

## Influence of bread volume on glycaemic response and satiety

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The role of carbohydrates in health and disease has received a high profile in recent years, in particular the glycaemic index (GI) as a physiological classification of carbohydrate foods. A common carbohydrate source in the UK is white bread, which is considered to have a high GI value and low satiety value. In the present study, the possibility of favourably altering the GI of white bread by manipulating bread structure (loaf form) was investigated. In a randomised repeated-measures design, ten subjects were tested for glycaemic and satiety responses to four loaves of varying volume, but of consistent macronutrient content. Peak plasma glucose levels and GI values were shown to be significantly reduced by lowering loaf volume ( $P=0.007$ ,  $P<0.001$  respectively). In addition, a greater satiety index (SI) was seen with decreased loaf volume ( $P<0.001$ ). In conclusion, the present study demonstrates that reducing the volume of white bread, which is generally considered to be high-GI and low-SI, can favourably alter metabolic and appetite responses. Relatively small differences in the GI of regularly consumed starch foods have been shown to have beneficial effects on health.

### Glycaemic index: Satiety: Loaf volume: Gastric emptying rate

In recent years, there has been considerable discussion regarding the effect of modifying dietary carbohydrate on blood lipid profiles, the metabolic syndrome, insulin resistance and risk of type 2 diabetes (Astrup & Raben, 1995; Ebbeling *et al.* 2003). Differences in postprandial glucose response to various carbohydrate-containing foods have been demonstrated in healthy and diabetic subjects (Jenkins *et al.* 1981). The glycaemic index (GI) is a physiological classification widely accepted for carbohydrate foods, with implications in health and disease. The GI is defined as the incremental area under the blood glucose curve (IAUC) of a 50 g available carbohydrate portion of a test food expressed as a percentage of the response to 50 g available carbohydrate of a standard (reference) food taken by the same subject, on a different day (Food and Agriculture Organization & World Health Organization, 1998). The principle is that the slower the rate of carbohydrate absorption, the lower the rise of blood glucose level and the lower the GI value (Brand *et al.* 1991). Indeed, high-GI foods are characterised by fast-release carbohydrate and higher blood glucose levels. A GI value  $\geq 70$  is considered high, a GI value 56–69 inclusive is medium and a GI value  $\leq 55$  is low, where glucose = 100 (Brand-Miller *et al.* 2003a).

A number of factors have been shown to influence the glycaemic response to carbohydrate foods including food form and particle size (Granfeldt *et al.* 1991), the structure of the starch component (Noakes *et al.* 1996), degree of starch damage through food processing (Jenkins *et al.* 1986), the inclusion of whole kernels (Hallfrisch & Behall, 2000), viscous fibres (Alvarado *et al.* 1999) and resistant starch

(RS; Langkilde *et al.* 2002). Mechanisms of reduction in glycaemic response appear to be changes in gastric emptying rate and/or starch amylolysis, involving starch gelatinisation and retrogradation.

The compactness of food influences starch digestion, demonstrated in studies on glycaemic response to pasta compared with white wheat bread (Hoebler *et al.* 1999). In studies of bread dough, light microscopy, scanning and electron microscopy show adhesion of neighbouring networks of protein in flour particles in the transformation of water and flour into viscoelastic dough, where the gluten protein fills the space between the starch granules (Amend & Belitz, 1991). Of equal importance is the extent of the physical barrier created by the protein network, influencing the relative accessibility of starch to amylase (Hoebler *et al.* 1999; Hayta & Alpaslan, 2001).

Of the starch foods in the UK, bread forms probably the most basic staple. The UK bread and morning-goods market, one of the largest sectors in the food industry, produces almost twelve million loaves and packs per d (Federation of Bakers, 2005). Moreover, white bread sales constitute more than 70% of sales in the UK. The result of long-term development in the bread-making process is a highly favoured white bread, of relatively high GI and of low satiety value (Wolever *et al.* 1994; Foster-Powell *et al.* 2002).

