



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

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

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16 Running head

17 Protein hydrolysate suppresses human appetite

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24

25 **Abstract**

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

35

36 **Key words**

37 Dietary peptide, Soybean beta-conglycinin, Appetite, Cholecystokinin

38

39 **Introduction**

40 Dietary protein is thought to have the highest satiety effect when compared with
41 isoenergetic fats and carbohydrates in human subjects and rats (Bensaid 2002,
42 Reid 1997, Tome 2004). Cholecystokinin (CCK) is one of the gut hormones which
43 induce satiety sensations and reduce food consumption in humans (Kissileff
44 1981, Lieverse 1995), and the gut hormone mediates protein-induced satiety
45 (Moran 2004, Nolan 2003).

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

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

56 In previous human studies, it has been demonstrated that satiating effects of
57 dietary proteins varies with the amount and source of protein, and also with the
58 experimental conditions (Lang 1998, Lang 1999, Diepvens 2008, Veldhorst
59 2009a). Gelatin and alpha-lactalbumin were reported to have higher satiating
60 effects than casein, soy or whey proteins (Veldhorst 2009b). In these human
61 studies, 15 to 50 g of protein sources were mixed with fats and carbohydrates in
62 a meal such as breakfast or lunch with total energy of around 2 MJ (~ 500 kcal).
63 Such study design could help to observe apparent changes in appetite scores.
64 However, there are few studies using relatively small amount of protein (< 5 g)
65 mixed in a vehicle with lower energy content than in 'a meal' (Gustafson 2001). It
66 is valuable to examine the effect of relatively small amount of dietary peptides on
67 human appetite sensations. In the present study, subjects ingested 1.5-3 g of
68 beta-conglycinin hydrolysates and evaluated appetite sensations by using visual
69 analogue scale (VAS) method.

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

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

75

76 **Materials and Methods**

77 **Materials**

78 Beta-conglycinin hydrolysate (BconB) was prepared as follows.

79 Beta-conglycinin (provided by Fuji oil Co., Ltd., Tokyo, Japan) was suspended in
80 de-ionized water and the pH was adjusted to 6.0. Bromelain (a food processing
81 protease from pineapple stem, Biocon Japan Ltd., Nagoya, Japan) was added to
82 the suspension (0.1% against substrate) and shaken for 60 min at 55°C. The
83 suspension was boiled for 20 min to stop the enzyme reaction. The supernatant
84 was collected by centrifugation and filtration, and then lyophilized. Soy protein
85 hydrolysate (HN: Hinute) was provided by Fuji oil Co., Ltd. The average
86 molecular mass was estimated at 4,400 Da for BconB and 3,100 Da for HN by a
87 size exclusion chromatography performed in a FPLC system (AKTA explorer
88 10S and Superdex Peptide 10/300 GL column, GE Healthcare Bio-Sciences AB,
89 Uppsala, Sweden).

90

91 **Subjects**

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

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

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

103

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

106 Thirty volunteers (14 male and 16 female) with a mean age of 24 ± 1 (range
107 21-35 years old) participated in experiment 1. After the lunch, subjects fasted for
108 3 hours, but they were allowed to drink water until 16:00. At 16:25, basal appetite
109 profiles were rated before the ingestion of the test beverage. Appetite profiles
110 including hunger, fullness and prospective consumption were rated on a 100 mm
111 Visual Analogue Scales (VAS) anchored with 'not at all' and 'extremely'. BconB
112 (3 g), HN (3 g) or BconB (1.5 g) + HN (1.5 g) was dissolved in 100 ml of a low
113 calorie (46 kJ/100 ml) beverage (POCARI SWEAT STEVIA, Otsuka
114 Pharmaceuticals Co, Ltd., Tokyo, Japan). The dose of 3 g in 100 ml was
115 selected because this dose did not disturb the taste of the beverage and
116 subjects could consume test beverage in a short period (5 min). Subjects
117 ingested the test beverage at 16:30 after the instruction to drink the whole
118 beverage (100 ml, 96 kJ) within 5 min. Appetite profiles were rated every 15 min
119 until 60 min after the ingestion. Subjects were not allowed to have additional
120 drinks or foods during the experiment.

121

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

123 Forty-six volunteers (19 male and 27 female) with a mean age of 25 ± 1 (range

124 21-52 years old) participated in experiment 2. Grapefruit-flavored test jellies

125 (totally 264 kJ) consisted of agar, sugar and 3 g of peptides (prepared by a local

126 sweets company, Yamashitakan Santaxream, Ebetsu, Hokkaido, Japan). After

127 the lunch, subjects fasted for 3 hours, but they were allowed to drink water until

128 16:00. Before the ingestion of the test jelly, basal appetite profiles were rated as

129 described above. Subjects ingested the jelly (100 g, 264 kJ) and 50 ml of water

130 at 16:30, and were instructed to consume the serving within 5 min. Just after the

131 consumption, palatability of the test jelly was graded according to five ranks.

132 Appetite profiles were rated every 15 min until 60 min after the ingestion.

133 Subjects were not allowed to have additional drinks or foods after during the

134 experiment.

