. Assays were carried out using a sublethal dose of model onion or garlic extract. Briefly, 3 × 10^5 AGS cells were cultured overnight in 12-well tissue culture plates containing 2 mL of F12 media + 10% FCS. Fresh medium was added to cells 1 h prior to treatment. Model onion and garlic extracts were prepared as described earlier. Following incubation at room temperature for 5, 20, or 120 min, extracts were added to AGS cells (20 μg isoalliin/mL media, at 1 μg/uL) and incubated for 10 min at 37 °C prior to stimulation with TNFα (10 ng/mL) (Invitrogen). TNFα treatment alone was used as a positive control. Duplicate samples of 100 μL of medium were removed for IL-8 assays at 0, 1, 2, 3, 4, 6, and 8 h following TNFα stimulation. Addition of TNFα was set as time t = 0.

IL-8 concentrations were measured by ELISA following standard procedures as outlined in the manufacturer's instructions (R&D Systems). Additional human CXCL/IL-8 capture antibody and anti-hIL8 detection antibody were obtained from Pharmaco NZ Ltd., and streptavidin−HRP was obtained from R&D Systems. In brief, 50 μL of sample medium was used for the ELISA assay, and these were carried out in duplicate. Once reactions were stopped, sample color was measured at 450 nm using a SpectraMAX 190 (Molecular Devices)

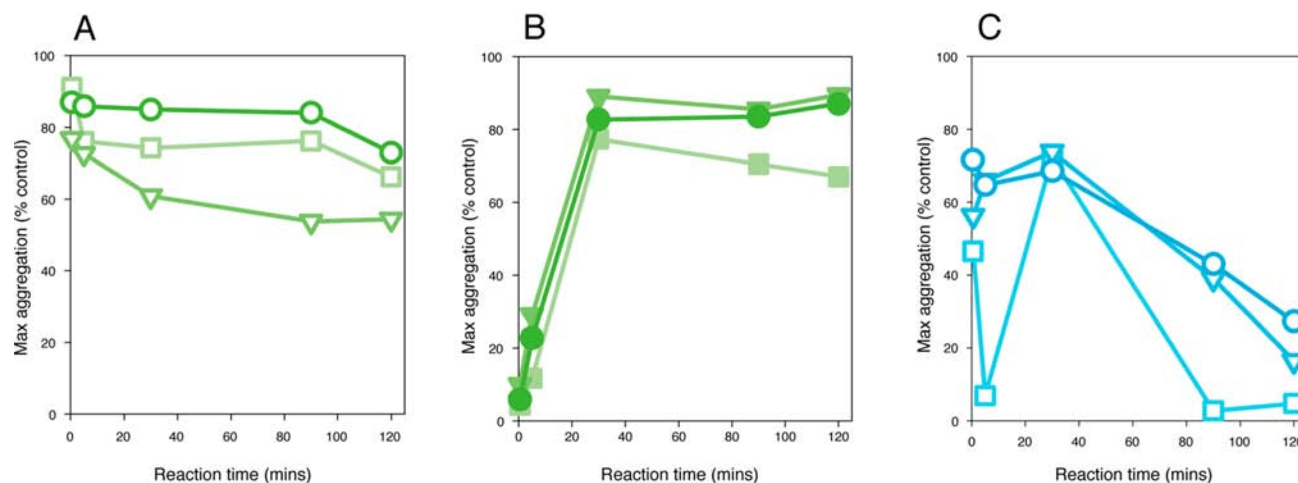

Figure 2. Platelet aggregation results of PRP from three individuals pretreated with model juice: (A) model garlic juice; (B) model onion juice; (C) model tearless onion juice. Model juice was incubated for 30 s and 5, 30, 90, or 120 min prior to addition to PRP. Collagen was used as agonist.

plate reader. As a control, untreated AGS cells were stimulated with TNFα.