Relatively small differences in the GI of regularly consumed starch foods have shown beneficial effects on health, including reduced CVD risk and glycaemic control (Frost

*et al.* 1998; Liu *et al.* 2000; Wolever & Mehling, 2002; Brand-Miller *et al.* 2003b). Thus, investigations into ways of reducing the GI and increasing the satiety index (SI) of white bread are of important application. In light of the above, the aim of the present study was to investigate the relationship between food structure and composition and glycaemic response and satiety of white bread.

## Methods

### Subjects

Ten healthy subjects (four male and six female; age 50.4 (SD 9.1) years; BMI 23.9 (SD 2.0) kg/m<sup>2</sup>) were recruited to the study. Interested subjects were asked to complete a health-screening questionnaire to check against ill health, including clinically abnormal glucose metabolism (fasting blood glucose > 6.0 mmol/l) and any medical conditions or medications that might affect glucose regulation, gastric emptying, body weight, appetite or energy expenditure. Mean fasting plasma glucose level was 5.3 (SD 0.1) mmol/l.

Anthropometric measurements were made in the fasting state, using standardised methods, on the morning of the first test. Height was recorded to the nearest centimetre using a stadiometer (Seca Ltd, Birmingham, UK), with subjects standing erect and without shoes. Body weight was recorded to the nearest 0.1 kg using the Tanita BC-418 MA (Tanita UK Ltd, Yiewsley, Middlesex, UK), with subjects wearing light clothing and no shoes. BMI was calculated using the standard formula: weight (kg)/height (m)<sup>2</sup>.

Ethical approval was obtained from the University Research and Ethics Committee at Oxford Brookes University (Oxford, UK). Subjects were given full details of the study protocol and the opportunity to ask questions. All subjects gave written informed consent before participation.

### Test breads

White bread loaves were made from 500 g white wheat flour (Carr's white strong bread flour), 8 g NaCl, 6 g sugar, 287 ml water, 6 g butter, 7 g skimmed milk powder and 8 g dehydrated yeast without additives (Fermipan®; DSM Bakery Ingredients, Dordrecht, The Netherlands).

Bread loaves of different volumes were prepared using a standard, readily available home bread-making machine (Breadman Pro; Russell Hobbs, Manchester, UK). Using the dough-cycle function to ensure consistency of mixing and

kneading intensities, while making possible manual manipulation of proving times, different bread volumes were produced of identical macronutrient content, including both water and yeast. Proving and baking conditions for each test bread are shown in Table 1. Dough was proved once (rise 1), shaped, reproofed (rise 2) and then baked in a conventional oven (200°C) for 20 min. Rises 1 and 2 took part as part of the bread-making machine cycle. For the highest volume loaf, the dough was removed from the bread maker at the end of the cycle, punched back and left to rise for one final proving, at room temperature (Table 1).

Following baking, the temperature at the centre of each loaf was determined using a kitchen food thermometer (Kitchen Craft Ltd, Weymouth, Dorset, UK). In addition, the weight of each loaf was measured at 5 min intervals for approximately 1 h, until complete cooling, as a crude assessment of water loss and movement through the loaf matrix. Volume measurement was carried out by seed displacement methodology, using a volume-weight calibration (Table 1).

### Study protocol

The method used to measure glycaemic response and to calculate the GI value was in line with procedures recommended by the Food and Agriculture Organization & World Health Organization (1998). In addition, on the day preceding a test, subjects were asked to restrict their intake of alcohol and caffeine-containing drinks and to refrain from intense physical activity (for example, long periods at the gym, excessive swimming, running, aerobics). To minimise the possible influence of the second-meal effect, subjects were asked to refrain from eating an extra-large evening meal or have an unusually high food intake the day preceding a test (Wolever, 1990; Granfeldt *et al.* 2006). Where possible, subjects ate a similar meal type on the evening before testing. All foods were tested in subjects after a 12 h overnight fast.

