The effect of agar jelly on energy expenditure, appetite, gastric emptying and glycaemic response.

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Abstract

Background and purpose: Agar contains a high amount of soluble fibre and has been shown to delay gastric emptying (GE) without impacting on glycaemic response (GR). The current study aimed to further the limited data on the effect of agar on metabolism by assessing the effects on GE and GR as well as appetite and diet induced thermogenesis (DIT).

Methods: In this randomised control trial eleven healthy volunteers were tested on two occasions following an overnight fast. Following baseline and resting measurements, volunteers were fed either a fruit flavoured drink (liquid) or consumed a fruit flavoured jelly (jelly). The two were exactly the same in composition except the jelly contained 4g of agar crystals. Both contained 50g of available carbohydrate. DIT was measured using indirect calorimetry, GE using the ^{13}C sodium acetate breath test, appetite using visual analogue scale and GR using finger prick blood samples.

Results: The jelly significantly delayed GE across all time points - latency phase ($p= 0.07$), lag phase ($p= 0.04$), half time ($p<0.0001$), ascension time ($p=0.025$). The jelly also increased all appetite parameters – hunger ($p=0.006$), fullness ($p=0.035$), desire to eat ($p=0.03$) and prospective consumption ($p=0.011$). However, there were no significant differences in either GR or postprandial DIT between the liquid and jelly.

Conclusion: Agar delays GE and increases appetite but does not change GR or DIT most probably due to the increase in viscosity caused by the agar jelly.

Key words: jelly; appetite; gastric emptying; glycaemic response

Introduction

Agar is a red algal polysaccharide containing ~80% soluble fibre commonly used in the Japanese diet. Agar forms a viscous gel when heated in water. The addition of soluble dietary fibres to foods increases the viscosity of that food which has implications for their digestion and absorption. Many other soluble fibre foods have been shown to reduce glycaemic response (GR) by delaying gastric emptying (GE) with GE accounting for 34% of the variability in peak blood glucose responses after a 75g glucose load [1]. For example high molecular weight barley β-glucan increases the viscosity of a soup which decreases its GE rate and reduces its GR [2]. For agar, only two previous studies on its effects on glycaemic control have been undertaken. The first indicated that a 12 week dietary intervention resulted in a decreased insulin response compared to a control group in diabetic patients but no difference in blood glucose [3]. The second by Sanaka et al [4] found that following the ingestion of 2.5g of agar, GE was delayed yet there were no decreases in postprandial blood glucose concentration. It is likely that the delay in GE observed is due to the increased viscosity of the agar. Previous research from our laboratory has also shown that GR and GE do not always correlate [5].

Soluble dietary fibre has been shown to have beneficial effects on glycaemic and insulin responses and cholesterol levels. It has also been shown to increase satiety that may encourage body weight maintenance [6, 7]. Several explanations are possible for this. One potential mechanism is that low GR foods have been shown to be more satiating via the glucostatic theory [8, 9]. The delayed GE is another explanation as the prolonged gastric distension due to the

retention of food in the stomach causes an enhanced and elongated period of satiety [5, 10, 11]. However there is potential that satiety may be altered by gut hormones as previous research has indicated that dietary fibre can increase GLP-1 and decrease ghrelin [12, 13]. To date no studies have examined the effect of agar on appetite even though it has been shown to influence GE [4] and cause significant body weight reduction [3].

In order to ascertain the true potential benefit of agar as a functional food in the management of metabolic diseases, it is necessary to ascertain the effect that the food can have both on energy expenditure as well as appetite. Diet induced thermogenesis (DIT) is the amount of energy required for absorption and metabolism of food and represents ~10% of total daily energy expenditure [14]. Although DIT consists of only a small proportion of total energy expenditure, if a food that has the potential to cause a rise in DIT is eaten repeatedly over time, it could prove beneficial in controlling the development of obesity. Soluble fibre has been shown to increase appetite, however there is also limited evidence suggesting that fibre may decrease DIT [2, 15, 16] with suggestions that this may be due to decreased palatability causing a reduced cephalic response (early initiation of digestion prior to ingestion that results in fast release of insulin that peaks between 1-4 minutes). If agar is to be a viable food ingredient for weight maintenance or loss it is important to ascertain the degree to which agar may decrease DIT and the influence of this on energy balance.

