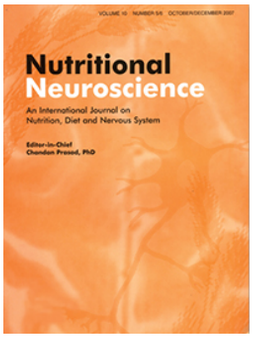

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Satiety and memory enhancing effects of a high-protein meal depend on the source of protein

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Objective: High- protein diets have become increasingly popular with various touted benefits. However, the extent to which protein quantity and source affects cognitive functioning through altering postprandial amino acid profiles has not been investigated. Further, whether all protein sources are similarly anorexigenic is uncertain. The objective of this study was to determine the influence of protein level and source on Barnes maze performance, satiety and plasma amino acid levels in male Sprague-Dawley rats.
Methods: Rats were entrained to a meal-feeding schedule consisting of a 30 minutes meal, equivalent to 20% of average daily intake, one hour into the dark phase then *ad libitum* access to food for 5 h. On test days, rats received one of three isocaloric diets as their first meal, hereafter referred to as Egg White (EW), Wheat Gluten (WG), or Basal, and then were measured for cognitive performance, feeding behavior, or plasma amino acid levels via jugular catheter. Percentage energy from protein was 35% for both EW and WG and 20% for Basal with equal amounts provided by EW and WG proteins.
Results: Rats provided EW performed similarly to Basal on the Barnes maze, whereas WG performed worse. EW increased satiety, whereas WG reduced satiety relative to Basal. Both EW and WG increased postprandial concentrations of large neutral and branched chain amino acids relative to Basal, but in EW, concentrations were slower to peak, and peaked to a higher level than WG.
Discussion: Results demonstrate the importance of protein source for cognition and satiety enhancing effects of a high-protein meal.

Keywords: Satiety, Cognitive function, Learning, Memory, Egg white protein, Wheat gluten protein, Protein type, Food intake

Introduction

High- protein diets have become widely popular in recent decades. For the majority of individuals, protein intake is heavily skewed towards the later meals of the day. While 40–42% of protein is consumed at dinner and 31% consumed at lunch, only ~15–16% protein is consumed at breakfast.[1] Increasing protein levels consumed at breakfast has been demonstrated to result in higher metabolic activation and heightened state of alertness in comparison to isoenergetic carbohydrate or fat meals.[2] Conversely, high-carbohydrate breakfasts were reported to induce greater sleepiness and calmness, when measured several hours later.[3] The protein component of breakfast requires increased consideration for the effects it could have on cognitive performance,[4] as well as body weight management.[5]

The effect of dietary protein on subsequent cognitive function has received limited attention. Most of the literature on meal-driven effects on cognitive function have focused on glycemic load and glycemic index of the meal.[6–8] However, dietary proteins can also influence post meal glycemia[9] and amino acid precursors to monoamines,[10] to thereby alter availability of fuel and neurotransmitter needed for cognitive function. In particular, concentrations of large neutral amino acids (LNAA) may affect brain functions by altering levels of aromatic amino acids, which are required by neurons for monoamine neurotransmitter synthesis. Evidence suggests that high- protein meals,

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and more specifically tyrosine administration, can improve cognitive performance,[11] possibly by supplying precursor amino acids for catecholamine neurotransmitter synthesis. However, the extent to which different protein sources can impact cognition by virtue of having different LNAA composition is not known. To identify specific dietary components, Edefonti and colleagues recommended comparison of isoenergetic breakfast interventions that differ in a single nutrient component.[4] We are aware of no studies evaluating protein source at the first meal of the day on subsequent cognitive performance.

The relationship between dietary protein and satiety is more firmly established. Dietary protein has been observed to increase satiety and suppress short-term food intake beyond what would be expected by an isoenergetic amount from carbohydrates and fats.[12] This effect appears to be most apparent with high- protein meals consumed at breakfast time. A high- protein meal provided at breakfast caused greater satiety compared to equally high- protein meals provided at lunch or dinner.[13] Food intake at lunch was lower following higher protein breakfasts (30 or 39 g protein) *vs* low protein (3 g) or no breakfast in normal to overweight women.[14] In overweight 8–12 yr old girls, a higher protein breakfast increased energy expenditure and fat oxidation as compared to a lower protein breakfast which corresponded with increased satiety ratings.[15] Meals having increased dietary protein evidently have positive effects on subsequent appetite and food intake, especially when consumed at breakfast.

Although it is established that a high-protein diet can enhance satiety, the extent to which the source of the protein matters is uncertain. Whey and casein, two milk derived protein sources, have more commonly been studied with regard to their effects on satiety, with whey protein reported as more satiating than casein.[16,17] It is less conclusive how other protein sources compare in their capacity to induce satiety. While whey protein preloads exerted greater satiety in comparison to both soy protein and egg white (EW) protein in one study,[18] a separate study reported satiety ratings to be the same for whey and EW protein.[19] Overweight subjects provided with either an egg breakfast (18.4 g protein) or bagel-based breakfast (13.6 g protein), showed greater satiety following the egg-based meal.[20] Crowder and colleagues, on the other hand, found no difference in apparent satiety between a mixed animal-based *vs.* plant-based protein breakfast providing 27 g of protein (~25% of calories) when fed to normal weight or overweight young women.[21] It has also been demonstrated in rats that ingestion of high- protein diets in which protein came from different milk proteins have differential effects on subsequent food intake.[22] Studying protein-induced satiety in rat models allows for careful control over various external influences which can impact food intake.[23]

Table 1 Composition of amino acids in test protein (grams/100 grams protein)

	Egg white	Wheat gluten
Ala	6.2	3.6
Arg	5.7	4.5
Asp	8.6	5.0
Cys	2.4	2.5
Glu	13.5	33.1
Gly	3.6	4.1
His	2.2	2.5
Ile	5.9	3.5
Leu	8.4	6.9
Lys	6.0	2.9
Met	3.8	1.2
Phe	6.1	4.7
Pro	3.6	10.9
Ser	7.1	4.6
Thr	4.3	2.9
Trp	1.5	1.1
Tyr	3.9	1.7
Val	7.2	4.3

