Consuming eggs for breakfast influences plasma glucose and ghrelin, while reducing energy intake during the next 24 hours in adult men

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Abstract

We hypothesized that consuming eggs for breakfast would significantly lower postprandial satiety and energy intake throughout the day. Using a crossover design, 21 men, 20 to 70 years old, consumed 2 isoenergetic test breakfasts, in a random order separated by 1 week. The macronutrient composition of the test breakfasts were as follows: (EGG, % CHO/fat/protein = 22:55:23) and (BAGEL, % CHO/fat/protein = 72:12:16). Fasting blood samples were drawn at baseline before the test breakfast and at 30, 60, 120, and 180 minutes after breakfast. After 180 minutes, subjects were given a buffet lunch and asked to eat until satisfied. Subjects filled out Visual Analog Scales (VAS) during each blood draw and recorded food intake the days before and after the test breakfasts. Plasma glucose, insulin, and appetite hormones were analyzed at each time point. Subjects consumed fewer kilocalories after the EGG breakfast compared with the BAGEL breakfast (\(P<.01\)). In addition, subjects consumed more kilocalories in the 24-hour period after the BAGEL compared with the EGG breakfast (\(P<.05\)). Based on VAS, subjects were hungrier and less satisfied 3 hours after the BAGEL breakfast compared with the EGG breakfast (\(P<.01\)). Participants had higher plasma glucose area under the curve (\(P<.05\)) as well as an increased ghrelin and insulin area under the curve with BAGEL (\(P<.05\)). These findings suggest that consumption of eggs for breakfast results in less variation of plasma glucose and insulin, a suppressed ghrelin response, and reduced energy intake.

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Abbreviations: ANOVA, analysis of variance; AUC, area under the curve; BAGEL, bagel-based high-carbohydrate breakfast; BMI, body mass index; EGG, egg-based low-carbohydrate breakfast; GLP-1, glucagons-like peptide 1; MUFAs, monounsaturated fatty acids; PYY, peptide YY; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; SI, satiety index; VAS, Visual Analog Scale.

1. Introduction

It is estimated that by the year 2030, obesity will cost the United States approximately 900 billion dollars annually [1]. One possible strategy to help control body weight is to implement a diet based on highly satiating foods that would make it easier to control energy intake without experiencing intense hunger. Holt et al [2] found that consuming foods with a higher satiety index (SI) resulted in reduced energy intake during an ad libitum buffet 2 hours later. Eggs have a 50% higher SI compared with white bread and cereal [3], which suggests that consuming eggs for breakfast may be able reduce appetite and energy intake throughout the day. Indeed, Vander Wal et al [4] have shown that compared with an isoenergetic bagel-based breakfast, an egg-based breakfast resulted in increased satiety and reduced short-term energy intake. Although the SI and Visual Analog Scales...
(VAS) are practical measures of appetite, it is imperative to develop a full understanding of how these foods influence the hormonal signals regulating energy homeostasis.

The orexigenic gastrointestinal peptide ghrelin has been implicated as a key figure in appetite regulation [5]. Ghrelin is synthesized in the stomach [6] and promotes hunger through interaction with the hypothalamic arcuate nucleus [7]. Ghrelin levels rise preprandially and decrease postprandially [8] suggesting a role in meal initiation. Previous research has shown that ghrelin levels are strongly suppressed after protein intake compared with carbohydrates [9,10]. In accordance with these data, protein is thought to be the most satiating macronutrient [11,12].

The hormones insulin, leptin, glucagons-like peptide 1 (GLP-1), and peptide YY (PYY) also play prominent roles in the regulation of food intake and energy balance [13-16]. Insulin and leptin are secreted in peripheral tissues and suppress food intake by inhibiting neuropeptide Y [17] and activating the melanocortin system [18]. Glucagons-like peptide-1 is released from L cells in the small intestine in response to energy intake. Peptide YY is secreted from L cells in the ileum and reduces appetite by delaying gastric emptying [19]. Egg intake may control hunger through a reduction of the postprandial insulin response. Consumption of eggs can reduce the plasma insulin response, thus decreasing carbohydrate oxidation and increasing fat oxidation [20-22]. In addition, eggs may promote satiety by preventing large deviations in plasma glucose and insulin levels [20-22].