The objective of the current study was to further the limited data on the effect of agar on metabolism as a potential functional ingredient to aid weight loss. The aims are to measure the effect of agar on GR, GE, appetite and DIT.

Methods

Subjects

Twelve healthy subjects were recruited for the study by means of advertisements and personal communications. One volunteer discontinued his participation for personal reasons leaving 11 volunteers in total (table1).

Before inclusion in the study, potential participants were briefed on all aspects of the experiment and were given the opportunity to ask questions. This was followed by a health assessment, which included anthropometric measurements and a health questionnaire (giving details of food allergies/intolerances, metabolic diseases, special dietary needs, and smoking habits). Those who fulfilled all the acceptable criteria (age 18-60 years; BMI<30 kg/m^2; blood pressure 110 - 120/75 - 85 mmHg; fasting blood glucose <6mmol/L; not on prescription medication; no self-declared genetic or metabolic diseases) were included in the study. On the day before each test, subjects were asked to restrict their intake of alcohol and caffeine-containing drinks and to refrain from strenuous physical activity.

The study was conducted at the Functional Food Centre at Oxford Brookes University. All participants gave written informed consent before starting and the study was initiated after the approval by the Oxford Brookes University Research Ethics Committee according to the guidelines laid down in the Declaration of Helsinki. On each test day subjects came to the Functional Food Centre between 7 and 9am on the morning after an overnight fasting (10-12

hours before testing time). Subjects were instructed to keep all physical exertion on the morning of testing to a minimum.

Study design

Volunteers participated in a randomised, controlled crossover study where they consumed either a jelly or a liquid on separate days in a random order. On the day prior to testing, volunteers were asked to record their food and repeat it prior to the subsequent test.

Energy expenditure

On arrival in the laboratory, volunteers were asked to rest for 30 minutes in a supine position on a bed before baseline measurements of EE were taken. Resting metabolic rate (RMR) was determined in the morning between 7 and 9 am. RMR was measured at one-minute intervals for 30 minutes under the ventilated hood indirect calorimetry system (Deltatrac™ II Metabolic Monitor, Datex-Ohmeda Inc., Finland). The analyzer was calibrated on each test day with standardized gases containing 5% CO_2 and 95% O_2.

DIT was determined for 15 minutes in every 30 minutes for 180 minutes after test meal ingestion [17]. The first 5 minutes of every 15 minute time period was discarded to allow for stabilisation within the Deltatrac hood and the average of the remaining ten minutes was used. This time period was recommended to be appropriate to measure the thermic effect of foods [17]. Energy expenditure was calculated using the equations of Lusk [18]. DIT was calculated as the increase in energy expenditure/min above pre-meal values for 3 h after meal intake. The incremental area under the curve (iAUC) was then calculated using the trapezoidal rule.

Appetite

One hundred millimeter continuous line visual analogue scales (VAS), were utilized to measure subjective feelings of hunger, fullness, desire-to-eat and prospective food consumption, at baseline (0 min) and then every 15 minutes for the first hour and every 30 minutes for the following three hours after the commencement of eating the test food [19]. The VAS ratings were quantified by measuring in millimeters, the distance between the left end of the scale and the point marked by the participant. The 'change in the subjective feeling' was calculated by computing the difference between the response at a time point and the baseline value (at 0 min). Using the 'change in subjective feeling' data, temporal curves were constructed for each of the four VAS questions for the testing time. The iAUC (using the trapezoidal rule) was then calculated for each of these curves.

Blood glucose measurements

The protocol used to measure the blood glucose response was adopted from that described by Brouns et al [20] and is in line with procedures recommended by the Food and Agricultural Organization (FAO)/World Health Organization (WHO) [21]. Blood was obtained by finger prick using the Unistik 3 single-use lancing device (Owen Mumford, Woodstock, UK). Before a finger prick, subjects were encouraged to warm their hand to increase blood flow. To minimize plasma dilution, fingertips were not squeezed to extract blood but were instead gently massaged starting from the base of the hand moving toward the tips. The first 2 drops of expressed blood were discarded, and the next drop was used for testing.