The objective of this study was to compare the effects of a high- protein breakfast composed of EW protein versus wheat gluten (WG) protein on cognitive performance, satiety, and plasma amino acid profile. EW and WG proteins are two of the most common sources of protein at breakfast, and to the best of our knowledge, have not been directly compared in regard to their effects on cognitive function and feeding behavior. EW protein is considered to be a higher quality protein, containing higher levels of essential amino acids (Table 1).[24] Therefore, we hypothesized that rats fed a meal containing EW protein would have a greater tyrosine to LNAA ratio and greater overall concentration of amino acids in circulation than rats fed WG, and that this would be associated with enhanced learning and memory performance on the Barnes maze and a smaller subsequent meal (an indication of increased satiety).

Methods

Animals

Male Sprague-Dawley rats (~125 g; Charles River) were singly housed in plexiglass cages (30 × 30 × 38 cm), except as noted in Experiment 2, in a temperature-controlled room (26 ± 2°C) with 12 h light:dark cycle (lights off at 1200). Rats were given ad libitum access to water and were acclimated to a feeding schedule for 1 week prior to the start of an experiment. Food was removed one hour prior to the onset of the light cycle for an overnight fast. At one hour into the dark cycle (1300 h), rats were given 30 minutes to consume the meal, representing 20% average daily intake. Fewer than 7% of rats did not finish their meal in the allotted 30 minutes, and were removed from experiments. Basal diet was available ad

libitum between 1800 and 2300 h. Separate cohorts of rats were used for each experiment. Studies were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Illinois and University of North Carolina Greensboro, and were in accordance with the Guide for Care and Use of Laboratory Animals (National Research Council).

Diet preparation

Diets were prepared to be comparable to AIN-93G (Table 2). All diet components were purchased from Dyets, Inc. (Bethlehem, PA) except EW powder and maltodextrin which were purchased from Harlan (Indianapolis, IN). Protein in the basal diet was a 50:50 mix of EW and WG at 20% of calories. Protein in the other diets was 35% of calories and provided by spray dry EW or WG (WG).

Experiment 1: learning and memory

The first experiment was conducted to test the effect of WG and EW on learning and memory on the Barnes maze. Rats received one of the three test diets ($n = 9$ per diet) at 1300 h, the entrained meal- feeding period, and were allowed 30 minutes to complete the meal. A fourth group received no meal (NB, $n = 6$) to serve as a negative control.[8,25] Testing began 30 – 60 minutes following ingestion of the test meal. The maze platform was black, circular, 122 cm in diameter, with 20 evenly spaced holes (10 cm diameter) 2 cm away from the edge, with only one hole leading to a removable black escape tunnel. The test paradigm consisted of a habituation session on the first day during which rats were placed in the escape tunnel for one minute with the lights turned off. This was followed by four consecutive days of acquisition trials with four trials per day in which rats were allowed 180 seconds per trial to find the escape tunnel. Rats that were unsuccessful at locating the escape tunnel by the end of the 180 seconds were gently guided to the correct hole by the investigator. Rats were returned to their home cage for 15 minutes between trials. A 90 sec probe trial (no escape tunnel) was performed the day following the last day of acquisition. Bright lights and a standing fan were turned on during the task to encourage animals to find the escape tunnel and were immediately turned off once the rat entered the escape tunnel. The maze was kept in the same position in the room and visual cues, including intentional images placed on the walls, were never moved between trials. Animal movements in the maze were tracked using TopScan software (Clever Sys Inc., Reston, VA) analysis of ceiling-mounted video feed.

Table 2 Composition of test diets

Grams	Basal	EW	WG
Egg white powder[a]	123	430.5	
Wheat gluten powder[b]	131		458.5
Corn starch	372	256	238
Maltodextrin	120	83	77
Sucrose	75.68	52	48
Cellulose	53.5	53.5	53.5
Soybean oil	90	90	90
Mineral mix (EW)[c]	17.5	35	
Mineral mix (WG)[d]	17.5		35
Vitamin mix[e]	10	10	10
Choline bitartrate	2.5	2.5	2.5
L-Lysine	1.9		6.8
L-Threonine	0.5		1.8
	1015	1013	1021
kcal%			
Protein	20%	35%	35%
Carbohydrate	60%	45%	45%
Fat	20%	20%	20%

[a]Egg white solids, spray-dried, #160230 (83.2% protein, 4.5% carbohydrate), Evigo, Madison, WI, USA.
[b]Wheat gluten, #402100 (76.1% protein, 1.1% fat, 13.7% carbohydrate), Dyets, Inc., Pittsburg, PA, USA.
[c]AIN-93G-MX for egg white diets (40.1% sucrose), #210038, Dyets, Inc., Pittsburg, PA, USA.
[d]AIN-93G-MX for wheat gluten diets (18.4% sucrose), #210039, Dyets, Inc., Pittsburg, PA, USA.
[e]AIN-93VX, #310025 (97.4% sucrose), Dyets, Inc., Pittsburg, PA, USA.