Four bread loaves with different volumes (test breads) were administered to subjects in a randomised, repeated-measures design, with each subject acting as his/her own control. All test breads were compared with a standard food (glucose) and were tested in equivalent amounts (50 g) of available carbohydrate. As blood glucose responses vary within subjects from day to day, the standard food was tested three times in each subject. Thus, subjects tested each test bread once and the standard food three times in random order on separate days, with at least a 1 d gap between measurements to minimise carry-over effects. All test breads and the standard

**Table 1.** Proving and baking conditions of test breads\*

	Rise 1 (min)	Pb† (s)	Rise 2 (min)	Pb† (s)	Rise 3 (min)	Volume (ml)		Temperature (°C)	Weight (g) after cooking	
						Mean	SD		0 min	50 min
Bread 1	10	10	2	–	–	1100	100	140	793	784
Bread 2	30	10	12	–	–	1700	150	160	780	767
Bread 3	60	10	30	–	–	2400	150	170	773	758
Bread 4	40	10	25	10	50	3000	150	190	760	744

Pb, punchback time.

\*Rises 1 and 2 in bread-making machine cycle; rise 3 outside machine, following third 'punchback'.

† Manual punching down of risen dough.

food were served with 200 ml water. A further 200 ml water was given during the subsequent 2 h. Subjects were asked to eat the test breakfast within a 10–12 min period to reduce the influence of chewing on particle size (Hoebler *et al.* 1998).

#### Blood glucose measurements

Finger-prick blood samples were taken for capillary blood glucose analysis. Recent reports suggest that capillary blood sampling is preferred for reliable GI testing (Food and Agriculture Organization & World Health Organization, 1998; Wolever & Mehling, 2003). A fasting blood sample was taken at 0 min and the standard food or test bread was consumed immediately after this. Further blood samples were taken at 15, 30, 45, 60, 90 and 120 min after starting to eat.

Blood was obtained by finger-prick using the Glucolet 2 multi-patient lancing system (Bayer HealthCare, Leverkusen, Germany). Before a finger-prick, subjects were encouraged to warm their hand to increase blood flow. Fingers were not squeezed to extract blood from the fingertip as this may dilute with plasma. Blood glucose was measured using Ascensia Contour<sup>®</sup> automatic blood glucose meters (Bayer HealthCare). The blood glucose meters were calibrated daily using control solutions from the manufacturer and were also regularly calibrated against a clinical dry chemistry analyser (Reflotron<sup>®</sup> Plus; Roche Diagnostics Ltd, Lewes, East Sussex, UK) and the HemoCue Glucose 201 + analyser (HemoCue<sup>®</sup> Ltd, Dronfield, Derbyshire, UK).

Fig. 1 shows the Pearson regression and Bland–Altman analyses (Bland & Altman, 1986) for a random selection of 106 blood samples simultaneously measured using the

Ascensia Contour<sup>®</sup> and the HemoCue Glucose 201 + analyser. There was a very strong correlation ( $r$  0.978;  $P < 0.001$ ) and good agreement (mean difference  $-0.3$  (95% CI  $-0.3$ ,  $-0.2$ ) mmol; limits of agreement  $-0.75$  and  $0.21$ ) between blood glucose measurements using the automatic analyser and the HemoCue analyser.

#### Calculation of glycaemic index

The IAUC, ignoring the area beneath the baseline, was calculated geometrically for each test bread (Food and Agriculture Organization & World Health Organization, 1998). The IAUC for each test bread eaten by each subject was expressed as a percentage of the mean IAUC for the reference food eaten by the same subject:

$$GI = (\text{IAUC test bread} / \text{IAUC reference food}) \times 100.$$

The GI of each test bread was taken as the mean for the whole group.

