Mastication of almonds: effects of lipid bioaccessibility, appetite, and hormone response

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ABSTRACT
Background: Epidemiologic and clinical data indicate that nuts can be incorporated into the diet without compromising body weight. This has been attributed to strong satiety properties, increased resting energy expenditure, and limited lipid bioaccessibility.

Objective: The role of mastication was explored because of evidence that the availability of nut lipids is largely dependent on the mechanical fracture of their cell walls.

Design: In a randomized, 3-arm, crossover study, 13 healthy adults (body mass index, in kg/m²: 23.1 ± 0.4) chewed 55 g almonds 10, 25, or 40 times. Blood was collected and appetite was monitored during the following 3 h. Over the next 4 d, all foods were provided, including 55 g almonds, which were consumed under the same chewing conditions. Complete fecal samples were collected.

Results: Hunger was acutely suppressed below baseline (P < 0.05), and fullness was elevated above baseline longer (P < 0.05) after 40 chews than after 25 chews. Two hours after consumption, fullness levels were significantly lower and hunger levels were significantly higher after 25 chews than after 10 and 40 chews (P < 0.05). Initial postingestive glucagon-like peptide-1 concentrations were significantly lower after 25 chews than after 40 chews (P < 0.05), and insulin concentrations declined more rapidly after 25 and 40 chews than after 10 chews (both P < 0.05). Initial postingestive glucagon-like peptide-1 concentrations were significantly lower after 25 chews than after 40 chews (P < 0.05), and insulin concentrations declined more rapidly after 25 and 40 chews than after 10 chews (both P < 0.05). Initial postingestive glucagon-like peptide-1 concentrations were significantly lower after 25 chews than after 40 chews (P < 0.05), and insulin concentrations declined more rapidly after 25 and 40 chews than after 10 chews (both P < 0.05). All participants had higher fecal energy losses after 10 and 25 chews than after 40 chews (P < 0.005).

Conclusion: The results indicate important differences in appetitive and physiologic responses to masticating nuts and likely other foods and nutrients. This trial was registered at clinicaltrials.gov as NCT00768417.

INTRODUCTION
The high prevalence of obesity in the United States (1) has prompted recommendations for adherence to low-energy-dense diets in an effort to decrease energy intake and manage body weight (2, 3). Despite the lack of conclusive evidence supporting the link between energy-dense diets and body weight (4), such diets exclude most nuts. However, epidemiologic studies indicate an inverse association between the frequency of nut consumption and body mass index (BMI; in kg/m²) (5, 6). Additionally, clinical trials have shown little or no change in body weight with regular intake of nuts in free-living populations (7–11). This issue is important because there is a qualified health claim linking daily consumption of nuts with reduced cardiovascular disease risk (12), which has resulted in an enhanced interest in promoting nut intake. The claim is based on evidence that nut consumption improves blood lipid profiles (5, 6, 13–15), but other benefits have also been noted, such as moderation of postprandial glycemia, reduced risk of diabetes (16–19) and cancer (20–22), and improved bone health (17).

Much of the energy contributed by nuts is offset by compensatory reductions in energy intake from other sources (8). However, an additional mechanism responsible for the less-than-predicted influence of nuts on body weight stems from an estimated 10%–20% of the energy from nuts being lost in the stool (23). This has been attributed to the resistance of nut parenchyma cell walls to microbial and enzyme degradation in the gastrointestinal (GI) tract (24, 25). Consequently, lipids that are not liberated through the mechanical disruption of the cell walls are inaccessible for absorption in the gut. This raises the possibility that masticatory efficiency influences energy balance through changes in lipid availability.

The role that masticatory efficiency plays in energy balance is complex. Increased chewing could liberate more lipids from the nut and thereby increase the amount of energy available to the body, which contributes to positive energy balance. In contrast, the increased presence of lipids in the small intestine may result in an increased secretion of several hormones, such as cholecystokinin (CCK) (26, 27), glucagon-like peptide-1 (GLP-1) (27), and peptide YY (PYY) (28). Higher plasma concentrations of CCK (29), GLP-1 (30), and PYY (31) are associated with greater sensations of satiety. Consequently, the additional amount of energy available from the increased liberation of lipids may be offset by a stronger satiety response.