Experiment 2: feeding behavior

Rats ($n = 12$) were allowed three days to acclimate to a Comprehensive Laboratory Animal Monitoring Systems (CLAMS) (Oxymax; Columbus Instruments International, Columbus, OH, USA) before test diets were randomly assigned. CLAMS provided continuous monitoring of food intake following ingestion of treatment meals for assessment of subsequent satiety. Each CLAMS cage provided access to a food cup that rested on an electronic scale that continuously reported the weight of the contents to the computer. Each time food was removed, the weight change on the scale was recorded as a bout of feeding. At 1 h into the dark phase, the test diets were available for 30 minutes or until the allotted 20% of average daily intake was consumed. Food cups were replaced 15 minutes after the end of the first meal with the basal diet and rats were allowed *ad libitum* access to feeders until one hour prior to the light phase. This test was repeated for a total of three consecutive days. After 3 days, each rat received one of the other diets (randomized and counterbalanced) for an additional 3 days of testing. Meals were defined as the sum of bouts of food intake greater than 0.03 grams and inter-meal time interval between adjacent bouts of at least 10 minutes.[26]

Experiment 3: preference study

A follow up study was conducted to assess if the difference in food intake after the test meal could be related to food preference. Rats ($n = 24$) were randomly assigned to one of the three diet groups (Basal, EW, or WG) for the 30 minute test meal. Fifteen minutes following the test meal, rats were given a choice between

two diets and food intake was measured for 90 minutes. A cross over design was used such that rats in each treatment group received each combination of diets (EW/Basal, EW/WG, or Basal/WG) over three consecutive days of testing. The food was placed in glass jars attached to opposite corners of the cage in random fashion and food intake measured by the difference in jar weight after correcting for spillage. Rats were provided ground chow (Harlan Teklad 8640, Madison, WI) to consume *ad libitum* later in the day for 5 h before food was removed 1 h prior to light onset.

Experiment 4: plasma amino acids

Plasma samples were collected as previously described[27] from rats adapted to the feeding schedule and fitted with jugular vein catheters. Plasma was collected at 30 minute intervals, with the first sample taken 30 minutes before and the final sample taken 210 minutes after rats received one of the three test diets ($n = 8$ Basal; $n = 12$ WG; $n = 12$ EW). Whole blood (250 microliters) was collected into heparinized microtubes, centrifuged for 10–15 seconds at 6000 rpm and 100 microliters of plasma harvested. Red blood cells were re-suspended in an equal volume of 0.9% sterile saline solution and returned to each animal through the jugular vein catheter. Plasma were kept on ice until stored at −80 Celsius until amino acid analysis.

Amino acids were analyzed by HPLC with fluorometric detection[28] using an Ultimate 3000 RSC System (Thermo Scientific). Plasma was deproteinated with 3.5% perchloric acid and centrifuged for 10 minutes at 8000 rpm before injection onto a 250 × 4.0 mm C18 (5 μm) column after derivatization with o-phthaldialdehyde. Amino acids were separated using a binary gradient with sodium phosphate and acetonitrile/methanol based buffers. Data were collected and analyzed using Chromeleon Data System software (Thermo Fischer).

Statistical analysis

Data were analyzed using SAS v9.2 (SAS Institute Inc., Cary, NC). Average latency (s), path length (mm), and speed (mm/s) to enter the escape tunnel in the Barnes maze (averaged across the four trials per day) were analyzed by repeated measures ANOVA with Days as the within-subjects factor and Treatment (Basal, NB, EW, WG) as the between-subjects factor. For the probe trial, duration spent in the target quadrant was compared to chance performance (i.e. 25%, four quadrants) using a one-sample *t*-test separately for each group to determine whether the rats in that group remembered the location of the escape tunnel. Next, performance was compared across groups by analyzing the duration in the target quadrant (s), latency (s), and speed (mm/s) to the target quadrant by one-way ANOVA with Treatment (Basal, NB, EW, WG) as the factor. Main effects of protein source on subsequent food intake were analyzed the same way except Treatment included only three levels, (NB was only included in the first experiment). Preference data were analyzed using repeated measures two-way ANOVA as follows. Food intake of one of the two diets provided during the preference trial was analyzed as a function of the Test meal administered at 1300 h (between-subjects factor with three levels: EG, WG, Basal), the Food type consumed during the preference test which was entered as the response variable for that line of data (within subject factor also three levels: EG, WG, Basal), and the interaction between test meal and Food type. Using this analysis, a main effect of the Test meal indicates the extent to which the treatment diets affected food intake after collapsing across all three different types of food offered during the preference test (as a measure of general satiety). A main effect of Food type indicates the extent to which certain diets were preferred or rejected during the preference test. The interaction indicates the extent to which the preference for the Food types was altered depending on which diet the rat was given as the Test meal. Concentrations of amino acids were analyzed by repeated measures ANOVA with Time-point (9 levels) as the within-subjects factor and Treatment (Basal, EW, WG) as the between-subjects factor. Large neutral amino acids (LNAA; phenylalanine, tyrosine, tryptophan, leucine, isoleucine, and valine) and branched chain amino acids (BCAA; leucine, isoleucine, and valine) were summed first. Least square differences (*t*-tests, with mean square error from ANOVA model) were used to evaluate pair-wise differences between means. Values were presented as means ± SEM. An alpha level of $P < 0.05$ was considered statistically significant.

Results

Experiment 1: learning and memory performance

Rats in all four treatment groups learned to locate the target escape hole as indicated by decreased latency ($F_{3,78} = 49.32$; $P < 0.0001$; see Fig. 1A) and path length ($F_{3,78} = 26.70$; $P < 0.0001$; see Fig. 1B) to the target hole across the four training days. A trend was detected for a Treatment × Day interaction for path length ($P < 0.1$). On day 1, rats provided the EW or Basal treatment meal tended to travel a shorter path than the NB and WG groups, whereas on subsequent days, the treatments did not differ from each other (see Fig. 1B). There was a significant main effect detected for speed ($F_{3,78} = 3.17$; $P < 0.05$; graph not shown). Rats fed WG traveled approximately 10% faster in the maze as compared to the other groups which did not differ from each other.

