Title

Acute effect of soybean beta-conglycinin hydrolysate ingestion on appetite sensations in healthy humans

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Running head

Protein hydrolysate suppresses human appetite

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Abstract

A hydrolysate prepared from soybean beta-conglycinin reduced food intake through cholecystokinin release in rats; however, effects of the hydrolysate on human appetites are unknown. In this study, healthy volunteers ingested 3 g of the beta-conglycinin hydrolysate (BconB) and/or a soy protein hydrolysate (HN) contained in a beverage or in a jelly. Appetite profiles (hunger, fullness and prospective consumption) and palatability of test jellies were recorded. Fullness was rated higher, and hunger was rated lower after BconB ingestion as compared to HN ingestion. These results demonstrate that 3 g of BconB is effective to enhance fullness and reduce hunger sensations in healthy humans.

Key words

Dietary peptide, Soybean beta-conglycinin, Appetite, Cholecystokinin

Introduction

Dietary protein is thought to have the highest satiety effect when compared with isoenergetic fats and carbohydrates in human subjects and rats (Bensaid 2002, Reid 1997, Tome 2004). Cholecystoknin (CCK) is one of the gut hormones which induce satiety sensations and reduce food consumption in humans (Kissileff 1981, Lieverse 1995), and the gut hormone mediates protein-induced satiety (Moran 2004, Nolan 2003).

It has been demonstrated that the duodenal infusion of a hydrolysate prepared from soybean beta-conglycinin suppressed food intake by stimulation of CCK secretion in rats (Nishi 2003a). Beta-conglycinin is the 7S globulin fraction of soy

protein, and its content is estimated to be more than 20% of soy protein. Suppression of food intake was caused by 5 mg of beta-conglycinin hydrolysate but not by soy protein hydrolysate or wheat gluten hydrolysate at the same dose; indicating that beta-conglycinin hydrolysate is a potent suppressor of food intake in rats. These results raise the possibility that ingestion of beta-conglycinin hydrolysate induces satiety and reduce appetite via CCK secretion in human subjects.

In previous human studies, it has been demonstrated that satiating effects of dietary proteins varies with the amount and source of protein, and also with the experimental conditions (Lang 1998, Lang 1999, Diepvens 2008, Veldhorst 2009a). Gelatin and alpha-lactalbumin were reported to have higher satiating effects than casein, soy or whey proteins (Veldhorst 2009b). In these human studies, 15 to 50 g of protein sources were mixed with fats and carbohydrates in a meal such as breakfast or lunch with total energy of around 2 MJ (~ 500 kcal). Such study design could help to observe apparent changes in appetite scores. However, there are few studies using relatively small amount of protein (< 5 g) mixed in a vehicle with lower energy content than in ‘a meal’ (Gustafson 2001). It is valuable to examine the effect of relatively small amount of dietary peptides on human appetite sensations. In the present study, subjects ingested 1.5-3 g of beta-conglycinin hydrolysates and evaluated appetite sensations by using visual analogue scale (VAS) method.

Peptic hydrolysates generally have strong bitter taste for humans. Here, beta-conglycinin was hydrolyzed with another protease, bromelain, to improve the taste of the hydrolysate for human consumption. The purpose of the present

study is to examine whether the ingestion of beta-conglycinin hydrolysate in a liquid or a solid vehicle induces satiety in healthy human subjects.

Materials and Methods

Materials

Beta-conglycinin hydrolysate (BconB) was prepared as follows. Beta-conglycinin (provided by Fuji oil Co., Ltd., Tokyo, Japan) was suspended in de-ionized water and the pH was adjusted to 6.0. Bromelain (a food processing protease from pineapple stem, Biocon Japan Ltd., Nagoya, Japan) was added to the suspension (0.1% against substrate) and shaken for 60 min at 55°C. The suspension was boiled for 20 min to stop the enzyme reaction. The supernatant was collected by centrifugation and filtration, and then lyophilized. Soy protein hydrolysate (HN: Hinute) was provided by Fuji oil Co., Ltd. The average molecular mass was estimated at 4,400 Da for BconB and 3,100 Da for HN by a size exclusion chromatography performed in a FPLC system (AKTA explorer 10S and Superdex Peptide 10/300 GL column, GE Healthcare Bio-Sciences AB, Uppsala, Sweden).