The greater oral mechanical effort required to prepare whole nuts for deglutition may enhance satiety through neural mechanisms as well. Chewing is a key stimulus of cephalic phase

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responses (32, 33), and sensory stimulation may promote the release of appetitive hormones such as insulin (34), ghrelin (35), CCK (36), PYY (37), and GLP-1 (38). Furthermore, studies in rats suggest that mastication enhances satiation through histaminergic activation of the ventromedial hypothalamus and paraventricular nucleus (39). The present study sought to explore the effects of mastication efficiency on lipid bioaccessibility, satiety, and hormone responses.

**SUBJECTS AND METHODS**

**Subjects**

Participants were recruited starting in April 2006 via public advertisements. Eligibility was established through completion of screening questionnaires eliciting health and demographic information. To be eligible, subjects had to be nonsmokers, have a BMI of 20–25, be between 18 and 50 y of age, have a full set of healthy teeth, not be pregnant or lactating, have low dietary restraint (3-factor eating questionnaire restraint score ≤13) (40), have no allergy to nuts, have no endocrine or eating disorders, be weight stable (<3 kg change over the past 3 mo), and not be taking medications likely to confound study outcomes. All participants signed an informed consent form approved by the Institutional Review Board and received monetary compensation.

**Experimental design and procedures**

*Testing sessions, appetitive ratings, and blood collection*

The study followed a crossover design with 3 treatment periods of 4 consecutive days. There was a minimum of a 1-wk washout between treatment periods. On day 1 of each treatment period, participants reported to the laboratory in the morning after an 8-h fast. They were required to rate their appetitive sensations using a visual analogue scale (VAS) presented on a personal data assistant. Standard appetite questions, as described by Hill and Blundell (41), were used. The VAS had end anchors ranging from “not at all” to “extremely” and questions such as, “How strong is your feeling of hunger (fullness, desire to eat, etc.) right now?”

After completion of the VAS, an indwelling catheter was inserted in a vein in the antecubital space of the arm, and a baseline blood sample was taken. The participant was then presented with 55 g (≈2 oz; 1324 kJ, 27 g fat) (42) of raw, whole almonds. Depending on the treatment, participants chewed the almonds in 5-g portions (≈4 almonds) 10, 25, or 40 times before swallowing. A 15-min time allotment was given to consume the almonds.

Immediately after almond consumption (time point = 0), a 15-mL blood sample was drawn. Blood samples were drawn 15, 30, 45, 60, 90, 120, and 180 min after almond consumption. All samples were collected into EDTA-coated evacuated tubes, immediately cooled on ice, and transferred to a refrigerated centrifuge for separation of plasma before storage at −80°C. Active plasma ghrelin, total GLP-1, and PYY3-36 were measured with commercially available radioimmunoassay (RIA) kits (GHRA-88HK, GLP1T-36HK, and PYY-67HK; Millipore, Billerica, MA). The ghrelin RIA kit had a lower and upper detection limit of 7.8 and 2000 pg/mL, respectively. The intraassay CV was 6.7%, and the interassay CV was 9.6% at a sample concentration of 138.6 pg/mL. The lower and upper detection limits for the GLP-1 assay were 3.0 and 333 pmol/L, respectively, the intraassay CV was 29%, and the interassay CV was 10% at a sample concentration of 53 pmol/L. The lower detection limit for the PYY assay was 20 pg/mL, the upper limit was 1280 pg/mL, and the intra- and interassay CVs were 11% and 15%, respectively, at a sample concentration of 84 pg/mL. Plasma glucose and insulin concentrations were assayed with the Cobas Integra 400 Analyzer and Elecsys 2010 Immunoassay System (Roche Diagnostics Inc, Somerville, NJ). The insulin assay had a lower and upper detection limit of 1.4 and 6945 pmol/L, respectively, and the intraassay CV was 1.9% and the interassay CV was 2.7%. Before each blood collection, participants again completed the appetitive questions. After the last blood draw, the catheter was removed, and the participant was served lunch.