#### Assessment of satiety

At the same time as the finger-prick blood samples (i.e. 0, 15, 30, 45, 60, 90 and 120 min), the subjective feeling of satiety was measured using an equilateral seven-point rating scale (Holt *et al.* 1995). The satiety response for each test bread was quantified as the incremental area under the response curve (AUC), ignoring area beneath the baseline. SI scores were obtained by dividing the satiety AUC for the test bread by the group mean satiety AUC for glucose and multiplying by 100. Thus, glucose had an SI score of 100% and the SI scores of the test breads were expressed as a percentage of glucose.

Alertness ratings were included as a distraction from the importance of satiety ratings; in addition, all marks of ratings were covered manually immediately to prevent subjects referring to previous ratings (Holt *et al.* 2001).

#### Statistical analysis

Statistical analysis was performed using SPSS software (version 11.0.1; SPSS Inc., Chicago, IL, USA). Data are presented as means and standard deviations. To examine the correlation and agreement between the automatic analyser and the HemoCue Glucose 201 + analyser, Pearson's correlation coefficient and the method of Bland & Altman (1986) were used. Repeated-measures ANOVA, with Bonferroni's correction, was used to compare glycaemic response and satiety rating between the four different bread loaf volumes. Statistical significance was set at  $P < 0.05$ .

#### Results

Fig. 2 shows the mean IAUC for the test breads. Other parameters such as the fasting, peak rise, IAUC and GI values are presented in Table 2.

Peak rise in glucose was significantly different ( $P < 0.001$ ). Peak rise for bread 1 was significantly lower than corresponding values for breads 2, 3 and 4 ( $P = 0.19$ ,  $P = 0.002$  and  $P = 0.001$ , respectively). Bread 2 also produced a significantly lower peak rise glucose than bread 4 ( $P = 0.049$ ).

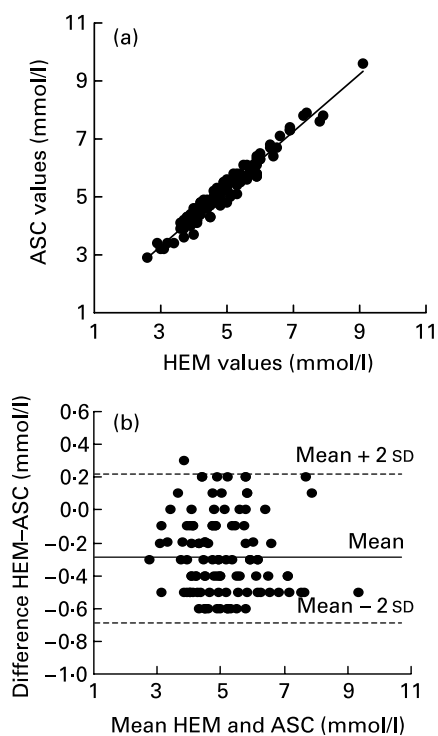


Fig. 1. Pearson regression (a) ( $y = 0.9912x + 0.3143$ ;  $R^2$  0.9562) and Bland–Altman analyses (b) of blood glucose measurements between the Ascensia Contour (ASC) and HemoCue 201 + analyser (HEM).

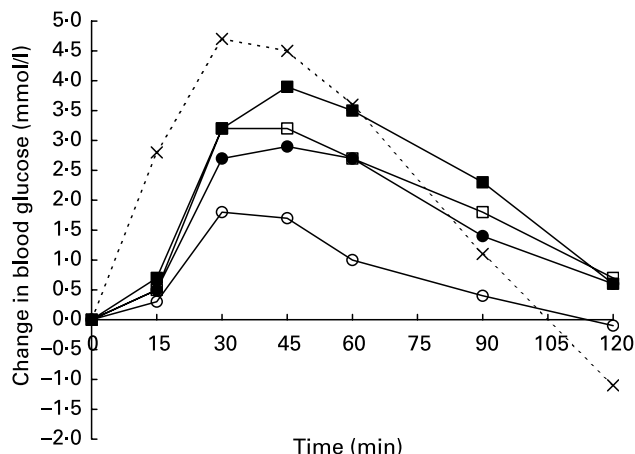


Fig. 2. Incremental area under the blood glucose curve for glucose and test breads: glucose (×); bread 1 (○); bread 2 (●); bread 3 (□); bread 4 (■).