IL-8 was calculated on the basis of a standard curve generated for each plate. All sampling times and treatments were assayed in duplicate and experiments repeated on three separate occasions. Data were analyzed using the restricted maximum likelihood (REML) statistical model fitted to log-transformed treatment means but with different residual variance for each sampling time.

Rat Feeding Trial Experimental Design. The experiment comprised five dietary treatments and eight rats per treatment. Outbred Sprague–Dawley laboratory rats were used in the feeding trial and were sourced from and housed in the Food Evaluation Unit (Plant and Food Research, Palmerston North, New Zealand). The feeding trial was carried out in a room maintained at a temperature of 22 ± 1 °C and humidity of 60 ± 5% with air exchange of 12 times per hour and a 12 h light/dark cycle. Forty male rats were housed in family groups and fed a commercial pelleted rodent diet from 3 to 7 weeks of age. The study was approved by the AgResearch Grasslands Animal Ethics Committee (Palmerston North, New Zealand) according to the Animal Welfare Act 1999.

Rat Feeding Trial Experimental Procedure. The 40 rats were transferred to individual hanging cages at 7 weeks of age and fed a lactic casein-based diet (Supporting Information) for 14 days. The rats were randomly allocated to the experimental dietary treatments and weight adjusted to ensure all groups (n = 8) had similar mean starting body weights. Experimental dietary treatments were administered by oral gavage (0.2 mL) once a day for 14 days. The rats continued to be fed the lactic casein diet ad libitum. Body weight and food intake were recorded for each animal for each 7 day period.

Rat Feeding Trial Treatments. The dietary treatments were control deionized water (200 μL), inactivated onion juice (60 μL of water, 140 μL of microwaved onion juice), model onion juice (28.3 μL of water, 140 μL of microwaved onion juice + 12.7 μLof rLFS + 19 μL of alliinase), model garlic juice (41 μL of water, 140 μL of microwaved garlic juice + 19 μL of alliinase), and model tearless onion juice (41 μL of water, 140 μL of microwaved onion juice + 19 μL of alliinase). The dietary allium treatments were given immediately upon mixing.

Rat Blood Glucose Measurement. Immediately prior to blood collection for platelet preparation, a drop of whole blood was used to determine blood glucose concentrations using an Accu Chek Performa device (Roche Diagnostics, Auckland, New Zealand).

Rat Platelet Preparation. At the end of the experimental period, the rats were euthanized via ether overdose, and blood was collected via cardiac puncture using a 19G needle and syringe. Blood (10 mL) was collected using sodium citrate as the anticoagulant at a ratio of 9:1. Blood was centrifuged at 200g for 15 min at 22 °C (Jouan CR4i, Thermo Scientific, New Zealand), and the cloudy PRP was transferred to a fresh tube. The remaining red cell fraction was further centrifuged at 1171g for 10 min at 22 °C to yield the clear PPP fraction, which was transferred to a fresh tube. Platelet counts were estimated as for human blood. PRP was normalized with PPP to give a final platelet concentration of approximately 200 × 10^6 cells/mL.

Rat Platelet Aggregation. Aggregation was activated by the addition of 25 μL of ADP (80 μM, final 8 μM). Alongside the duplicate normalized PRP samples, an undiluted PRP sample and a blank control were also run. Aggregation was recorded for 5 min, and results were expressed as a percentage of maximum aggregation.

Platelet aggregation (% max), body weight (g), and blood glucose concentrations (mM) were analyzed by one-way ANOVA, and the means were compared using the least significant difference (LSD) at the 5% level. Data are expressed as the mean ± SEM.