**Meal preparation and composition**

To accurately determine the effect of mastication on lipid excretion, participants were fed 3 controlled meals and a snack each day. The 4-d-cycle menu comprised foods typical of a Western-diet, but excluded all nuts. Standard meals provided a mean of ≈10,266 kJ and a macronutrient composition of 35% fat, 15% protein, and 50% carbohydrate (Nutrition Data System for Research Software 2007; University of Minnesota, Minneapolis, MN). Meals were eaten in the laboratory, with no additional food or beverages allowed outside of the laboratory. All meals were prepared in the laboratory kitchen, and each portion was weighed before serving. Participants were required to consume all provided food and beverages. Duplicate portions of the menu were homogenized, frozen, freeze-dried, and stored. The samples were analyzed for gross energy by bomb calorimetry with a Parr 1281 Bomb Calorimeter (Parr Instruments, Moline, IL) and total fat content was measured with automated Soxhlet extraction with an Ankom XT15 Extraction System (Ankom Technology, Macedon, NY).

**Stool collection**

On the first morning of the study, participants ingested 3 capsules of green food coloring with their almond load. On the fourth morning, participants consumed capsules with red food coloring. Participants were instructed to collect all stool passed until the red marker appeared. Samples were pooled by participant and treatment. Fecal composites were made by the addition of 2 parts water followed by homogenization. Aliquots of the samples were then frozen, freeze-dried, and stored until analyzed. The energy content of the samples was determined by bomb calorimetry with a Parr 1281 Bomb Calorimeter (Parr Instruments), and the fat content was measured by automated Soxhlet extraction (Ankom XT15 Extraction System).

**Mastication and almond particle size**

On a separate visit, participants masticated 5-g almond portions for a specified number of times (10, 25, or 40), but expectorated rather than swallowed. They then rinsed their mouths with three 30-mL portions of deionized water and expectorated any remaining almond particles. The expectorated samples were collected through a series of 8 sieves that yielded the following particle size ranges: >3.35, 3.35–2.00, 1.99–1.00, 0.99–0.50,
0.49–0.25, 0.24–0.125, 0.124–0.063, 0.062–0.032, and <0.032 mm (WS Tyler, Mentor, OH). The expectorated samples were washed with 250 mL deionized water. The water was allowed to drain completely through all sieves, and the samples were then dried at 54°C for 6 h. This method has previously been used to eliminate water from similarly sized almonds (43, 44). The fully dried samples from each individual sieve were weighed and recorded.

Statistical analysis

Statistical analyses were performed by using the Statistical Package for the Social Sciences (SPSS), version 15.0 (SPSS Inc, Chicago, IL). The criterion level for statistical significance was \( P < 0.05 \) (2-tailed). Treatment effects were tested by repeated-measures analysis of variance, followed, where appropriate, by a post hoc Bonferroni test to correct for multiple comparisons. All data are expressed as means ± SEMs. Sign tests were used to examine the distributions of fecal energy and fat. Relations between particle size, fecal output, hormone responses, and appetitive sensations were determined by Pearson’s correlation analyses.

RESULTS

Participant characteristics

Twenty participants signed consent forms and began the protocol; however, individuals who failed to report to the laboratory for meals (\( n = 2 \)), supplied incomplete fecal collections (\( n = 1 \)) and appetite data (\( n = 2 \)), or who did not finish the protocol for personal reasons (\( n = 2 \)) were excluded. Participants included in the final analysis (\( n = 13; 5 \) women and 8 men) had a mean BMI of 23.1 ± 0.4 (range: 19.6–24.9) and were 24 ± 1.8 y of age (range: 19–43 y).

Almond particle size assessment

The number of chews was negatively correlated with the total percentage of recovered particles, as calculated by the percentage weight of all recovered particles in the sieves relative to the total weight of the masticated almond \( (r = -0.57, P < 0.05) \). There was a significantly higher proportion of recovered particles after 10 chews than after 25 and 40 chews \( (P < 0.001) \) (Figure 1A). A significantly lower proportion of almond particles <3.35 mm were recovered after 10 chews \( (P < 0.05) \) (Figure 1B). Previous work by our group with this sorting technique resulted in 95–97% recovery when complete collections were made (43). Thus, the balance is primarily attributable to particles <0.032 mm and free lipid that passed through the small screen. The recovered particle mass <0.032 mm was negatively correlated with the total percentage recovery for all treatments \( (r = -0.91, P < 0.01) \). The mass of particles sized >3.35 mm was positively correlated with the total percentage recovery for all treatments \( (r = 0.70, P < 0.01) \) and negatively correlated with particle sizes <0.032 mm after 10 \( (r = -0.65, P = 0.016) \) and 25 \( (r = -0.77, P = 0.002) \) chews. However, there were no significant correlations between particle size and appetitive ratings, fecal fat excretion, or hormone concentrations.