The mechanisms underlying the interactions between gut hormones and appetite are still being investigated. The purpose of this study was to examine the effect of 2 typical breakfasts on appetite hormones, satiety, and energy intake. Our hypothesis was that an egg-based, low-carbohydrate breakfast would promote satiety through suppression of appetite hormones, resulting in reduced energy intake at lunch and the subsequent 24-hour period. Understanding the role of typical breakfasts in the American diet on suppressing appetite will advance our knowledge of the interrelationship between food, satiety, and weight maintenance.

2. Methods and materials

2.1. Materials

All food was purchased at Big Y supermarket (Mansfield, Conn). Human Gut Hormone Panel Lincoplex kits were obtained from Linco (Linco Research, Inc, St Charles, Minn). Total plasma ghrelin kits were obtained from Phoenix Pharmaceuticals (Burlingame, Calif). Aprotinin, sodium azide, and phenylmethylsulfonyl fluoride were obtained from Sigma Chemical (St Louis, Mo).

2.2. Subjects

Twenty-one men between the ages of 20 and 70 years were recruited from the University of Connecticut and the surrounding community. Subjects were excluded from participation if they were diabetic or allergic to eggs. All protocols were approved by the institutional review board from the University of Connecticut and subjects signed a consent form.

2.3. Study design

The study used a crossover design with each subject having the 2 breakfasts in a randomized and balanced order separated by 1 week. Both test breakfasts were isocaloric and provided approximately 1657 KJ of energy. The egg-based breakfast (EGG) consisted of 3 scrambled eggs and 1.5 pieces of white toast. The macronutrient composition of this breakfast was approximately 22% energy from carbohydrates, 23% energy from protein, and 55% energy from fat. The bagel-based breakfast (BAGEL) consisted of 1 white bagel, 1/2 tablespoon of low-fat cream cheese, and 6 oz of low-fat yogurt. The macronutrient composition of this breakfast was approximately 72% energy from carbohydrates, 16% from protein, and 12% from fat. The subjects could have a maximum of 12 oz of water with breakfast. The breakfasts were given in a random order, with half of the subjects starting with EGG and half starting with BAGEL. Subjects were given detailed instructions by trained personnel regarding how to keep a 24-hour diet record to assess food intake before and after the intervention. In addition, protocols were explained to all participants making sure that no bias was introduced toward a particular breakfast.

After an overnight fast, subjects reported to the Nutritional Science Department at 7 AM with a 24-hour food record of the previous day. Baseline subjective hunger values were determined through the use of a VAS. Next, a flexible catheter was inserted into a forearm vein, and blood samples were collected using a 3-way valve connected to the end of the catheter to assess appetite hormone levels. After the baseline blood sampling, a test breakfast was served and completed within 15 minutes. Blood samples and VAS were measured at 30, 60, 120, and 180 minutes after breakfast was completed.

After 180 minutes, the catheter was removed, and subjects were fed a buffet lunch that consisted of white bread, turkey, American cheese, low-fat mayonnaise, and apples. The planned meal was limited in choices to better evaluate food intake in situ. Subjects were encouraged to eat until they were comfortably satisfied, and the amount of food consumed during lunch was measured. Total water intake did not exceed 48 oz for the testing period. After lunch, subjects were asked to record what they ate for the next 24 hours after the intervention. One week later, subjects returned to the laboratory to follow the same procedure with the other test breakfast. All subjects were carefully instructed on how to do dietary records by experienced personnel including registered dietitians.
2.4. Blood collection

After an overnight fast, blood samples were collected at baseline as well as 30, 60, 120, and 180 minutes after breakfast into EDTA-containing tubes. Plasma was separated by centrifugation at 2000 × g for 20 minutes, and aprotinin (0.5 mL/100 mL), sodium azide (0.1 mL/100 mL), and phenylmethylsulfonyl fluoride (0.1 mL/100 mL) were added for preservation. Plasma was stored in individual aliquots at −80°C for analysis of appetite hormones, insulin, and glucose.