135

136 Statistical analysis

137 Appetites profiles were expressed as absolute changes (mmVAS) from the

138 basal (0 min). All data are expressed as mean \pm SEM. Two-way repeated

139 ANOVA was performed to assess the effect of treatment, time and interaction of

140 treatment and time (treatment x time) on appetite profiles (Fig. 1 and 2).

141 One-way ANOVA was performed to assess the possible difference among the

142 palatability of test jellies. After detecting a significant ($p < 0.05$) main effect in the

143 ANOVA, significant differences among mean values were determined by the

144 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

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

181 The palatability of test jellies was evaluated by subjects just after consumption.
182 Subjects scored 3.41 ± 0.20 for HN jelly, 3.55 ± 0.19 for HN+BconB jelly, and
183 3.88 ± 0.17 for BconB jelly, respectively. This demonstrates that these jellies are
184 relatively favorable for subjects participated in the present study. Palatability was
185 slightly increased in jellies containing BconB, but the differences were not
186 statistically significant (ANOVA p value was 0.18).

187

188 **Discussion**

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

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

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

217 level after 45 min.

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

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

231 Several studies demonstrated suppressive effects of dietary protein on the
232 human appetite. Various proteins (casein, soy protein, whey and gelatin) were
233 used; however, the effectiveness and potency of these proteins on appetite
234 control are controversial (Bowen 2006, Diepvens 2008, Hall 2003, Lang 1998,
235 Veldhorst 2009a-c). Proteins (15-50 g) were ingested as a mixed meal which
236 contains also carbohydrates and fats, and such meal had more than 1000 kJ in
237 total energy. In contrast, subjects received 3 g of hydrolysate (50 kJ) mixed in a
238 beverage (totally 96 kJ) or in a jelly (totally 264 kJ) in the present study.

239 Therefore changes in appetite ratings in our study are relatively small as
240 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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340
341
342

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 \pm 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 \pm 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

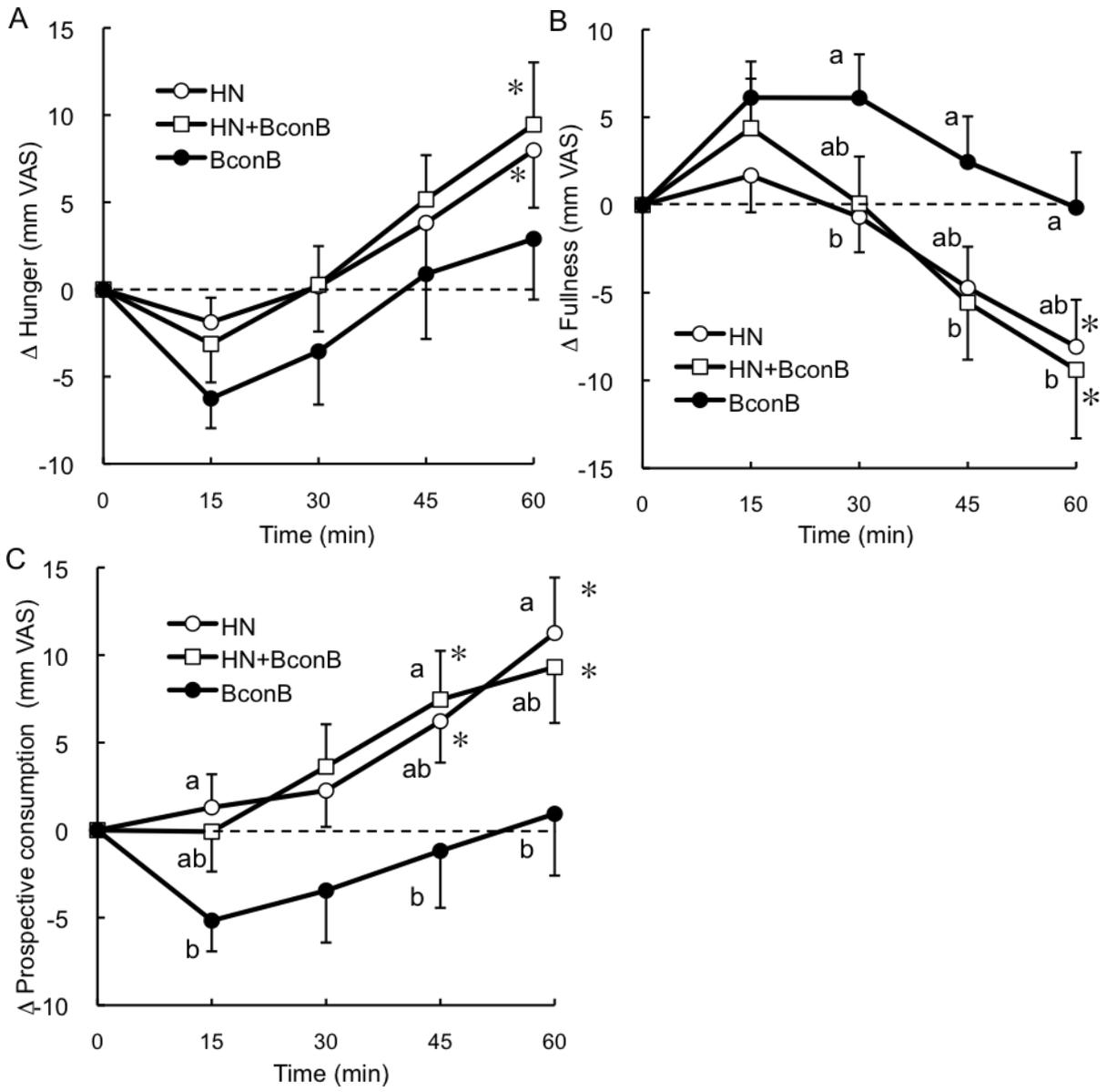
367 (B) were 0.68 for treatment, < 0.01 for time and 0.99 for treatment x time; the
368 values for prospective consumption (C) were 0.10 for treatment, < 0.01 for time
369 and 0.99 for treatment x time. Asterisk (*) signs indicate significant differences
370 from basal score ($p < 0.05$).