Fecal excretion

There was a significant main effect of chewing on fecal energy excretion \( (P = 0.015) \). Mean energy excretion was significantly higher after 10 chews than after 40 chews \( (P = 0.011) \) (Figure 2). Similarly, total fecal fat excretion determined by gram weight was significantly higher after 10 chews than after 25 \( (P = 0.018) \) and 40 \( (P = 0.044) \) chews. Total fecal fat excretion as a percentage of crude fat was significantly higher after 10 than after 40 chews \( (P = 0.015) \). In comparison with the measured energy content of the diet, there was a significant loss of energy after 10 than after 40 chews \( (P = 0.01) \). The energy loss was primarily attributable to increased fecal fat excretion. Relative to the lipid load, the proportion of lipid lost in the stool after 10 chews \( (43.7\% ± 4.0\%) \) was greater than the proportion lost after 25 \( (32.7\% ± 2.7\%; P = 0.006) \) and 40 \( (30.8\% ± 4.4\%; P = 0.015) \) chews. In absolute terms, percentage fecal fat excretion increased by 11.1% ± 3.4% after 25 chews and by 12.9% ± 4.5% after 40 chews compared with losses after 10 chews.

There was a significant inverse association between the number of chews and energy/g dry fecal weight \( (r = -0.53, P < 0.05) \). Total dry fecal weight was significantly lower after 40 chews \( (175.1 ± 10.7\ g) \) than after 10 chews \( (152.8 ± 10.8\ g; P = 0.05) \). Total fecal energy and fat losses were significantly

![Figure 1](https://academic.oup.com/ajcn/article-abstract/89/3/794/4596705/796-FIGURE-1)
correlated with the 10 (r = 0.68, P = 0.011), 25 (r = 0.58, P = 0.039), and 40 (r = 0.73, P = 0.004) chew treatments. Fecal energy excretion was higher in 10 of 13 participants after 10 than after 25 chews (P < 0.05) and in 13 of 13 participants after 10 than after 40 chews (P < 0.05). All 13 participants also had higher fecal energy losses after 25 chews than after 40 chews (P < 0.005).

Appetitive ratings
Baseline appetitive ratings were not significantly different across treatments. Both the hunger- and fullness-by-time interactions were significant (P = 0.001 and P = 0.021, respectively) (Figure 3). Data are presented as a change from baseline. Postprandial subjective hunger ratings (0–90 min after almond consumption) were suppressed below baseline longer with 40 chews than with 25 chews (P = 0.031) (Figure 3A). These ratings were significantly different from baseline after 40 chews (P < 0.05). Conversely, fullness remained elevated above baseline longer with 40 chews than with 25 chews (P = 0.041) and showed a trend toward significance after 10 chews (P = 0.054) (Figure 3B). Fullness ratings were also significantly different from baseline 60 min after almond consumption (P < 0.05). Preprandial subjective fullness ratings (2 h after almond consumption and preceding the subsequent eating occasion) were significantly lower and hunger levels were significantly higher after 25 chews than after 10 and 40 chews (P < 0.05).

Postconsumption hormone responses
There were no significant treatment effects on active plasma ghrelin or PYY. However, the GLP-1-by-time interaction showed a trend toward significance (P = 0.055), and initial postigestive concentrations of GLP-1 were lower after 25 chews than after 40 chews (P = 0.016) and significantly lower than baseline (P < 0.05) (Figure 4). Final concentrations after 10 chews were significantly lower than baseline (P < 0.05). Data are presented as changes from baseline. Although not significant, rank ordering of mean treatment response values showed that GLP-1 concentrations were higher after 40 chews followed by 25 and 10 chews. Whereas no significant treatment effects were observed for plasma glucose, the insulin-by-time interaction was significant (P = 0.025) (Figure 5A, B). Post hoc analysis showed a more precipitous decline in insulin concentration from 45 to 180 min after almond consumption after 25 and 40 chews than after 10 chews (both P < 0.05).