2.5. Subjective satiety and dietary analysis

A VAS was completed at baseline (0 minute), 30, 60, 120, and 180 minutes during the intervention. Subjects rated their hunger, fullness, desire to eat, and how much they thought they could eat on 10-cm line scales. Questions such as “How hungry do you feel” and “Would you like to eat something right now” are above a 10-cm line anchored by opposing phrases like “I am not hungry at all” and “I have never been more hungry” or “yes, very much” and “no, not at all.” Subjects marked a single spot on the line and the value was quantified and analyzed.

All dietary records were analyzed using the Nutrition Data System 8.0 (Minneapolis, Minn). Subjects recorded 24-hour intakes before and after each of the test breakfasts (4 records total). The 24-hours records were analyzed for contribution from carbohydrates, proteins, fats, and dietary cholesterol.

2.6. Fasting glucose and plasma ghrelin concentrations

Fasting glucose concentration was analyzed using an automated lactate/glucose analyzer (2300 STAT; YSI, Yellow Springs, Ohio). Plasma total ghrelin concentration was measured using an enzyme immunoassay kit (Phoenix Pharmaceuticals, Inc, Burlingame, Calif) according to the manufacturer’s protocol. Total ghrelin concentration was measured using a spectrophotometer at an absorbance of 450 nm with software capable of 4 parameter logistics to quantify peptide concentration.

2.7. Plasma leptin, insulin, GLP-1, and PYY concentrations

Plasma leptin, insulin, GLP-1, and PYY were measured from fasting plasma using the human gut hormone panel Lincoplex kit, which is a multiplex assay kit based on the Luminex xMAP technology (Linco Research, Inc, St Charles, Mich) [23]. This multiplex assay kit allows simultaneous quantification of these peptide hormones from Antibody-Immobilized beads. The sensitivity for this assay was 5.2, 8.4, 44.5, and 157.2 pg/mL for GLP-1, PYY, insulin, and leptin, respectively.

2.8. Statistical analyses

Repeated-measures analysis of variance (ANOVA) were used to determine differences in appetite hormones, subjective satiety, and energy intake between EGG and BAGEL. Area under the curve (AUC) was calculated as the sum of the areas under and over the baseline.

3. Results

Participant enrollment began in September 2007, and the study concluded in March 2008. Table 1 shows the baseline characteristics of the subjects in terms of body mass index (BMI), age, and plasma lipids indicating that all subjects were normolipidemic and with healthy BMI.

3.1. Dietary intake

All subjects consumed the same amount of kilocalories for breakfast, approximately 1675 Kj. There were no differences in energy intake during the 24 hours before EGG and BAGEL. Subjects ingested significantly more kilocalories during ad libitum lunch after BAGEL compared with EGG (P < .01) as illustrated in Table 2. In addition, subjects consumed a greater amount of fat (P < .05), carbohydrate (P < .01), protein (P < .01), cholesterol (P < .01), and saturated fatty acid (SFA; P < .01) during the lunch after BAGEL. Interestingly, the percent distribution of energy was not different between breakfasts. Percent energy distribution was 45.6:36.1:17.7 and 45.8:35.7:17.7 for carbohydrate/fat/protein after the EGG and the BAGEL breakfasts, respectively.