RESULTS AND DISCUSSION

Human Platelet Aggregation Response to Model Juices. Due to the time required to develop and confirm the status of transgenic plants, as well as issues in finding suitable containment facilities for GM crops in New Zealand, we devised model systems to carry out initial investigations, including the use of the purified isoalliin compound and inactivated onion juice with purified alliinase and rLFS enzymes. Following the addition of the enzymes alliinase ± rLFS to inactivated onion juice, aliquots were removed and analyzed for their effects on platelet aggregation. Model onion, tearless onion, and garlic juices containing between 5 and 20 μg of alliin or isoalliin were added to 225 μL of PRP to determine the effective range. At 5 μg very little effect on aggregation was observed for any of the model juices tested with either ADP or collagen as agonist. At 20 μg inhibition was high in all cases, making determination of differences difficult and suggesting direct adverse effects on platelets or agonist (data not shown). Alliin or isoalliin at 5 and 10 μg was subsequently selected for the assays. Aggregation initiated with ADP as agonist showed very little variation in the presence of any of the model juices (5 and 10 μg of alliin or isoalliin) when compared to control, except at early time points, indicating that a primary compound, possibly the abundant and highly volatile LF, had an effect on ADP (data not shown). Collagen-mediated aggregation was also inhibited at early time points for model onion juice, again possibly due to LF, but this inhibition was not observed for extracts left to react for >30 min even with 10 μg of isoalliin (Figure 2B). In comparison, model tearless onion juice showed much less early inhibition (possibly due to a lack of LF) but,