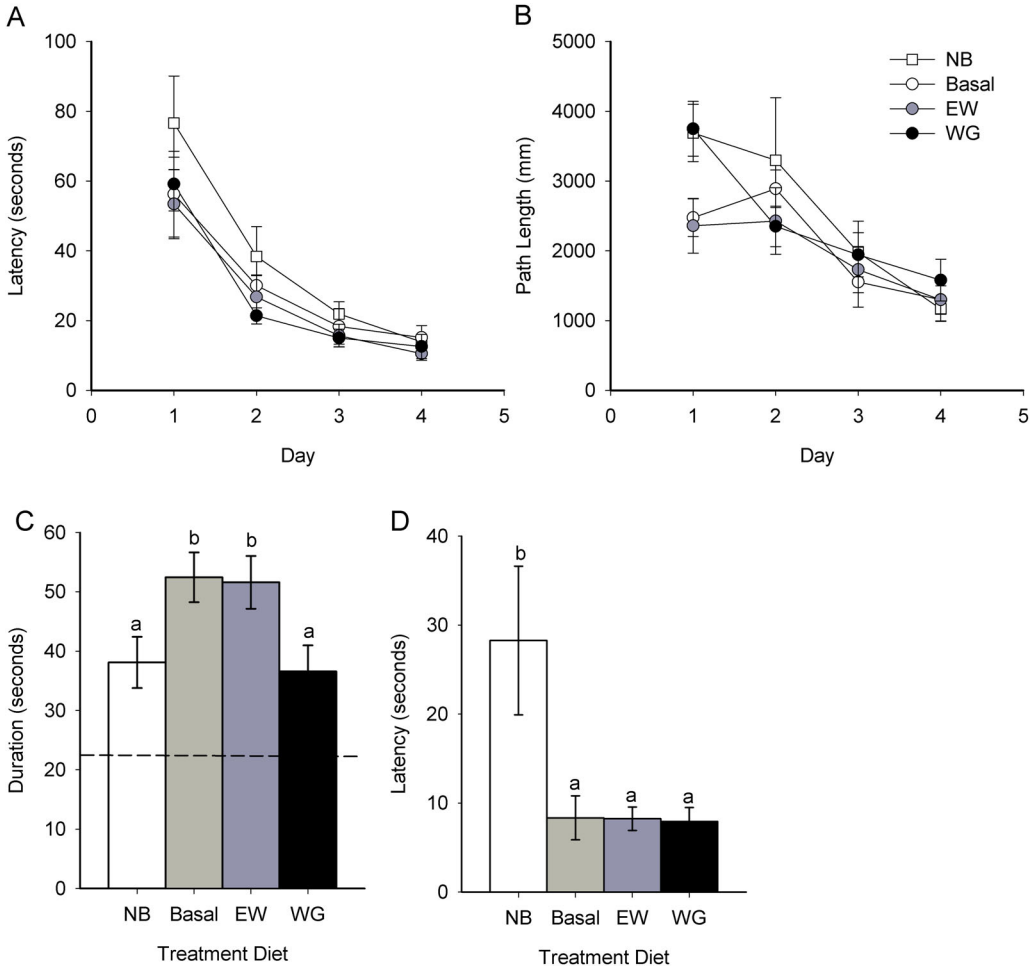

Figure 1 Learning and memory performance on the Barnes Maze. (A) Average latency (seconds) to the escape tunnel across days ($n = 6$-9/treatment). (B) Same as A for path length (mm). (C) Average duration (sec) spent in target quadrant during the 90 second probe trial ($n = 6$-9/treatment). (D) Average latency (sec) to reach the location of the previously correct target hole during probe trial. Bars represent mean ± SEM. * indicates a significant main effect of treatment ($P < 0.05$). Bars with different superscripts are significantly different by posthoc *t*-tests ($P < 0.05$). NB, No Breakfast; WG, Wheat gluten diet; EW, Egg white diet.

In the probe trial, all treatment groups spent significantly more time in the target quadrant than would be expected by chance (22.5 seconds; 25% of 90 sec trial), indicating that the animals learned spatially where the hidden escape hole was located (all $P < 0.0001$; see Fig. 1C). In addition, there was a significant main effect of diet on duration spent in the target quadrant ($F_{3,24} = 3.18$; $P < 0.05$; see Fig. 1C). Rats provided the Basal and EW diets spent more time in the target quadrant than rats in the other two treatment groups (all $P < 0.05$). A treatment effect on latency to the target hole was also detected ($F_{3,24} = 5.92$; $P < 0.005$; see Fig. 1D). The NB rats that had not been provided a meal took longer to get to the hole where the escape tunnel had been on previous testing days as compared to the other three groups ($P < 0.05$). A treatment effect was also detected for speed ($F_{3,26} = 7.97$; $P < 0.001$; graph not shown). Rats provided the WG diet as the treatment meal traveled approximately 20% faster during the probe trial as compared to the other treatment groups.

Experiment 2: feeding behavior

Rats consumed the 4 g test meals at different rates ($F_{2,54} = 105.4$; $P < 0.01$). They consumed the Basal diet the quickest and EW diet the slowest (Table 3). When feeders were reopened after the test meal, rats from all three treatment groups immediately approached the feeder, though the size of the first meal following the test meal was different ($F_{2,21} = 4.39$; $P < 0.05$) across groups. Rats that had with the WG group consuming significantly more (7.1 ± 0.7 g) than the Basal group (4.5 ± 0.5 g; $P < 0.001$) or the EW (3.6 ± 0.4 g; $P < 0.001$) (see Fig. 2). However, no significant differences in the length of the subsequent meal were detected. Over the course of the *ad libitum* period, rats provided WG consumed fewer total meals ($F_{2,69} = 13.60$; $P < 0.0001$) compared to EW ($P < 0.0001$) or Basal ($P < 0.01$). Additionally, WG fed rats had larger meals ($F_{2,69} = 8.97$; $P < 0.0005$, Table 3) compared to Basal ($P < 0.05$) and EW-fed rats ($P < 0.0005$). Table 3 provides a summary of additional food intake measurements collected from CLAMS cages.