Table 1
Age, BMI, and plasma lipids of study participants at baseline

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Means ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>35 ± 16</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.4 ± 3.39</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>79.2 ± 53.1</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>142.2 ± 24.2</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>77.2 ± 13.1</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>49.2 ± 9.7</td>
</tr>
</tbody>
</table>

Values are means ± SD, n = 21 subjects. LDL-C indicates low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

Table 2
Energy, fat, carbohydrate, protein, cholesterol, SFA, MUFA, and polyunsaturated fatty acid (PUFA) intakes during an ad libitum lunch after the egg and the bagel-based breakfasts

<table>
<thead>
<tr>
<th>Parameter</th>
<th>EGG</th>
<th>BAGEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (KJ)</td>
<td>2273 ± 862</td>
<td>2742 ± 992</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>22 ± 11</td>
<td>26 ± 12*</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>63 ± 25</td>
<td>76 ± 30†</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>23 ± 10</td>
<td>29 ± 13†</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>62 ± 36</td>
<td>76 ± 33†</td>
</tr>
<tr>
<td>SFA (g)</td>
<td>9 ± 4</td>
<td>11 ± 5†</td>
</tr>
<tr>
<td>MUFA (g)</td>
<td>6 ± 3</td>
<td>7 ± 3</td>
</tr>
<tr>
<td>PUFA (g)</td>
<td>5 ± 6</td>
<td>6 ± 6</td>
</tr>
</tbody>
</table>

Values are means ± SD for n = 21 subjects. Data were analyzed using repeated-measures ANOVA. Values in the same column with * indicate significantly different at P < .05; values in same column with † indicate significantly different at P < .01.
Subjects consumed significantly fewer kilocalories during the 24 hours after EGG compared with BAGEL (\(P < .05\); Table 3). During this time period, subjects consumed less fat (\(P < .01\)), protein (\(P < .05\)), cholesterol (\(P < .05\)), SFA (\(P < .01\)), and monounsaturated fatty acid (MUFA; \(P < .05\)) after EGG as well. The percent energy from fat was also higher after BAGEL (\(P < .05\)).

### 3.2. Plasma glucose, ghrelin, and insulin concentrations

As expected, the EGG resulted in a lower AUC for glucose compared with the BAGEL (Fig. 1, \(P < .05\)). Plasma glucose levels were significantly higher 30 and 60 minutes after the BAGEL compared with the EGG. After 180 minutes, plasma glucose concentrations returned to baseline levels after the EGG, whereas the plasma glucose concentrations for BAGEL were still below baseline levels.

Accordingly, EGG resulted in a reduced AUC for insulin compared with BAGEL (Fig, 2, \(P < .05\)). Plasma insulin concentration was significantly higher 60 minutes after BAGEL compared with EGG. At 180 minutes, plasma insulin concentration was significantly higher after EGG.

Interestingly, plasma ghrelin levels did not decrease after breakfast. However, there was a reduced AUC ghrelin after EGG compared with BAGEL (Fig. 3, \(P < .05\)). Circulating ghrelin levels were significantly higher 30 minutes after BAGEL compared with the EGG breakfast.

### 3.3. Plasma leptin, GLP-1, and PYY levels

Due to budget constraints, plasma leptin, GLP-1, and PYY were only analyzed at baseline, 30 minutes, and 180 minutes. There were no differences in these hormones in any of the time points or between breakfasts (Table 4).
3.4. Subjective satiety

Based on VAS, subjects had a reduced AUC for hunger after EGG and were significantly less hungry 180 minutes after EGG compared with BAGEL (Fig. 4, *P* < .01). In addition, subjects had an increased AUC for satisfaction after EGG and were significantly more satisfied 180 minutes after EGG compared with the BAGEL breakfast (Fig. 5, *P* < .01). There were no other significant differences in the other components of VAS between the 2 different breakfasts.

4. Discussion

Postprandial appetite ratings after egg intake have been previously investigated [3,4]; however, the experimental data regarding hormonal responses remain limited. In this study, EGG significantly suppressed insulin, glucose, and ghrelin AUCs compared with BAGEL. In conjunction with these hormonal responses, individuals had reduced hunger and increased satisfaction after EGG, resulting in reduced energy intake at an ad libitum lunch buffet and in the 24-hour period after the test breakfast.