Subjects

Healthy volunteers were recruited by advertisements on notice boards at several faculties in Hokkaido University. Subjects with an allergy to soybean products or in poor health condition were not selected. All participants gave informed consent to take part in this study. All studies were randomized and double-blinded, characterized by three treatments for each subject that were

conducted on separate days, with a 3-4 day wash-out period.

The study protocol was approved by the Medical Ethics Committee of the faculty of Medicine, Hokkaido University, and by the Ethics Committee of the faculty of Agriculture, Hokkaido University. The study was performed in accordance with the ethical standards established in the 1964 Declaration of Helsinki.

Experiment 1~ Effects of BconB and HN mixed in a low-calorie beverage on appetite.

Thirty volunteers (14 male and 16 female) with a mean age of 24 ± 1 (range 21-35 years old) participated in experiment 1. After the lunch, subjects fasted for 3 hours, but they were allowed to drink water until 16:00. At 16:25, basal appetite profiles were rated before the ingestion of the test beverage. Appetite profiles including hunger, fullness and prospective consumption were rated on a 100 mm Visual Analogue Scales (VAS) anchored with ‘not at all’ and ‘extremely’. BconB (3 g), HN (3 g) or BconB (1.5 g) + HN (1.5 g) was dissolved in 100 ml of a low calorie (46 kJ/100 ml) beverage (POCARI SWEAT STEVIA, Otsuka Pharmaceuticals Co, Ltd., Tokyo, Japan). The dose of 3 g in 100 ml was selected because this dose did not disturb the taste of the beverage and subjects could consume test beverage in a short period (5 min). Subjects ingested the test beverage at 16:30 after the instruction to drink the whole beverage (100 ml, 96 kJ) within 5 min. Appetite profiles were rated every 15 min until 60 min after the ingestion. Subjects were not allowed to have additional drinks or foods during the experiment.

Experiment 2~ Effects of BconB and HN mixed in an agar-jelly on appetite.

Forty-six volunteers (19 male and 27 female) with a mean age of 25 ± 1 (range 21-52 years old) participated in experiment 2. Grapefruit-flavored test jellies (totally 264 kJ) consisted of agar, sugar and 3 g of peptides (prepared by a local sweets company, Yamashitakan Santaxream, Ebetsu, Hokkaido, Japan). After the lunch, subjects fasted for 3 hours, but they were allowed to drink water until 16:00. Before the ingestion of the test jelly, basal appetite profiles were rated as described above. Subjects ingested the jelly (100 g, 264 kJ) and 50 ml of water at 16:30, and were instructed to consume the serving within 5 min. Just after the consumption, palatability of the test jelly was graded according to five ranks. Appetite profiles were rated every 15 min until 60 min after the ingestion. Subjects were not allowed to have additional drinks or foods after during the experiment.

Statistical analysis

Appetites profiles were expressed as absolute changes (mmVAS) from the basal (0 min). All data are expressed as mean ± SEM. Two-way repeated ANOVA was performed to assess the effect of treatment, time and interaction of treatment and time (treatment x time) on appetite profiles (Fig. 1 and 2). One-way ANOVA was performed to assess the possible difference among the palatability of test jellies. After detecting a significant ($p < 0.05$) main effect in the ANOVA, significant differences among mean values were determined by the Fisher's LSD test ($p < 0.05$). Statistical analysis was performed by using

145 StatView 5.0 (SAS Institute Inc., USA, 1998).

146

147 **Results**

148 Changes in appetite profiles were monitored by VAS method in healthy
149 volunteers after the ingestion of beverages containing either 3 g of BconB, HN or
150 mixture of BconB and HN (HN+BconB). Ingestion of the beverage containing
151 BconB showed the largest reduction in hunger rating among three treatments
152 (Fig. 1A). Hunger ratings gradually increased 15 min after ingestion, and ratings
153 at 60 min in HN and HN+BconB group were significantly higher than basal level.
154 The rating at 60 min in BconB-treated subjects was lower than in other
155 treatments and was not significantly different from the basal level. Hunger
156 ratings returned to basal levels (dotted line in the figure) at 45 min in the BconB
157 group, while it returned at 30 min in the HN and HN+BconB group.
158 BconB-treated subjects showed higher fullness ratings than the other two
159 groups throughout the experimental period. Fullness ratings at 60 min in the HN
160 and HN+BconB groups were significantly lower than basal level. Fullness ratings
161 returned at 60 min in BconB group and at 30 min in HN and HN+BconB group.
162 Changes in prospective consumption were similar with those in hunger ratings in
163 each treatment. Prospective consumption ratings at 45 and 60 min after HN and
164 HN+BconB ingestion were significantly higher than basal levels. The ratings of
165 prospective consumption were lower in the BconB group than in other groups.
166 In experiment 2, significant reductions in hunger rating were observed at 15, 30
167 and 45 min after the consumption of the jelly containing BconB (Fig. 2). Hunger
168 ratings at 15 and 30 min after the consumption of HN+BconB jelly were also