Table 3 Feeding behavior following consumption of a test meal (20% of daily caloric intake) of basal, wheat gluten, or egg white diet

	Basal	Wheat gluten	Egg white	*P*-value
Test meal				
Meal duration (min, start to end)	15.3 ± 0.5	20.4 ± 1.1	29.4 ± 0.4	<0.01
Subsequent meal				
Meal duration (min, start to end)	44.4 ± 12.0	48.4 ± 8.2	22.6 ± 4.8	0.09
Meal duration (min, total of bouts)	39.9 ± 11.9	41.5 ± 8.0	18.8 ± 4.5	0.13
Time to feeder (min)	6.7 ± 3.3	7.9 ± 6.3	1.5 ± 0.5	0.50
Meal size (g)	4.5 ± 0.5	7.1 ± 0.7	3.6 ± 0.4	<0.01
Whole day				
Food intake (g)	18.1 ± 0.5	17.1 ± 0.6	18.2 ± 1.3	0.61
Time spent at feeder (min)	115.6 ± 12.0	93.5 ± 7.7	103.0 ± 7.2	0.24
Number of meals	7.9 ± 0.5	6.1 ± 0.4	9.1 ± 0.3	<0.01
Average meal size (g)	2.4 ± 0.1	3.2 ± 0.3	2.0 ± 0.1	<0.01
Average meal duration (min)	16.7 ± 2.6	18.9 ± 3.0	11.6 ± 0.9	0.08
Meal number	7.9 ± 0.5 [a]	6.1 ± 0.4 [b]	9.1 ± 0.3 [c]	<0.01
Meal size (g)	2.4 ± 0.1 [a]	3.2 ± 0.3 [b]	2.0 ± 0.1 [c]	<0.01
Meal duration (min)	16.7 ± 2.6	18.9 ± 3.0	11.6 ± 0.9	0.08

Results are represented as mean ± SEM.

Experiment 3: preference study

A significant main effect of Test meal ($F_{2,21} = 7.31$; $P < 0.005$) suggests the different treatment diets had different effects on amount consumed in the subsequent period (satiety measure) (see Fig. 3). Similar to experiment 2, animals fed EW consumed less total food during the subsequent period than when fed Basal or WG ($t = 2.56$, $P = 0.013$ and $t = -4.68$, $P < 0.0001$, respectively), with WG fed rats consuming more than rats given the Basal diet ($t = -2.18$, $P = 0.033$; see Fig. 3).

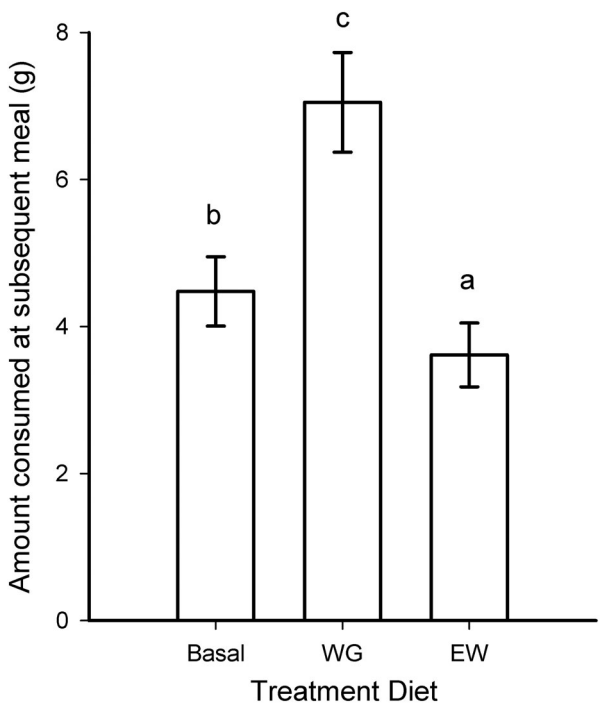

Figure 2 Effect of breakfast type (Basal, WG, or EW) on total amount consumed in subsequent meal (Experiment 2). Bars represent mean ± SEM. Bars with different superscripts are significantly different by posthoc *t*-tests ($P < 0.05$). WG, Wheat gluten diet; EW, Egg white diet.

A main effect of Food type ($F_{2,41} = 24.07$; $P < 0.0001$) and interaction between Food type and Test meal ($F_{4,41} = 5.59$; $P < 0.005$) suggests that the different diets were unequally preferred and that preferences depended on the test meal given. Basal and WG diets were equally preferred, as seen by the nearly equal consumption when both diets were provided during the preference testing period (see Fig. 3A). When Basal was provided as the treatment meal and EW was provided as one of the choices during preference testing, less EW was consumed (WG *vs* EW $t = -3.37$, $P = 0.002$ and Basal *vs.* EW $t = 4.43$, $P < 0.0001$; see Fig. 3B and C). Similarly, when WG was provided as the treatment meal and EW was provided as one of the choices during preference testing, less EW was consumed (WG *vs* EW $t = -6.88$, $P < 0.0001$ and Basal *vs.* EW $t = 3.60$, $P = 0.001$; see Fig. 3B and C). However, it is important to note that when EW was provided for the treatment meal, no difference in amount consumed between choice diets was observed during the preference testing period (WG *vs* EW $t = -0.90$, $P = 0.373$ and Basal *vs.* EW $t = 1.17$, $P = 0.250$; see Fig. 3B and C).

Experiment 4: plasma amino acid analysis

Concentration of leucine in the plasma monotonically increased for approximately 90 min after initiation of feeding, but the rate and magnitude of the increase depended on the diet. The steepest rise in leucine occurred following the WG meal; the increase following EW was more gradual and was marginal for Basal. Average level of leucine across the entire time period was lower in the Basal group than the high- protein diets, and tended to be higher in WG than EW (Fig. 4A). This was indicated by a significant main effect of Time ($F_{8,291} = 34.3$; $P < 0.0001$), main effect of Treatment ($F_{2,37} = 9.18$; $P = 0.0006$), and significant interaction between Treatment and Time ($F_{16,291} = 3.43$; $P < 0.0001$). Posthoc differences