371

372 **FIGURE 1**

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374



375

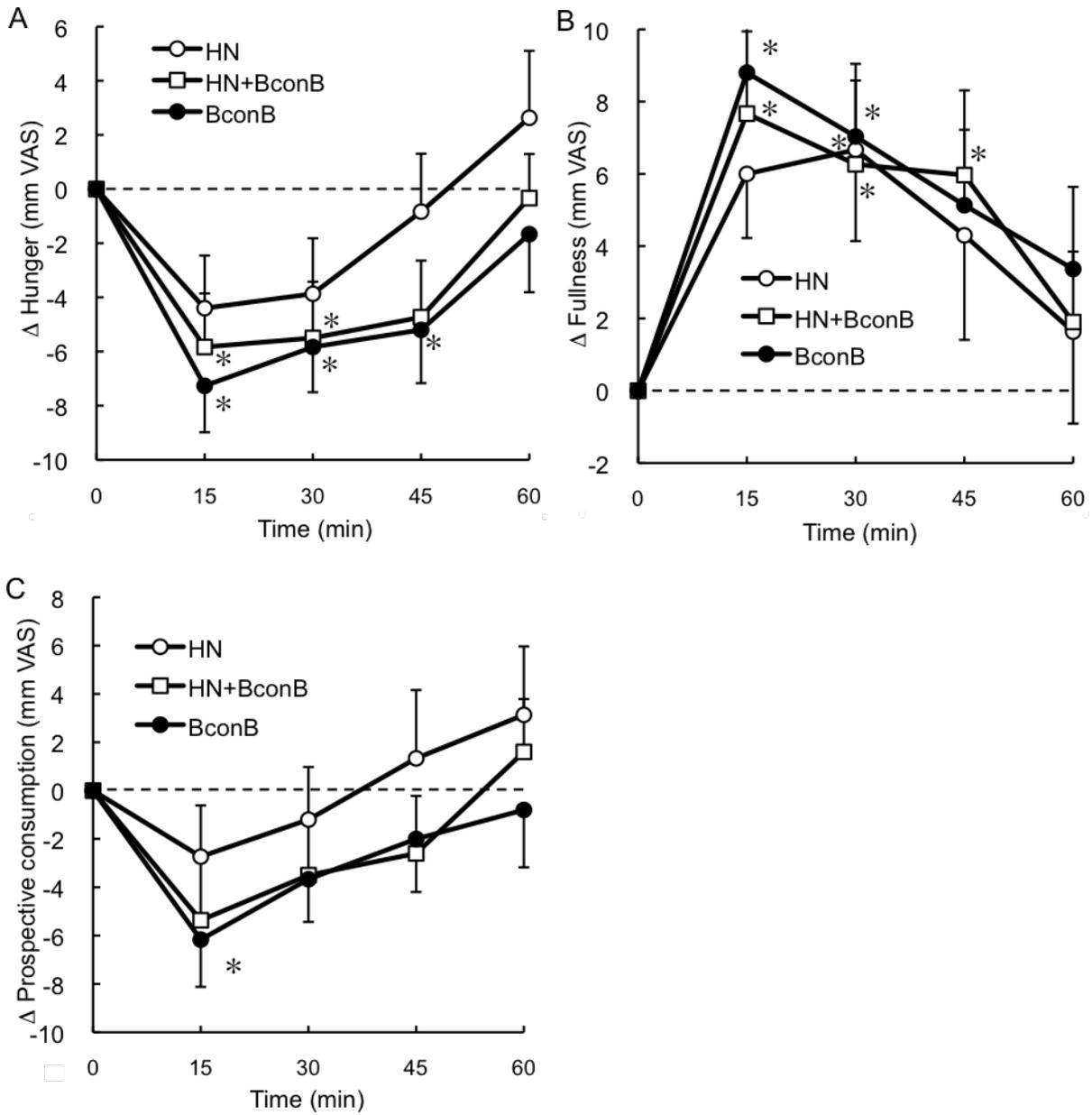
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377

378 **FIGURE 2**

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