Blood glucose was measured using the HemoCue 201+ Glucose analyzer (HemoCue Ltd, Dronfield, UK). The HemoCue is a reliable method of blood glucose analysis [22]. Fasting blood samples were taken at −5 and 0 minutes, and the test food was consumed immediately afterward. The participants consumed the test food and the water at a comfortable pace, within 15 minutes. Further blood samples were then taken at 15, 30, 45 60, 90, 120, 150 and 180 minutes after consuming the test meal.

The GR data were converted to "the change in GR" values. The change in GR was calculated by computing the difference between the blood glucose concentration at a time point and mean baseline blood glucose concentration (based on 2 baseline values taken 5 minutes apart). The total blood glucose response was expressed as the iAUC ignoring the area beneath the baseline and was calculated geometrically using the trapezoidal rule [20, 23, 24]. It was this change in GR that was used for all further analyses, including iAUC calculations, blood glucose response curve construction, and statistics

Gastric emptying

Sodium acetate labelled with 1-^{13}C was used in this study to measure GE as acetate is hydrophilic, poorly absorbed in the stomach, and rapidly metabolized after absorption. Sodium [1-^{13}C] acetate is considered a reliable and valid method for identifying changes in GE of semi solids [25]. Breath samples were collected by blowing gently into a 10-mL Exetainer (Labco, Buckinghamshire, UK) with a drinking straw and replacing the cap just before the end of exhalation. Breath samples were analyzed using isotope ratio mass spectrometry (ABCA, Sercon, Crewe, UK), and the results were expressed relative to V-PDB, an international standard

for known ^{13}C composition. $^{13}CO_2$ values were expressed as the excess amount in the breath above baseline and converted into moles. Data are then displayed as percentage of $^{13}CO_2$ dose recovered per hour and cumulative percentage $^{13}CO_2$ recovered over time. CO_2 production was assumed to be 300 mmol/m^2 body surface area per hour. Body surface area was calculated using a validated weight-height formula [26]. This was then fitted to a GE model developed by Ghoos et al [27]. For all the data, r^2 coefficient between the modelled and raw data was calculated and r^2 was < 0.95 for all tests. From this model, several parameters were measured. Lag phase and half time were calculated using the formulae derived by Ghoos et al [27]. Lag phase is the time taken to maximal rate of $^{13}CO_2$ excretion [28] and is equivalent to the time of the inflection point [29]. Half time is the time it takes 50% of the ^{13}C dose to be excreted [28]. Latency phase [29] is the point of intersection of the tangent at the inflection point of the $^{13}CO_2$ excretion curve representing an initial delay in the excretion curve. Ascension time [29] is the time course between the latency phase and half time, representing a period of high $^{13}CO_2$ excretion rates.

Test meal

The test meal consisted of either a liquid or a jelly. The liquid contained 150ml of apple and mango juice (Tesco, Cheshunt, Hertfordshire, UK) and 35g glucose powder (Lloyds, Coventry, UK). The jelly contained 150ml of apple and mango juice (Tesco, Cheshunt, Hertfordshire, UK) and 35g glucose powder (Lloyds, Coventry, UK) and 4g agar (80.9% fibre) (Clearspring Ltd, London, UK). The jelly was made by heating the juice and agar in a saucepan until it was boiling, adding in the glucose powder and ^{13}C sodium acetate and then simmering and stirring for five minutes at which point the agar was dissolved. The jelly was then cooled and allowed to set. The same procedure was repeated for the liquid to control for evaporation between the two

test meals. Both meals were served with 150 ml of drinking water. Available carbohydrates of the meals were calculated using the FAO/WHO procedure [21] according to the nutrition information available from the food manufacturers. The test meals were calculated to provide 50g of available carbohydrates.