There was a significant effect of bread volume on GI value ( $P < 0.001$ ). The GI value for bread 1 was significantly lower than each of the higher volume breads: bread 2 ( $P < 0.001$ ), bread 3 ( $P = 0.002$ ) and bread 4 ( $P < 0.001$ ). In addition, the GI values of breads 2 and 3 were significantly lower than for bread 4 ( $P = 0.011$  and  $P = 0.048$ , respectively).

Satiety response for each of the test breads is shown in Fig. 3. SI values were 202 (SD 48), 235 (SD 51), 117 (SD 54) and 155 (SD 62) for breads 1, 2, 3 and 4, respectively. There was a significant effect of bread volume on satiety response ( $P < 0.001$ ). In particular, the SI of breads 1 and 2 was significantly higher than bread 3 ( $P = 0.005$  and  $P < 0.001$ , respectively) and the SI of breads 2 and 3 was significantly higher than bread 4 ( $P = 0.035$  and  $P = 0.045$ , respectively).

## Discussion

The present study has shown that manipulation of bread dough proving time, resulting in lower loaf volume, can lead to reduced glycaemic response. Reducing bread volume from 3000 ml to 2400, 1700 and 1100 ml led to 14, 28 and 62% reductions in GI values, respectively. In addition, peak rise in glucose was significantly reduced by lower loaf volume, similar to the lente features of pasta (Granfeldt *et al.* 1991). The GI values of three of the breads (breads 2, 3 and 4) were above 70. This may be due to the use of a home-style bread maker, involving long proof times. The influence of manipulation of proof times was a salient feature of the present study.

Any condition or process leading to a breakdown or disruption of the starch granule will lead to more readily digestible starch, with a resultant higher blood glucose response (Granfeldt *et al.* 2000). This is due to a greater susceptibility of the granule to enzymic degradation by salivary and pancreatic amylases. Gelatinisation of the starch granule is an important concept in terms of enzyme access and bioavailability of glucose (Bornet *et al.* 1989; Rashmi & Urooj, 2003). Importantly, any impact upon starch, which results in a limited swelling and gelatinisation, such as lowered bread loaf core temperature, and a denser bread matrix, as seen in the present study, will result in reduced postprandial glycaemic responses. Variation in starch–protein interactions in the loaves with differences in loaf temperature and matrix density may also play a part.

A reduced rate of starch hydrolysis due to greater starch–protein interactions is possible in the denser bread, where a lower loaf temperature may not allow breakdown of such interactions. Moreover, a slower rate of gastric emptying with denser, more compact bread cannot be ruled out. Granfeldt *et al.* (1991) showed an absence of any slower rate of amylolysis of thin linguine pasta using such methods, concluding that the reduced glycaemic response was more likely to be due to reduced gastric emptying rate.

All initial macronutrients in the original bread recipe were consistent; however, RS was not measured. It is possible that the reduced gelatinisation with reduced volume produced differences in RS. The substitution of digestible starch with RS may reduce the appearance of glucose in the blood and result in a lower GI value (Achour *et al.* 1997; Langkilde *et al.* 2002). Short-term consumption of RS has been demonstrated to enhance postprandial insulin sensitivity in healthy subjects (Robertson *et al.* 2003) and several ingredients with high levels of RS are becoming available commercially.