After BAGEL, circulating glucose levels were significantly increased 30 and 60 minutes compared with EGG. These results were expected due to the high-carbohydrate content of BAGEL, and accordingly, plasma insulin levels were significantly elevated at 60 minutes compared with EGG. Ludwig [21] outlined the sequence of physiologic events that occur after consuming a high-carbohydrate meal. In the early postprandial period, ingestion of high-carbohydrate food results in rapid absorption of carbohydrate and hyperglycemia. This stimulates insulin secretion, leading to increased glucose uptake into muscle and adipose. This elevated insulin response is followed by a period of hypoglycemia and reduced metabolic fuel (eg, free fatty acids), leading to increased feelings of hunger designed to reestablish energy balance. The results of the current study corroborate this chain of events. Subjects experienced significant transient increases in glucose and insulin concentrations up to an hour after BAGEL. At 120 minutes, subjects experienced a sharp decline in plasma glucose and insulin concentration after both breakfasts. However, at 180 minutes, serum glucose levels rebounded back to baseline after EGG, whereas plasma glucose concentration was below baseline at this point after BAGEL. In addition, serum insulin levels were significantly higher at 180 minutes after EGG compared with BAGEL. The elevated insulin levels observed 180 minutes after EGG probably factored into the reduced kilocalorie intake during the ad libitum lunch and possibly during the subsequent 24 hours. According to the VAS, subjects were significantly less hungry and more satisfied after EGG compared with BAGEL, which bolsters
the importance of postprandial glucose and insulin concentrations in regard to appetite and energy intake.

Ghrelin is the only known appetite-stimulating hormone. Circulating ghrelin levels climax before meals and reach a nadir shortly after energy intake [8,24], suggesting a role in meal initiation. The results of the current study were unexpected. Although, the AUC for ghrelin was reduced after EGG as expected, peak ghrelin levels for both BAGEL and EGG occurred at 60 minutes, rather than baseline. Previous research has shown that psychologic determinants of appetite play a noticeable role in ghrelin secretion [25-27]. Frecka and Mattes [27] suggest that ghrelin secretion could be connected to habitual meal patterns. In the current study, subjects were fed breakfast at 7 AM on testing days, but the self-reported dietary intakes for the 24 hours before the testing period showed that almost all subjects generally consumed breakfast between 8 and 9 AM. Natalucci et al [28] observed an increase and spontaneous decrease in ghrelin concentration in fasting individuals during typical meal times. It is possible that plasma ghrelin levels were disposed concentration occurring at 60 minutes, instead of before breakfast at baseline.

In addition, although ghrelin levels were significantly different between groups at 30 minutes, they were never significantly different from baseline values. These results differ from other studies that find a significant reduction in ghrelin levels after energy intake compared with baseline [9,30]. Sugino et al [25] found that ghrelin surges and nadirs were attenuated in sheep fed ad libitum compared with those that were fed 2 or 4 scheduled meals a day. Twenty-four-hour dietary records both preintervention and postintervention indicated that subjects tended to consume several smaller meals throughout the day rather than 3 square meals. It is possible that this eating pattern further established ghrelin responses, resulting in more stable ghrelin levels throughout the day.

The most plausible explanation for the ghrelin profile may relate to the amount of energy consumed at breakfast. Postprandial suppression of ghrelin is proportional to the amount of energy ingested [31]. Callahan et al [31] found that a meal less than 2093 KJ was much less effective at suppressing ghrelin levels compared with higher energy meals. In the current study, the test breakfasts were approximately 1675 KJ, which may help explain the limited ghrelin suppression observed. Although the energy content of the breakfasts were not enough to promote the normal decrease of ghrelin levels typically observed after feeding, the AUC for ghrelin was suppressed after EGG compared with BAGEL.