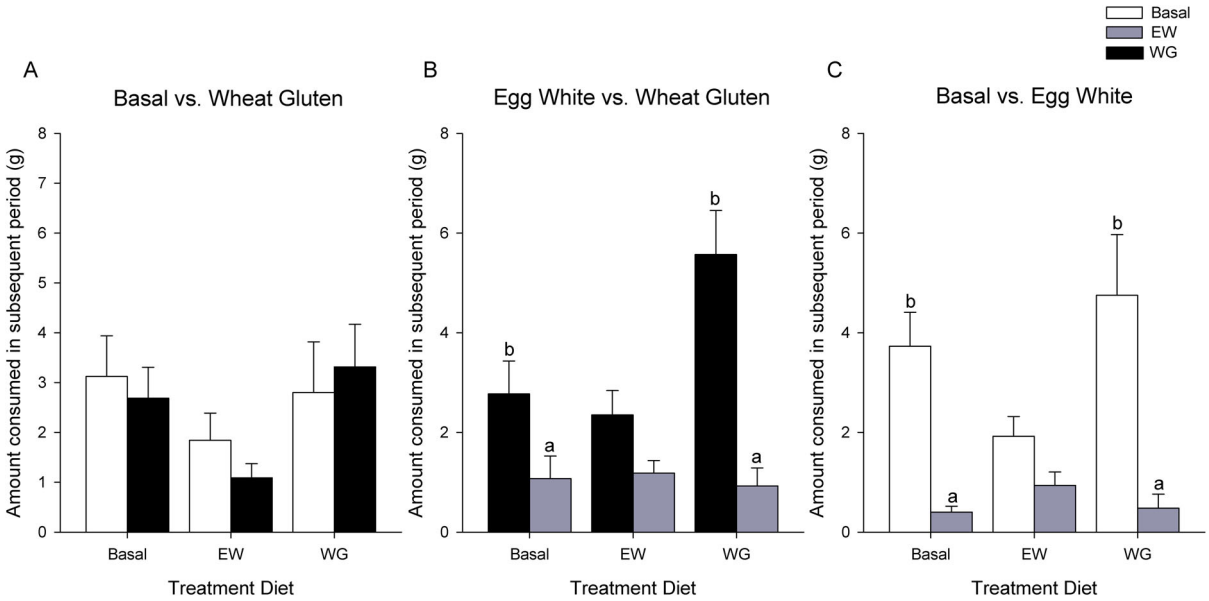

Figure 3 Food intake during preference testing ($n = 8$/pairing/breakfast). (A) Amount of Basal and WG diet consumed following each breakfast treatment. (B) Amount of EW and WG diet consumed following each breakfast treatment. (C) Amount of Basal and EW diet consumed following each breakfast treatment. Bars represent mean ± SEM. Bars with different superscripts are significantly different by posthoc *t*-tests ($P < 0.05$). WG, Wheat gluten diet; EW, Egg white diet.

between means collapsed across time indicated Basal was different from WG ($P = 0.0001$) and EW ($P = 0.0078$) and a trend for a difference between WG and EW ($P = 0.055$).

Similar to leucine, concentration of tyrosine in the plasma monotonically increased for approximately 90 min after initiation of feeding, but the rate and magnitude of the increase depended on the diet. Tyrosine peaked at 60 min following initiating of feeding in WG animals, whereas it peaked at around 90 min in EW fed animals and 120 min in Basal fed rats. Average level of tyrosine across the entire period was highest after EW, followed by WG then Basal (Fig. 4B). This was indicated by a significant main effect of Time ($F_{8,291} = 17.9$; $P < 0.0001$), main effect of Treatment ($F_{2,37} = 3.75$; $P = 0.033$), and significant interaction between Treatment and Time ($F_{16,291} = 2.13$, $P = 0.008$). Posthoc differences between means collapsed across time indicated concentration of tyrosine was higher in EW than Basal ($P = 0.01$), and tended to be higher in WG than Basal ($P = 0.068$), but not different between EW and WG.

Concentration of LNAA and BCAA in the plasma also monotonically increased for approximately 100 min after initiation of feeding, with the rate and magnitude of the increase depending on the diet. For both these groups of amino acids, concentrations peaked at 60 min following initiation of the WG meal, whereas it peaked at around 90 min in EW fed animals and 120 min in Basal fed rats. Average level of LNAA and BCAA across the entire period was highest after EW, followed by WG then Basal (Fig. 4 C and D). For LNAA, this was indicated by a significant main effect of Time ($F_{8,287} = 41.3$; $P < 0.0001$), main effect of Treatment ($F_{2,37} = 6.8$; $P = 0.003$), and significant interaction between Treatment and Time ($F_{16,287} = 3.28$; $P < 0.0001$). For BCAA, this was indicated by a significant main effect of Time ($F_{8,291} = 45.4$; $P < 0.0001$), main effect of Treatment ($F_{2,37} = 7.2$; $P = 0.002$), and significant interaction between Treatment and Time ($F_{16,291} = 3.8$; $P < 0.0001$). Posthoc differences between means collapsed across time indicated LNAA and BCAA levels were higher in EW than Basal ($P = 0.001$ and $P = 0.001$, respectively), higher in WG than Basal ($P = 0.01$ and $P = 0.01$, respectively) and no differences between EW and WG. Posthoc differences between means indicated that EW was significantly greater than WG at all timepoints greater than 90 min except at the 180 min timepoint for LNAA (see Fig. 4C and D). No significant differences were detected in the ratios of tyrosine and tryptophan to LNAA between the three diet treatment groups (Fig. 4E and F).