DISCUSSION
Accumulating evidence indicates that nut consumption may have various health benefits, yet concern about their impact on body weight persists. Studies indicate that the incorporation of nuts into the diet does not promote weight gain because they are highly satiating, their energy-yielding nutrients have limited bioaccessibility, and they may promote energy expenditure (7–11, 15, 24, 25, 45). However, the mechanisms that account for their satiety and bioaccessibility properties are not clear. It was hypothesized that masticatory function and the subsequent variation in bioaccessibility may modulate these properties. The results of the present study indicate that mastication significantly influences energy absorption and appetitive responses, although the 2 were not related.
With controlled chewing, the present study achieved marked differences in mechanical disruption of the physical state of the almonds. This was documented, in part, through differences in resulting particle sizes. The consequence was a significant dose-response effect on fecal fat and energy excretion. This finding is consistent with reports that lipid bioaccessibility is primarily determined by the degree to which the cell walls of nuts are ruptured in the oral cavity (24). Limited further extraction of lipids occurs in the gastric and duodenal phases of digestion (25). This is not attributable to low bioavailability because energy absorption is markedly higher with the ingestion of nut butter or oil (45). In vitro models and mathematical modeling predict as much as 60% of the lipids in finely ground almonds and 85% of the lipids in 2 mm of natural almond cubes is not bioaccessible through processes such as chewing and gastric and duodenal digestion (25). In the only published human feeding study permitting such a calculation, fecal fat losses increased with nut consumption by the equivalent of 30%–40% of the lipid provided by the nut (45). Fecal fat contributed 29% of the total fecal energy in the control condition and 33.9% in the peanut consumption condition. The present study did not include a control arm to determine the fecal fat concentration after ingestion of the diet without almonds. Consequently, an absolute effect on fecal fat loss could not be determined. However, chewing the almonds 25 or 40 times led to 25.5% and 29.4% greater reductions in fecal fat relative to chewing only 10 times. In absolute terms, fat represented 39.8% of fecal energy excretion with 10 chews, 35.1% with 25 chews, and 35.3% with 40 chews. Although these substantive losses account, in part, for the limited effect that nut consumption has on body weight, they are lower than the modeling predictions. This suggests that further digestion and absorption occur distal to the duodenum. Fermentation in the colon may account for this discrepancy (46).

The increments in total energy loss were 15.6% and 20.5% with 25 and 40 chews compared with 10 chews. Thus, fat loss was proportional to that noted for total energy, which indicated that the primary effect on energy balance was attributable to the fat component of the almonds. Other studies have shown that protein bioaccessibility is limited, comparably to that of fat (25). However, protein accounts for a markedly smaller energy component of almonds (~14% of energy from protein compared with ~77% from fat). Part of the fat loss may also stem from a reduction in the absorption of free fatty acids because of the high fiber content of almonds (47).

The 3 levels of chewing tested coincided with observations of naturalistic eating, ie, 9–65 chews for carrots and 14–44 chews for Brazil nuts (48). To some degree, this range reflects individual differences in chewing efficiency. Chewing a fixed number of times results in particles of notable size difference (49); however, ad libitum chewed, pre-swallowed particle size is relatively consistent within an individual (48). Generally, participants in the present study indicated that the 25-chew condition was most comfortable. Under this condition, fat accounted for ~35% of fecal energy—a value exceeding the measured 30% of energy from fat contributed to the diet ingested.

Requiring participants to chew the almonds 40 times led to the strongest reduction in hunger and augmentation of fullness. Such

![Figure 4](https://academic.oup.com/ajcn/article-abstract/89/3/794/4596705)

**Figure 4.** Mean (±SEM) changes in glucagon-like peptide 1 (GLP-1) concentrations after almond consumption. The GLP-1–by-time interaction showed a trend toward significance ($P = 0.055$; repeated-measures ANOVA with post hoc Bonferroni multiple comparison tests). $n = 12$. The bracket indicates the 15-min time period allotted for almond consumption.

*Significant differences between 25 and 10 and 40 chews, $P < 0.05$.