Statistical analysis

Studies of the analysis of GR in humans have been based on 10 subjects, as reviewed by the FAO/WHO [21] to take into account the inter-individual variations and this number is similar to that used in the previous study on this topic [4]. Hence a sample size of 11 was considered adequate for the current study. Statistical analysis was performed using Statistical Package for the Social Sciences (version 20.0; SPSS, Chicago, IL, USA) and data and figures were processed in Microsoft Excel spreadsheet (2006, Reading, UK).

Differences in the iAUC for DIT, GR and satiety as well as the GE parameters were compared using paired sample t-test. A Kolmogorov-Smirnov test before analysis indicated that all the data sets were normally distributed. Significance was set at $p < 0.05$. Values are presented as means±standard deviation.

Results

Gastric emptying

There were significant differences in GE for all time points between the two meals – latency phase ($p = 0.007$), lag phase ($p = 0.04$), half time ($p < 0.0001$), ascension time ($p = 0.025$) with the

231 jelly causing a delayed GE in comparison to the liquid (Table 2). The differences in all time
232 points, indicates that GE was delayed both for initial emptying and later emptying following the
233 jelly (Figure 1).
234
235 *Appetite*
236 There were significant differences in appetite AUC for all appetite parameters – hunger
237 (p=0.006), fullness (p=0.035), desire to eat (p= 0.03) and prospective consumption (p= 0.011)
238 between the two meals with the jelly causing an increase in appetite in comparison to the liquids
239 (Table 3).
240
241 *Glycaemic response*
242 There was no significant difference in GR AUC between the two meals (Liquid: 161±54, Jelly:
243 168±45 mmol·min/l; p=0.738; Figure 2). There was no significant correlation between GE and
244 GR (p>0.05).
245
246 *Energy expenditure*
247 There were no significant differences in baseline energy expenditure between the two test days
248 (Liquid: 0.98±0.20; Jelly 0.99±0.21 kcal/min). There was no significant difference in total DIT
249 between the two meals (Liquid: 7.8±4.0, Jelly: 6.0±4.7 kcal; p=0.436; Figure 3).
250
251 **Discussion**
252

253 The current study demonstrated the ability of 4g of agar to delay GE and increase appetite but
254 without having a significant effect on either GR or DIT. Although other studies have shown a
255 significant effect of soluble fibre on GR [30, 31], the current study was unable to see any
256 difference in GR when agar was added to a liquid. This result is counterintuitive as it would be
257 expected that a delay in GE would result in a decreased availability of glucose for absorption and
258 hence a lower GR. However the result of the current study is in keeping with the one other
259 previous study on the effect of agar on GR [4]. Sanaka et al. [4] in their discussion highlight that
260 their findings imply there may be a tendency towards a reduced GR following the agar. They
261 believe this difference may have been masked by their low subject numbers. Although subject
262 numbers in the current study were the same as the previous one, the data clearly indicate no
263 difference or tendency towards a difference in GR with the addition of agar. This is especially
264 true given that the quantity of agar used in the current was larger than that used in Sanaka et al
265 [4]. Following an intervention consisting of 180g of agar per day for 12 weeks in diabetic
266 individuals, Maeda et al, [3] found a difference in the baseline HbA_{1C} and a decrease in insulin
267 AUC yet no difference in GR. However in the study no agar was added to the test meal itself.
268 Together, these data suggest that even very large amounts of agar do not change GR.
269
270 An interesting result from the current study is that GE was significantly delayed throughout the
271 emptying period yet this did not result in a lower GR and the two parameters were not correlated
272 with eachother. It has previously been shown that the delivery of nutrients into the duodenum for
273 absorption plays a significant role in the blood glucose response [1]. In previous work by our
274 group we have shown that soups have the ability to delay GE yet still significantly increase GR,
275 indicating that the two may not always be related. However, the soup data may be easily