Discussion

The main finding of the study is the differential impact of the two protein sources, EW and WG, on cognitive performance and satiety when consumed at the first meal of the day. WG impaired learning and memory on the Barnes maze relative to the Basal and EW groups, displaying similar performance as the NB negative control. EW performed no better than Basal, but better than NB. This finding suggests that a high-protein diet composed of EW improves memory relative to skipping a meal, but is no better than a lower protein diet as represented by the Basal control. Additionally, rats given EW ate less food at the subsequent meal than Basal fed rats, supporting

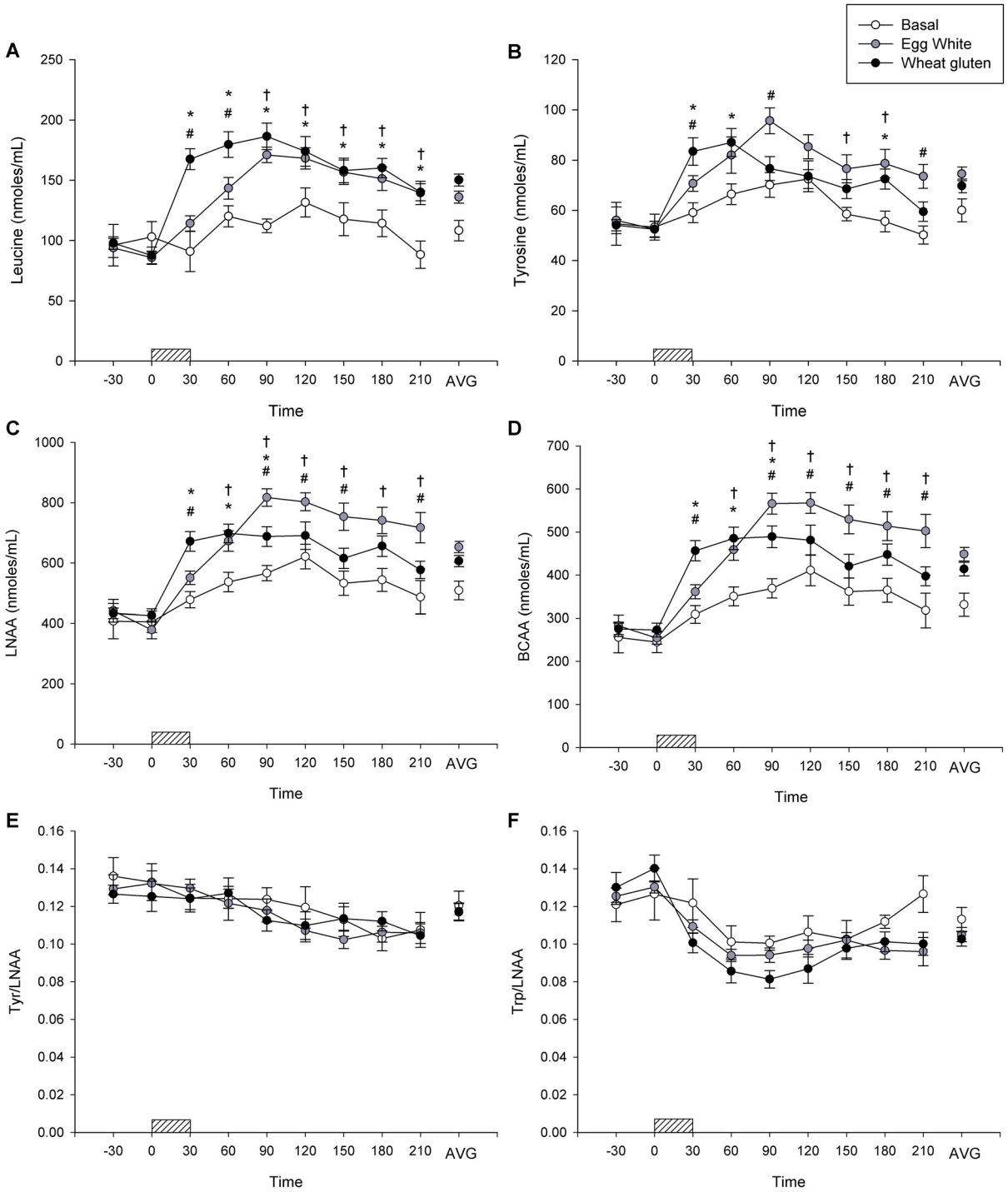

Figure 4 Effect of breakfast on postprandial plasma amino acid concentrations. (A) Average concentration of leucine ± SEM at each time-point, 30 minutes before the test meal, at the onset of the test meal (time-point 0), and 30 min increments thereafter. The box on the x-axis with the diagonal lines represents the time period (30 min) when the test meal was administered. Following the 210 time-point, the least square mean (AVG) collapsed across all time points is shown with the standard error from the ANOVA model. (B) Same as A for tyrosine. (C) Same as A for LNAAs. (D) Same as A for branched chain amino acid. (E) Same as A for ratio of tyrosine to LNAAs. (F) Same as A for ratio of tryptophan to LNAAs. Symbols indicates a significant different between treatment groups by posthoc *t*-tests ($P < 0.05$). *, Basal *vs*. Wheat gluten; #, Wheat gluten *vs*. Egg white; †, Basal *vs*. Egg white.

the hypothesis that a high- protein diet derived from EW enhances satiety. Surprisingly, WG rats ate more food at the subsequent meal than Basal fed rats, supporting an orexigenic mechanism for a high- protein diet composed of WG. Taken together, these data provide important evidence supporting EW as a superior protein source, in comparison to WG, for increasing satiety and for supporting normal cognitive performance.

Effect of protein source on cognition

Results of our study suggest that the source of protein in a high-protein diet influences cognitive performance. Significant differences were observed in the

path length for learning (Fig. 1B) and duration spent in the target quadrant of the Barnes maze during the probe test (Fig. 1C) with rats fed the EW and Basal performing significantly better than WG and NB. These results demonstrate that the EW meal enhanced memory for the escape tunnel relative to WG, but performed no better than rats fed the Basal control meal. The improved performance in the Basal and EW fed animals relative to NB is consistent with the results from human studies describing positive effects of breakfast on cognitive performance.[25,29] Additionally, these data show that ingestion of WG had deleterious effects on performance relative to Basal. Therefore, conclusions of previous studies that found diets with higher protein to carbohydrate ratios (4:1) enhanced short-term memory may need to be qualified with type of protein source provided to the human subjects.[2]

Although previous studies had suggested that the ratio of tyrosine to LNAAs and tryptophan to LNAAs increases uptake of precursors for catecholamine and serotonin neurotransmitter synthesis, respectively,[11] no differences in the ratio of these amino acids were observed in our study (Fig. 4E and F). Therefore, it is unlikely that ratios of these amino acids were related to the differential memory performance of the rats in our study. It is possible that the diets affected memory on the Barnes maze through alternative mechanisms than plasma amino acid profiles. Though it was not measured here, one possibility is that the different diets affected glucose availability which could impact cognitive performance.[2,6–8,30,31] Dietary proteins and amino acids can influence glucose availability directly by recycling carbons through gluconeogenesis, as well as indirectly by altering concentrations of glucagon and insulin.[32] Future studies should explore how the protein sources used in our study may differentially influence glucose availability.