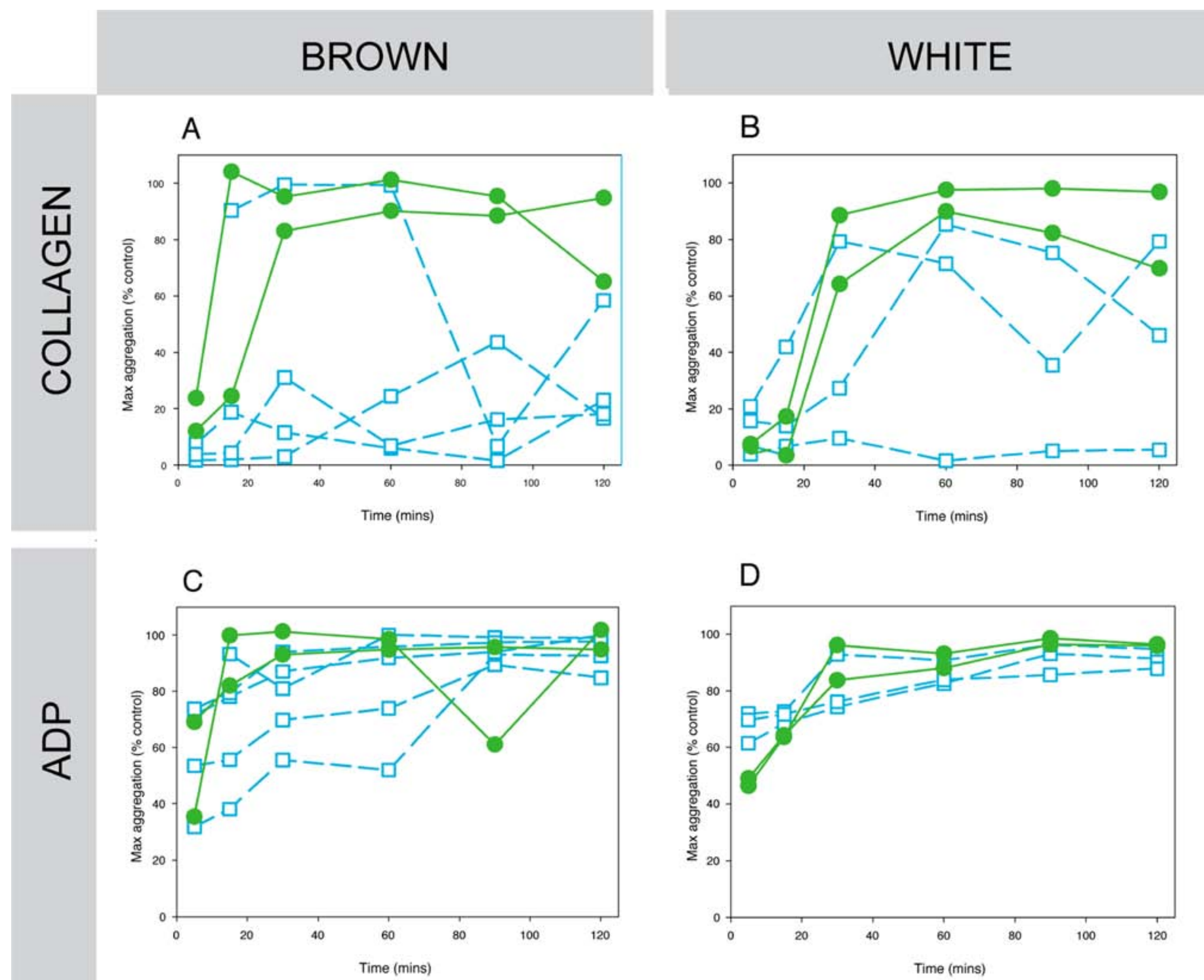

Figure 3. Platelet aggregation results of PRP from healthy individuals pretreated with extract from transgenic tearless onion (blue squares) or tearing onion (green circles): (A) brown onions with collagen as agonist; (B) white onions with collagen as agonist; (C) brown onions with ADP as agonist; (D) white onions with ADP as agonist. Onion juice was incubated for 5, 15, 30, 60, 90, or 120 min prior to addition to PRP.

after 30 min, increased greatly with 10 μg of isoalliin (Figure 2C). Model garlic juice showed a low level of inhibition at early time points, with inhibition increasing only slightly over time (Figure 2A). Results from similar studies on various allium species[5,14,15] indicate that the garlic thiosulfinate-derived compounds preferentially inhibit the collagen-mediated aggregation pathway. The in vitro studies above support this to some degree and also indicate a similar or greater response to thiosulfinates derived from tearless onion.

Human Platelet Aggregation Response to Real Juices. White and brown normal (tearing) and tearless phenotypes were used in real allium juice studies. When possible, three onions from each group were analyzed on isolated human platelets from our group of healthy volunteers, with no discrimination toward gender or age. Samples were prepared according to the method described earlier. Aliquots were taken at 5, 15, 30, 60, 90, and 120 min after incubation at room temperature, and effects on collagen and ADP-induced platelet aggregation were measured (Figure 3). Results generated from normal and tearing brown onions gave responses similar to those obtained using the reciprocal model system derived from microwaved onion juice (Figure 3A). The brown tearless onions produced a more extreme inhibition than observed in the reciprocal tearless model system for all but one onion. These data represent effects from juice that contained 10 μg of isoalliin, and when we subsequently tested volumes containing only 5 μg, the effects were similar but greatly reduced (data not shown). The transgenic brown tearless onions used for this assay show reduced collagen-mediated platelet aggregation. White tearless onions inhibited collagen-mediated aggregation, but this was much reduced compared with the brown tearless onion (Figure 3B). The transgenic white and brown tearless onions also gave an initial inhibitory response with ADP, indicating that some LF may still be present, but this rapidly decreased to control levels (Figure 3C,D). The reason for the reduced effectiveness of the white tearless onion in the presence of collagen is unknown, but may be due to differences such as total sulfur chemistry, fructan, or quercetin concentrations of the dehydration type onion compared with the brown fresh market type onion. The isoalliin concentrations found in the white tearing and tearless onions were generally greater than in the brown tearing and tearless onions. White onions tested here ranged between 1000 and 1700 μg/g fresh onion juice, whereas brown onions ranged between 640 and