significantly lower than the basal rate. Ratings in these two groups were lower than the basal level throughout the experimental period. In contrast, ingestion of HN-containing jelly did not cause significant changes in hunger ratings, and the rating returned basal level after 45 min. Fullness ratings were significantly higher than basal levels at 30 min in the HN-treated subjects, at 15 and 30 min in the BconB-treated subjects and at 15, 30 and 45 min in the HN+BconB-treated subjects. Subjects that ingested the BconB-containing jelly showed tendency to rate higher fullness than subjects that ingested the HN-containing jelly. Ratings of prospective consumption were lower in the BconB-treated and BconB+HN-treated subjects than the HN-treated subjects. BconB-treated subjects showed a significantly lower rating than basal levels at 15 min after the ingestion.

The palatability of test jellies was evaluated by subjects just after consumption. Subjects scored 3.41 ± 0.20 for HN jelly, 3.55 ± 0.19 for HN+BconB jelly, and 3.88 ± 0.17 for BconB jelly, respectively. This demonstrates that these jellies are relatively favorable for subjects participated in the present study. Palatability was slightly increased in jellies containing BconB, but the differences were not statistically significant (ANOVA p value was 0.18).

Discussion

Previously, it was demonstrated that a duodenal infusion of peptic hydrolysate of beta-conglycinin (BconP) suppressed food intake in rats via CCK secretion from the intestine (Nishi 2003a). However, it was not known whether beta-conglycinin hydrolysate exerts the same satiety-inducing or

appetite-suppressing effect in humans. Pepsin digestion generates peptide fragments that have bitter tasting amino acids (hydrophobic and aromatic) at their terminals, resulting in peptic hydrolysates with a strong bitter taste. Indeed, peptic hydrolysate of beta-conglycinin has a strong bitter taste. In order to have less bitter hydrolysates for human use, beta-conglycinin was hydrolyzed by several food processing proteases. After tasting several hydrolysates, we selected the bromelain hydrolysate because its bitterness was much weaker than peptic hydrolysate, and it was not easily distinguishable from a control hydrolysate (a soy protein hydrolysate; Hinute). In the present study, we investigated the effect of BconB ingestion on appetites in healthy human subjects.

In experiment 1, we used a low-calorie beverage as the vehicle for the hydrolysates. Although changes in appetite profiles are relatively small against the scale of VAS (100 mm in full), the ingestion of a BconB-containing beverage induced fullness and reduced hunger to a larger extent than that of the HN-containing beverage (Fig. 1). In addition, prospective consumption was reduced only by BconB ingestion. Subjects ingesting a mixture of BconB and HN (HN+BconB) rated intermediate appetite profiles between HN and BconB. For the first time, these results demonstrate that the ingestion of BconB dose-dependently induces satiety and reduces appetite in healthy human subjects. Our results indicate that 3 g of BconB is the minimum dose to exert this satiety effect. Also at this dose, statistically significant changes in appetite profiles were detectable even with a 96 kJ energy intake. The satiety effect of BconB sustained at least for 40 min, as appetite profiles were returned to basal

level after 45 min.

In experiment 2, subjects ingested BconB- and/or HN-containing jelly as a solid vehicle. Overall changes in appetite ratings were greater (Fig. 2) as expected, but differences among the three treatments were less clear as compared to experiment 1. This might be due to the increased energy intake (264 kJ) or due to the change of vehicle from a liquid (beverage) to a solid (agar-jelly). However, BconB-containing jelly was more effective at inducing satiety than the HN-containing jelly; these effects lasted more than 60 min. From these results, the satiety effect of BconB was confirmed.

Subjects did not note any differences in the palatability of the test jellies evaluated, indicating that the effect of BconB is independent of palatability or taste. Preferable gradings (3.5-4.0 in average) of test jellies suggest that the taste of hydrolysates were well masked (provably by grapefruit flavor) and the appetite-reducing effects were not caused by ‘bad or bitter’ taste.