276 explained by the increased viscosity of the soup causing a delay in GE, and the increased
277 bioavailability of the starch for digestion and absorption causing an amplified GR. In the current
278 study it is not as clear. It is possible that the initial rapid emptying of the liquid may have
279 induced an immediate and large insulinaemic response that may have blunted the GR to the
280 extent that the jelly and liquid GR could not be differentiated. Unfortunately the insulinaemic
281 responses were not measured in the current study.
282
283 The current study indicated that the agar has the ability to increase appetite significantly in
284 comparison to the control liquid. This is first study to the author's knowledge that has assessed
285 the appetite effect of agar. Food form plays a large role in appetite with an increased viscosity
286 being known to induce appetite [32, 33]. It is probable that the delayed GE may have resulted in
287 this decreased appetite due to the prolonged distension of the stomach and the delayed delivery
288 of nutrients into the small intestine. It has previously been shown that the gastric distension
289 results in a reduction in food intake via neural reflex arcs. Similarly, gastric distension has been
290 found to augment the reduction of nutrient intake affected by intravenous CCK-8 [34]. By
291 maintaining the food in the stomach for a longer period of time the stomach remains full and
292 distended for longer prolonging the period of satiety. However recent research by Cassidy et al.
293 [35] has indicated that a cognitive effect and the implied satiating properties of solid or viscous
294 food can induce sensations of appetite. Whether the effect of agar on appetite is due to cognition
295 or food form, or a combination of these, its role has implications for its use in the control of food
296 intake and weight maintenance.
297

This increase in appetite is considerably significant given that the DIT was the same following the two meals. Previous research on fibre and DIT has shown that fibre can moderately decrease DIT [15, 16]. However in the current study there was no discernible difference between the jelly with agar and the liquid. This is significant as it implies that any reduction in food intake due to the agar would not be compensated for by a decrease in DIT as has been found with other high fibre meals. However it is important to note that this potential energy deficit would need to be confirmed using a measurement technique that can quantify appetite such as *ad libitum* food intake, as opposed to the cognitive method of visual analogue scales used in the current study. In the current study quantification was not physically possible due to the measurement of DIT and GE requiring 3 hours minimum for testing. The jelly and liquid induced similar levels of appetite at three hours due to both groups being extremely hungry; hence if measurements of *ad libitum* food intake had been taken it is unlikely any differences would have been detected.

The current study highlights some interesting findings in terms of the implications for agar as a food ingredient that has the potential to increase appetite most likely due to the delayed emptying of food from the stomach. It would be expected that a delay in GE would result in a reduced rate of delivery of nutrients and hence a reduced GR, however the no difference in GR was observed even though GE was delayed. This requires further investigation. Further research into the insulinaemic response and *ad libitum* food intake in response to agar is warranted to support these findings and understand how agar is exerting its effects.

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Tables

Table 1: Volunteer characteristics.

Sex	7f 4m
Age (yr)	28.5±4.5
Height (m)	1.69±0.01
Weight (kg)	66.1±15.6
BMI (kg/m^2)	22.5±2.8

Table 2: Indicates volunteer's gastric emptying (GE) half time, lag phase, latency phase and ascension time of each of the meals - jelly and liquid. Data are given as mean ± SD (n=11) *p<0.05.

	Jelly	Liquid
Half time* (min)	105.5±8.6	93.7±9.6
Lag phase* (min)	62.6±9.6	48.5±11.6
Latency phase* (min)	12.7±5.6	19.4±5.3
Ascension time* (min)	86.1±7.9	81.0±8.1

Table 3: Indicates volunteer's (n=11) area under the appetite curve from visual analogue scales for hunger, fullness, desire to eat and prospective consumption of each of the meals - jelly and liquid. The higher the value indicates the greater the satiety. Data are given as mean ± SD (n=11) *p<0.05.

	Jelly	Liquid
Hunger (mm·min)*	5504±3244	3262±2506
Fullness (mm·min)*	5358±3238	3400±2645
Desire to eat (mm·min)*	5924±3681	3335±2888
Prospective consumption (mm·min)*	4726±3358	2585±2296

List of figures

Figure 1: Indicates volunteer's gastric emptying following both of the meals - jelly and liquid. Data are given as mean ± SD (n=11) *p<0.05.

Figure 2: Indicates volunteer's glycaemic response following both of the meals - jelly and liquid. Data are given as mean ± SD (n=11)

530 **Figure 3:** Indicates volunteer's diet induced thermogenesis following both of the meals - jelly
531 and liquid. Data are given as mean ± SD (n=11)
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