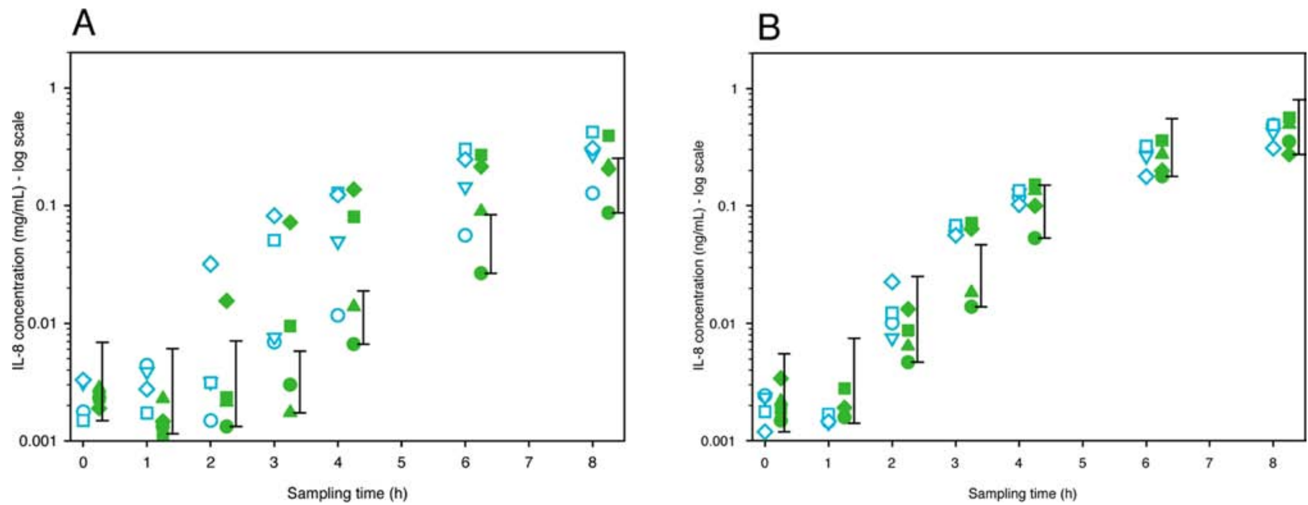

Figure 4. ELISA results for IL-8 following pretreatment of AGS cells with model onion juices: (A) model tearless and tearing onion juice; (B) model isoalliin derived tearless and tearing extracts. Model juice and purified isoalliin mixes were incubated for 5, 20, and 120 min prior to addition to AGS cells. I bars indicate least significant difference between means for the incubation time to the left of the bar. Tearless samples: 5 min (blue cirles), 20 min (blue triangles), 120 min (blue squeares), TNF control (blue diamonds). Tearing samples: 5 min (green circles), 20 min (green triangles), 120 min (green squares), TNF control (green diamonds).

Figure 5. (A) Mean (±SEM) food intake (g/day) during the last week of the treatment period for the rats given the experimental treatments (n = 8/group). (B) Mean (±SEM) weight gain (%/14 days) for the rats given the experimental treatments (n = 8/group) (*, significantly different from other groups ($P < 0.005$)). (C) Mean (±SEM) blood glucose concentrations (mM) obtained at the end of the 14 day experimental period from rats given the experimental treatments. (D) Mean (±SEM) platelet aggregation (%max) at the end of the 14-day experimental period for the rats given the experimental treatments (*, significantly different from other groups ($P < 0.05$)).

1000 μg/g fresh onion juice. More analysis of the genetic and phenotypic differences between the segregated onions is required to help determine this. These results support the role of alliums, and particularly propenyl cysteine sulfoxide-derived thiosulfinate products, in the inhibition of the collagen-mediated platelet aggregation pathway and as such strongly support the findings of Aoyagi.[5]