Several studies demonstrated suppressive effects of dietary protein on the human appetite. Various proteins (casein, soy protein, whey and gelatin) were used; however, the effectiveness and potency of these proteins on appetite control are controversial (Bowen 2006, Diepvens 2008, Hall 2003, Lang 1998, Veldhorst 2009a-c). Proteins (15-50 g) were ingested as a mixed meal which contains also carbohydrates and fats, and such meal had more than 1000 kJ in total energy. In contrast, subjects received 3 g of hydrolysate (50 kJ) mixed in a beverage (totally 96 kJ) or in a jelly (totally 264 kJ) in the present study. Therefore changes in appetite ratings in our study are relatively small as compared to previous studies. However, subjects having (only 3 g of) BconB

241 rated statistically higher fullness and less hunger than subjects having HN in
242 both experiments (Fig.1 and 2). A milk-derived peptide, caseinomacropeptide
243 (CMP) is a CCK-releasing dietary peptide (Beucher 1994, Pedersen 2000).
244 However, CMP at the dose of 0.4 g and 2 g in non-calorie beverage did not affect
245 human appetite in a previous study (Gustafson 2001). Thus, BonB is the first
246 dietary peptide which has significant satiating effects at a low dose (3 g) in
247 humans. It is interesting to know whether a preload of BconB would reduce
248 energy intake in future study.
249 Since blood samples were not collected from the participants, we can only
250 speculate the mechanism by which oral BconB induces satiety. However, it
251 would be possible that BconB induces satiety through stimulation of CCK
252 secretion from enteroendocrine I cells since we have previously demonstrated
253 that beta-conglycinin peptides stimulate CCK secretion in rat (Nishi, 2003a and
254 b) and enteroendocrine cell model (Hira 2009, Nakajima 2010). Further studies
255 are needed to investigate the role of CCK and other factors (hormones and/or
256 amino acids) in BconB-induced satiety in humans.
257 In summary, we examined whether ingestion of beta-conglycinin hydrolysate
258 (BconB) affected appetite in healthy humans. Subjects treated with
259 BconB-containing vehicles (either beverage or jelly) rated higher satiety and
260 lower hunger sensations as compared to subjects treated with soy protein
261 hydrolysate (HN)-containing vehicle. These effects are not correlated with the
262 palatability of test samples. These results demonstrate that oral BconB is
263 effective to induce satiety in healthy humans.
264

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343 **Figure legends**

344 Fig. 1. Changes in appetite profiles after the ingestion of beverages containing
345 test hydrolysates in healthy volunteers.
346 Appetites profiles were recorded by VAS method before and after ingestion of
347 the test hydrolysate. Test beverages (100 ml) contained either 3 g of soy protein
348 hydrolysate (HN: open circle), 3 g of beta-conglycinin hydrolysate (BconB:
349 closed circle) or mixture of 1.5 g of HN and 1.5 of BconB (HN+BconB: open
350 square). Values are expressed as means ± SEM. Two-way ANOVA p values for
351 Hunger (A) were < 0.05 for treatment, < 0.01 for time and 0.97 for treatment x
352 time; the values for fullness (B) were < 0.01 for treatment, < 0.01 for time and
353 0.56 for treatment x time; the values for prospective consumption (C) were <
354 0.01 for treatment, < 0.01 for time and 0.57 for treatment x time. Asterisk (*)
355 signs indicate significant differences from basal rating ($p < 0.05$). Plots at the
356 same time point not sharing the same letter differ significantly between
357 treatments ($p < 0.05$).
358
359 Fig. 2. Changes in appetite profiles after the ingestion of jellies containing test
360 hydrolysates in healthy volunteers.
361 Appetites profiles were recorded by VAS method before and after ingestion.
362 Test jellies (100 g) contained either 3 g of soy protein hydrolysate (HN: open
363 circle), 3 g of beta-conglycinin hydrolysate (BconB: closed circle) or a mixture of
364 1.5 g of HN and 1.5 of BconB (HN+BconB: open square). Values are expressed
365 as means ± SEM. Two-way ANOVA p values for Hunger (A) were < 0.05 for
366 treatment, < 0.01 for time and 0.96 for treatment x time; the values for fullness

(B) were 0.68 for treatment, < 0.01 for time and 0.99 for treatment x time; the values for prospective consumption (C) were 0.10 for treatment, < 0.01 for time and 0.99 for treatment x time. Asterisk (*) signs indicate significant differences from basal score ($p < 0.05$).