**Figure 5.** Mean (±SEM) changes in insulin (A) and glucose (B) concentrations after almond consumption. The insulin-by-time interaction was significant ($P = 0.02$; repeated-measures ANOVA with post hoc Bonferroni multiple comparison tests). There was a more rapid decline in insulin concentrations from 45 to 180 min after almond consumption after 25 and 40 chews than after 10 chews ($P < 0.05$). $n = 12$. The bracket indicates the 15-min time period allotted for almond consumption.

*Significant differences between 25 and 10 and 40 chews, $P < 0.05$.

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**Figure 6.** Changes in total energy loss (kJ) after almond consumption. The primary effect on energy balance was attributable to the fat component of the almonds. Other studies have shown that protein bioaccessibility is limited, comparably to that of fat (25). However, protein accounts for a markedly smaller energy component of almonds (~14% of energy from protein compared with ~77% from fat). Part of the fat loss may also stem from a reduction in the absorption of free fatty acids because of the high fiber content of almonds (47).
an effect was hypothesized based on a predicted greater release of lipid and protein with consequent secretion of satiety hormones in response to these nutrients. GLP-1 was measured in this study because long-chain unsaturated fatty acids are effective stimuli for its release (50) and it elicits satiety sensations in humans (30). Although GLP-1 concentrations were consistently higher over the 90-min postprandial period after 40 chews, the difference was not significant. Furthermore, there was no significant correlation between GLP-1 and particle size, fecal lipid content, or appetite ratings. PYY and ghrelin concentrations were also not altered by the different chewing conditions. Several alternative explanations are possible. First, the hypothesis may hold, but other unmeasured peptides, such as CCK, may have had a dominating effect. Second, mastication beyond the point of customary oral processing of a food may diminish its palatability and, consequently, attenuate hunger ratings because a direct association between palatability and hunger has been reported (51). Third, animal studies indicate that the mechanical act of chewing may augment satiety through neural activation of central satiety centers (39). Mastication is also an effective (33), and possibly necessary (32), stimulus for cephalic phase responses, which are hypothesized to modulate appetite and energy balance (52). Cephalic or sensory-based releases of insulin (34), ghrelin (35), CCK (36), PYY (37), and GLP-1 (38) have been documented. Whereas there are observations consistent with an effect of chewing in humans (53), this has not been readily replicated with gum used as a masticatory stimulus (54).

Hunger and fullness ratings returned to baseline values more quickly and significantly overshot this sensation level during the later time period with 25 chews, unlike the ratings after 10 and 40 chews. The basis of this difference is not clear, but note that the 25-chew condition was regarded as most closely mimicking customary oral processing. Whether the more extreme conditions of 10 and 40 chews led to effects akin to novelty-induced hypophagia (55) warrants consideration.

Almond ingestion blunts the glycemic response to foods (16), reportedly because of their low glycemic index value or high fat content. This property has been posited as a mechanism for the high satiety value of nuts (19). However, the extent to which the glycemic index or load value of foods is related to appetitive sensations is a matter of debate (19). In the present study, almond ingestion elicited weak glucose and insulin responses that were unrelated to the level of chewing. This is most likely reflective of their high fat and low available carbohydrate contents.

The present findings do not suggest that individuals concerned with weight management should chew their food less. Rather, they highlight the important effects of chewing on various factors that influence weight management (i.e., lipid absorption, release of gut peptides, and increased satiety). Such effects were primarily observed after 25 or 40 chews. Whereas these findings are applicable to the consumption of almonds, and perhaps other nuts, they may provide insight as to how food form may be manipulated to optimize different properties for given purposes. Consumption of whole nuts may reduce energy absorption and augment satiety—properties useful for weight management. Whereas whole nuts are a rich source of various nutrients, a greater availability of vitamins, unsaturated fat, protein, and antioxidants may be achieved with more mechanically processed nut forms. These components may contribute to a reduced process of a wide array of health disorders, including cardiovascular disease, diabetes, and cancer.

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The authors’ responsibilities were as follows—BAC: study design, testing, sample and data analyses, and report generation; JHH: study design, testing, and report generation; RDM: study design, data analyses, and report generation; ADF: hormone analysis; and RVC: hormone analysis and report generation. None of the authors had a personal or financial conflict of interest.

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