IL-8 Inflammatory Response. TNFα is a cytokine that is involved in systemic inflammation and can result in the activation of NF-κB, a central transcription factor in adaptive immunity and a central regulator of proinflammatory gene expression. Sulfur compounds within garlic extracts have been well-documented to have an inhibitory (anti-inflammatory) effect upon this pathway.[14,19] To test whether tearless onion extracts exhibit pro- or anti-inflammatory activity, effects on TNFα-induced expression of IL-8 in human AGS (stomach adenocarcinoma) cells were measured. Inactive onion juice and purified isoalliin showed no effect on IL-8 production when added to AGS cells prior to TNFα stimulation (data not shown). No significant effects were observed when purified isoalliin was reacted with alliinase $\pm$ rLFS for various times before addition to AGS cells (Figure 4B). However, a significant reduction in IL-8 production was observed for the first 3 h when either the model onion or model tearless onion juice was added to AGS cells after 5 and 20 min of reaction time (Figure 4A). The fact that a reduction was observed for the model juice in the presence and absence of rLFS indicates that the thiosulfinate or derived products of isoalliin were not responsible for the increase. Preliminary data from a set of white and brown tearing and tearless sibling pairs also showed a trend similar to that of the model juices (data not shown), the transgenic and nontransgenic onions also indicating that the response is likely not due to the thiosulfinate or derived products. The 5 min incubated extracts, tearless and tearing, reduced IL-8 concentrations the most for the longest with a reduction observed over all 8 h measured. Interestingly, the 120 and 20 min incubated extracts resulted in an increase in IL-8 production at the later stages of incubation, indicating a pro-inflammatory response.

Rat Feeding Trials. Rats in all treatment groups maintained a consistent food intake throughout the experimental period (Figure 5A, $P < 0.508$) with a mean intake of 21 $\pm$ 0.6 g/day and a range of 11.8−27.3 g/day. The mean body weights of the rats at the start of the treatment period (week 9) were similar at 346 $\pm$ 2.5 g and ranged from 327 to 394 g ($P = 0.338$). All animals gained weight during the 14 days over which the treatments were administered (weeks 9−11; Figure 5B); however, rats given model tearless onion extracts gained less weight than those fed the other treatments ($P < 0.005$). Rats fed the model onion and model garlic juice also gained less weight but not significantly less than the group fed microwaved juice or the control group. Blood glucose concentrations ranged between 6 and 10 mM (mean = 7.7 $\pm$ 0.2 mM) at the end of the 2 week treatment period, with no significant differences between groups (Figure 5C; $P = 0.261$).

Rats given the model tearless onion juice showed significantly lower platelet aggregation than those fed the other dietary treatments (Figure 5D, $P < 0.05$).

Discussion and Conclusions. Developing genetically modified alliums is a difficult undertaking, and the propagation conditions compromised our ability to maintain lines and limited us to using nonisogenic material for analysis. Despite this, the research has demonstrated in vivo and in vitro differences that strongly suggest that tearless onion germplasm possesses health attributes normally associated with thiosulfinates found in garlic. As such, the tearless onion phenotype could prove to be a valuable quality trait for the onion industry via nontransgenic routes such as tilling populations in a variety of genetic backgrounds.

Increased platelet aggregation and impaired platelet function occur in a range of vascular and metabolic disorders including diabetes and cardiovascular disease. Garlic and, to some extent, onion have been shown to reduce platelet aggregation in vitro and in vivo.[20−22] This activity has been attributed to the sulfur compounds, although this may vary with cultivar and growing conditions.[20] It has been proposed that the effect of garlic on platelet aggregation is a synergistic response to the many compounds present, and its effect is exerted through multiple biochemical pathways.[23,24] Garlic has been shown to be more potent than onion in lowering thromboxane concentrations,[25] and combined observations over many studies also suggest onions are less potent in their antiaggregatory effect than garlic.[26]

Methodological studies have previously reported that rat platelets aggregate well in the presence of ADP but not with collagen, calcium ionophore, fibrinogen, thrombin, ristocetin, epinephrine, plasma, or arachidonic acid.[27,28] Collagen has been shown to be an effective agonist in rats, but the response was present or absent depending on the source of collagen,[29] and the response varied with strain of rat.[30] For this reason we chose to use ADP as agonist in our rat feeding trials.