Interestingly, none of the breads in the present study produced mean plasma glucose levels below baseline fasting levels, often demonstrated in studies using white bread (Foster-Powell *et al.* 2002). This could be reflective of the nature of the milling of the flour used in the present study. The flour used is low-pressure-milled on air-floated rollers and is a more gentle milling action. Modern treatments of starch foods incorporate the generation of a number of forces upon the starch granule, such as shearing, compression and extreme heat treatment, facilitating more readily the important process of gelatinisation. The more gently milling action of the flour used in the present study may be a factor reducing damage to the starch granule. For the purpose of the present study, the bread recipe included Carr yeast also,

Table 2. Fasting plasma glucose and postprandial glucose characteristics (Mean values with their standard errors)

	Glucose		Bread 1		Bread 2		Bread 3		Bread 4	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Fasting glucose (mmol/l)	5.3	0.1	5.6	0.2	5.2	0.1	5.2	0.1	5.2	0.2
Peak rise (mmol/l)	5.3	0.4	2.4 <sup>a</sup>	0.2	3.4 <sup>b</sup>	0.5	3.9 <sup>b</sup>	0.5	4.4 <sup>c</sup>	0.5
IAUC	279	33	106	15	204	204	230	30	273	30
GI value	—	—	38 <sup>a</sup>	4	72 <sup>b</sup>	72	86 <sup>b</sup>	9	100 <sup>c</sup>	7

IAUC, incremental area under the blood glucose curve; GI, glycaemic index.

<sup>a,b,c</sup> Mean values within a row with unlike superscript letters were significantly different ( $P < 0.05$ ).

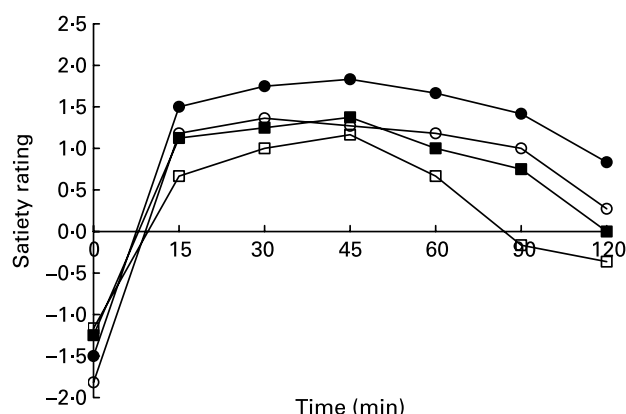


Fig. 3. Satiety response to test breads: bread 1 (○); bread 2 (●); bread 3 (□); bread 4 (■).

essentially without the action of vitamin C or amylases. This was used in order to more effectively reduce the volume of the bread with changes in rising times, otherwise overridden by the high-rising action of the yeast.

In the present study, an increase in satiety with decreased loaf volume was seen, although it was the second lowest volume (bread 2) that caused the greatest satiety response. The dense texture of bread 1 was disliked by many subjects, possibly influencing satiety scores. It is possible that differences in gastric fullness or extension, the presence of undigested or partially digested starch in the duodenum, jejunum or ileum, and postprandial glycaemia together determined satiety. A lower degree of gastric fullness with the higher volume bread is hypothesised to lead to a stronger urge to eat again following carbohydrate-rich meals containing such bread. Denser, coarse bread, for example, bread 2, may lead to increased satiety through a greater perceived fullness.

Automatic glucose meters are often used to measure glycaemic response due to their convenience. Velangi *et al.* (2005) found that glycaemic response determined by an automatic glucose meter (One Touch Ultra<sup>®</sup>) was variable. However, the automatic glucose meters used in the present study (Ascensia Contour<sup>®</sup>) showed a very strong correlation and good agreement with the HemoCue Glucose 201+ analyser.

The present study is the first to show a significant reduction in GI and significant increase in SI of white bread by reduction of bread volume to denser bread. Although the GI values of three of the four test breads remained high, i.e. > 70, relatively small differences in the GI of regularly consumed starch foods have been shown to have beneficial effects on health, including reduced CVD risk and glycaemic control (Frost *et al.* 1998; Liu *et al.* 2000; Wolever & Mehling, 2002; Brand-Miller *et al.* 2003b).

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