Effects of protein source on satiety

In the present study, we found that satiety from high-protein diets (35% protein) is strongly influenced by the protein source. Whereas EW enhanced satiety relative to Basal, WG reduced satiety, challenging the generality of the hypothesis that a higher protein diet enhances satiety and further highlighting that the satiety effect of a high- protein meal depends on the protein source. Despite EW protein being considered one of the highest quality protein sources,[24] studies in human subjects report that EW protein is either less satiating or no different than other protein sources.[18,19,33] However, there are considerable limitations in human clinical trials of this nature, due to lack of control over factors such as composition of preload and testing meals and also timing of meals. In that regard, animal models are useful to address such questions in a more controlled environment. In agreement with our study, Semon and colleagues showed that rats switched from a low protein (5.2%) diet to a 40% EW protein had the greatest satiety response as measured by a reduced food intake at later time- points (90 –180 min), relative to lactoalbumin, soy, and casein proteins.[34] However, the underlying mechanisms by which EW enhances satiety has yet to be determined.

A follow up study was performed to assess the possibility that differences in food intake after the test meal were impacted by innate preferences or palatability for one of the diets. Results showed the EW diet was least preferred, but also not rejected (Fig. 3). This is consistent with the observation that rats consumed the EW diet more slowly than the other diets, using most of the 30 min available for the test meal (Table 3). Results also showed that if the first meal was EW, rats consumed more of EW during the subsequent period than rats fed the either Basal or WG for the first meal, suggesting that the rats acclimated to EW. Others have also observed decreased initial acceptability of diets containing high levels of EW protein, which eventually subsided after prolonged exposure to the diet.[34] However, this does not explain why a rat initially fed a less preferred diet would eat less food in the subsequent meal, especially if the second meal was more palatable than the first. That is unless the less preferred diet had satiety enhancing properties that were operating independently of the preference effects. In addition, results show that regardless of the choice provided during the subsequent period, total intake was always lowest following EW. Taken together, we conclude that the satiety effect we observed from EW is most likely related to the postprandial effect of the EW meal rather than innate feeding preferences.

Measured concentrations of plasma amino acids were consistent with the hypothesis that the EW meal produced its satiating effect via postprandial processing. EW caused a higher concentration of LNAAs than the other diets, which is consistent with the idea that increased amino acids in circulation may have contributed to the satiety signaling. On the other hand, the amino acid results cannot explain how the WG diet was least satiating. LNAA and BCAA levels following WG were not the lowest of the three treatments, as would be predicted if amino acid levels alone accounted for the satiety responses. Alternative mechanisms for the opposite effects of EW and WG on apparent satiety compared to the lower protein Basal diet needs to be explored in future studies.

Postprandial changes in plasma leucine has been implicated in promoting satiety.[35,36] However, in our

study, postprandial plasma leucine levels were greater following the least satiating WG meal in comparison to the basal control diet and also in comparison to EW (up to 60 min post meal) (Fig. 4A), which does not support the leucine-satiety hypothesis. The EW protein source contained slightly higher levels of leucine (8.4 g leucine per 100 g protein), compared to the WG protein source (6.9 g leucine per 100 g protein) (Table 1), but that did not translate into higher postprandial circulating levels of the amino acid. Furthermore, the Basal diet which had the lowest levels of leucine produced the lowest levels in the blood but was more satiating than the WG diet. It is possible that higher doses of leucine than were used in our study are needed to produce the satiety enhancing effects of this amino acid. Taken together, results suggest that a combination of factors influence satiety and that although leucine should not be discounted, its role may be less important than previously speculated.

Alternative explanations besides the amino acid complement in the blood could have influenced satiety in our study. For example, protein sources coming from plants versus animals are known to differ in rates of digestion and absorption[37] with plant- based protein sources tending to be less bioavailable. A food with less bioavailability would be predicted to induce lower satiety. However, WG, which was the least satiating appeared to have similar bioavailability, with earlier peaking of amino acids in WG than EW (Fig. 4). These diets may also differentially induce post prandial levels of hormones known to be involved in satiety, such as ghrelin, CCK, PYY, and GLP-1.[16] The different protein sources may also cause differences in amino acid driven gluconeogenesis and diet-induced thermogenesis to influence satiety.[38] Another alternative is that the diets induced a differential rate of gastric emptying. Gastric volume alone can cause termination of eating independent of post-gastric, intestinal signaling.[39] Multiple different mechanisms likely converge and contribute to the satiety effects of the protein sources studied herein.

Conclusion

In the present study we found that the effects of a high-protein diet on cognitive and satiety outcomes depend strongly on the source of protein used in the diet formulation. Relative to the Basal diet, EW had no impact on cognitive performance on the Barnes maze and increased satiety, whereas WG impaired cognitive performance and reduced satiety. We interpreted these results as unlikely related to innate preferences for the diets and more likely related to postprandial changes in signaling, though the specific signaling mechanism remain unknown. Increased circulating amino acids could contribute to the enhanced satiety observed for EW. However, the mechanisms underlying the reduced satiety and impaired cognitive performance for WG does not appear related to the amino acid profile and remains unknown. Future work is needed to uncover the satiety enhancing mechanisms of EW, as well as the mechanisms underlying the negative effects that WG has on cognition and satiety.

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