The human aggregometry data presented here strongly support the COX-1 inhibiting activity of the cepathiolane compounds discovered by Aoyagi.[5] The rat feeding trial, however, also showed a degree of inhibition for ADP-mediated aggregation. The model garlic juice did not have as large a reduction in platelet aggregation as expected in any of the assays performed. This could be due to the formation of cepathiolanes in the LFS-silenced extracts having a greater activity than garlic-derived compounds such as allicin. In combination with the rat feeding trial, these findings indicate that compounds produced within either a pure system (from purified isoalliin) or tearless onions have the potential to act on whole platelets in vitro.

It was noted that rat blood is very sensitive to extraction. This factor and the use of ADP as the agonist may have reduced the observable difference in responses. In future studies, it may prove more suitable to use a larger model animal such as the pig, from which multiple blood samples could be obtained and a wide range of agonists used in the platelet aggregation assay to untangle the responses to these agonists and the functional foods. However, the larger size of the pig requires that a greater quantity of test material would need to be produced for in vivo feeding studies.

The IL-8 data showed no difference between the tearless and tearing onion juice, and this corresponded with preliminary studies using model tearing and tearless juice. Both extracts preincubated for 5−20 min lowered IL-8 production for the first 3 h. This corresponds to similar findings with garlic extracts.[31] The similarity between the tearing and tearless samples indicates that the mechanism of reduction is not via thiosulfinate or derived products. This is also supported by the initial isoalliin + alliinase studies, which showed no difference in IL-8 induction and raised the question then of what is responsible for the response seen in our and previous findings, where allicin or diallyl disulfide was implicated. It should be

noted that Keiss et al. reported both anti- and pro-inflammatory responses.[14] One hypothesis could be that extracts may affect the inflammatory response directly and indirectly via multiple pathways; however, further research is required to clarify this.

The rat live weight data demonstrated that rats gavaged with model onion, garlic, or tearless onion juice gained less weight than rats gavaged with water or microwaved onion juice. A decrease in body weight without any significant difference in energy intake after garlic consumption was reported by Lee et al. in diet-induced obese mice.[32] The thermogenic effect of garlic increased body temperature and thus energy expenditure.[32] Garlic consumption increases epinephrine and norepinephrine secretion, enhancing triglyceride and brown adipose tissue metabolism as well as increasing oxygen consumption, mitochondrial protein, and uncoupling protein in brown adipose tissue.[33,34] This effect was attributed to the organosulfur compounds diallyl disulfide, diallyl trisulfide, and alliin.[33] A more recent study by Kim et al. utilized garlic stem extracts and again showed a reduction in body weight without a reduction in food intake.[35] In this study, they have demonstrated a reduction in white adipose tissue hypothesized to be due to a modification of lipogenic pathways. The recent findings of Aoyagi[5] indicate that the cepathiolanes, through the action of the derived sulfur compounds in onion, garlic, and particularly tearless onion juice, can act as glucosidase inhibitors to inhibit or modulate carbohydrate uptake, which may in part be responsible for reduced weight gain. Whereas no significant differences in blood glucose were found in the present study, the affects of allium compounds on lipid regulatory pathways would be an interesting future investigation. Effects on major systemic pathways should also be investigated thoroughly due to conflicting reports of the potential for negative impacts, such as hemolytic anemia and drug interactions.[36−38] However, the reduced weight gain observed in this study is an exciting finding and could see a new role for tearless onions as functional foods for appetite control, antiobesity, or the amelioration of diabetes.[39]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

C.C.E., study concept and design, manuscript preparation; S.J.T., assistance in study design, protocol design, data generation, and manuscript preparation; P.R., IL-8 assays, data analysis; C.B., rat feeding trial design, setup, and manuscript preparation; S.O., rat feeding trial design, setup, and analysis; M.S., preparation of inactivated garlic and onion juice, preparation of alliinase enzyme; N.I.J., isoalliin and alliin analysis of onion samples.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

rLFS, recombinant lachrymatory factor synthase; ADP, adenosine diphosphate; COX-1, cyclooxygenase 1; PPP, platelet poor plasma; PRP, platelet-rich plasma

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