FIGURE 1

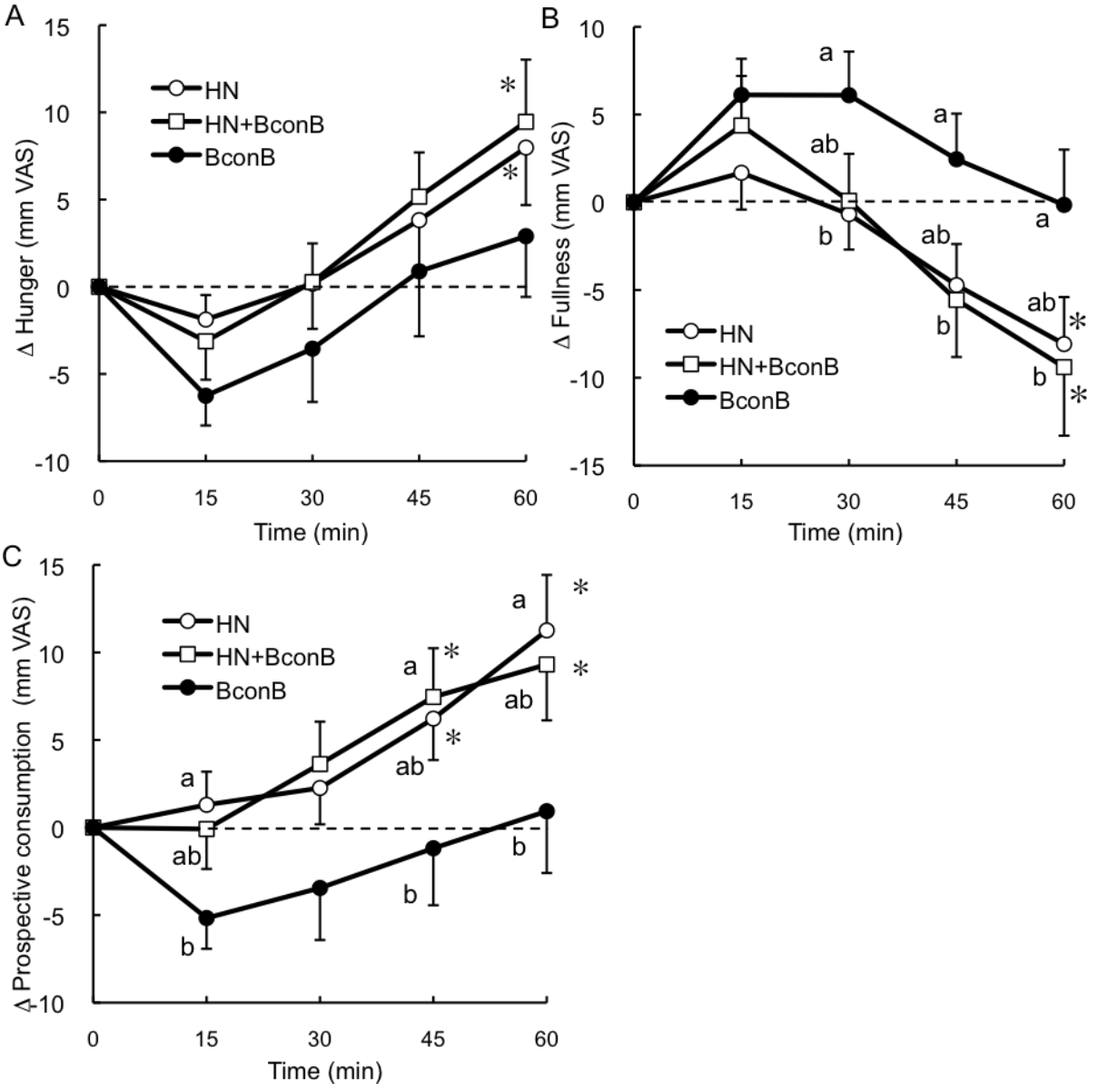
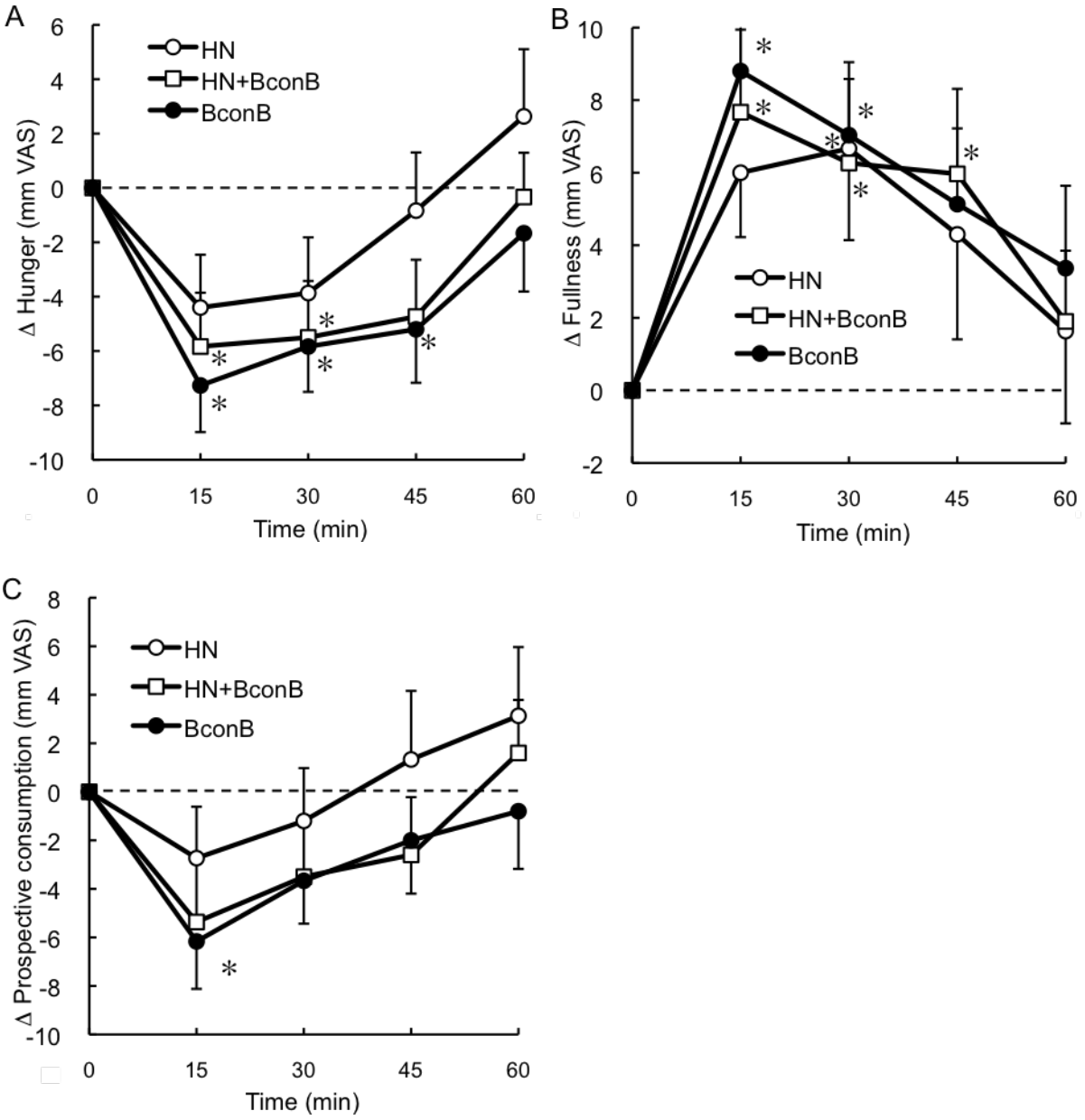

A
Δ Hunger (mm VAS)
Time (min)
HN
HN+BconB
BconB
B
Δ Fullness (mm VAS)
Time (min)
HN
HN+BconB
BconB
C
Δ Prospective consumption (mm VAS)
Time (min)
HN
HN+BconB
BconB

FIGURE 2


A
HN
HN+BconB
BconB
Δ Hunger (mm VAS)
Time (min)
B
HN
HN+BconB
BconB
Δ Fullness (mm VAS)
Time (min)
C
HN
HN+BconB
BconB
Δ Prospective consumption (mm VAS)
Time (min)