The mechanisms regulating ghrelin secretion during fasting and postprandially have yet to be elucidated. Cummings et al [8] found that ghrelin concentration was inversely related to plasma glucose and insulin levels, suggesting ghrelin secretion is controlled by these hormones. However, a double-blind, placebo-controlled crossover clamp study found that ghrelin concentrations are not directly regulated by glucose or insulin levels [32]. Increased plasma amino acid concentration may modify ghrelin secretion, possibly through direct luminal contact [32,33]. The protein contained within eggs may have contributed to the suppressed ghrelin AUC observed after EGG compared with BAGEL. In addition, fat digestion also plays an important role in ghrelin suppression [34]. The low-carbohydrate EGG breakfast contained a significantly higher amount of fat and protein compared with BAGEL, which may explain the significantly lower AUC of ghrelin for EGG.

Leptin, PYY, and GLP-1 have been shown to modulate food intake [13,14,16]. Leptin is released by adipocytes [35] and interacts with insulin to regulate energy homeostasis [14]. In accordance with previous research [36,37], leptin levels did not change postprandially, suggesting leptin has a diminished role during short-term energy regulation. Glucagon-like peptide-1 is released in response to a meal and may inhibit food intake by delaying gastric emptying [38]. Flint et al [39] found that plasma GLP-1 concentrations of ~80 pmol/L are able to exert suppressive effects on appetite after GLP-1 infusion. In the current study, peak GLP-1 concentrations were 25% less than those achieved by Flint et al. Perhaps the reduced energy content of the test breakfasts limited the GLP-1 response, suggesting that the reduction in energy intake is mediated through another pathway. Peptide YY is released in the ileum and signals the hypothalamus to inhibit appetite and promote satiety [40]. There were no differences in plasma PYY concentrations after the test breakfasts. Peptide YY is released in proportion to the amount of kilocalories ingested [41], so the energy intake at breakfast may have resulted in unexpected PYY levels despite the difference in macronutrient content. It seems that the reduction in energy intake at lunch and the subsequent 24 hours in the current study are likely unrelated to changes in leptin, GLP-1, or PYY concentrations.

One limitation of this study is the possible confounding effects of the macronutrient composition of the test breakfasts. Most kilocalories in EGG were coming from fat as well as protein. A future study could have 3 separate breakfasts, with each breakfast testing the effect of a different macronutrient on subsequent energy intake. This type of study design would isolate the effects of each macronutrient and determine whether the protein or fat played a greater role in promoting satiety in the current study. However, the breakfasts in the current study were chosen to reflect 2 typical American breakfasts and demonstrate the ability of egg consumption to promote increased satiety. In addition, previous research in our laboratory has shown that consumption of 3 eggs per day does not adversely affect the lipid profile [42]. A second limitation was the amount of energy consumed at breakfast. The 1675 KJ provided by the test breakfasts likely blunted the amplitude of the ghrelin curve and could also explain why there were no significant
changes in postprandial GLP-1 or PYY concentration. A third limitation was the timing of the breakfasts. The AUC for ghrelin in the current study did not resemble a “typical” circadian response. It was hypothesized that because the intervention took place an hour before the subjects’ habitual time of breakfast, the AUC for ghrelin was phase shifted so that the anticipatory rise in ghrelin occurred at 60 minutes rather than baseline. Perhaps a washout period would be necessary to entrain the subjects to a specific feeding regimen, that way individual circadian ghrelin responses could be partly controlled.

In summary, results from this study demonstrate that consuming eggs for breakfast can more effectively promote satiety and reduce subsequent energy intake. The EGG elicited reduced insulin, glucose, and ghrelin AUCs compared with BAGEL. These results suggest that foods with high-protein and high-fat content and low in simple carbohydrates may be incorporated into the diet to decrease energy intake by reducing hunger. The EGG was able to increase satiety, possibly by more effectively sustaining insulin concentration. In addition, this study supports the role of insulin as a key participant in appetite regulation independent of ghrelin and bolsters the role of ghrelin entrainment in human appetite regulation.

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References


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