Short communication

Evaluation of role of concentration and molecular weight of oat β-glucan in determining effect of viscosity on plasma glucose and insulin following an oral glucose load

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Data from clinical studies established that there was an inverse linear relationship between measures of postprandial blood glucose and insulin responses to an oral glucose load, consumed in a drink, and the logarithm of viscosity of the drink. These data have been re-analysed using concentration and molecular weight as the dependent variables. Molecular weight (M) of the β-glucans used was determined using high-performance size exclusion chromatography equipped with a triple detector system of right angle light scattering, viscometry and refractive index. A significant relationship between changes in peak blood glucose and a combination of logarithm of the concentration and logarithm of M was found.

β-Glucan: Molecular weight: Glycaemic response

The (1→3)(1→4)-β-D-glucan (oat β-glucan) polysaccharide present in oats has been accepted by the Food and Drug Administration in the USA as a component which can lower serum cholesterol levels (Anon, 1997). An oat β-glucan isolate was shown to lower serum cholesterol levels in hypercholesterolaemic subjects by 9 % (Braaten *et al.* 1994). Viscous soluble fibres like oat β-glucan and guar gum, in addition to reducing blood cholesterol levels, also attenuate postprandial blood glucose and insulin levels. Jenkins *et al.* (1978) reported that viscosity was important for the latter activity but the role of viscosity in lowering serum cholesterol levels in human subjects is less well established. Wood *et al.* (1994) modified the viscosity of a glucose drink by using partially hydrolysed β-glucan and by changing the concentration in the drink. In this drink model, a significant inverse linear relationship between glycaemic response to a 50 g oral glucose load and $\log_{10}$ viscosity (at 30 /s) of a solution of oat β-glucan or guar gum, consumed by healthy subjects was established. The relationship was true for blood glucose or insulin peak increments above baseline, excursions, or areas under the 2 h curve.

The shear thinning behaviour of random coil polysaccharides such as guar galactomannan and oat β-glucan requires that viscosity measurements be quoted for a specific shear rate, and comparison of products is difficult, with relative values dependent on the concentration and shear rates chosen for comparison (Wood *et al.* 1990). Viscosity of polymer solutions is mainly controlled by concentration in solution and molecular size and its distribution. These might be more fundamental variables with which to evaluate the ability of a polysaccharide to modify blood glucose and insulin response.

Beer *et al.* (1997) determined the molecular weight (M) of β-glucans using high-performance size exclusion chromatography (HPSEC) and analysed the eluted material with refractive index, right angle light scattering and viscometric detectors (Viscotek, Houston, TX, USA). This system has now been applied to determine the M of the β-glucan and partially hydrolysed products used to modify the viscosity of the drinks consumed in the study of Wood *et al.* (1994). This paper reports the 1994 blood glucose data, re-analysed using the variables of concentration and weight average molecular weight (M_w) instead of viscosity.

Abbreviations: HPSEC, high-performance size exclusion chromatography; M, molecular weight; M_w, weight average molecular weight.

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Materials and methods

General

The clinical studies, preparation and characterisation of the β-glucan and measurements of viscosity were described in Braaten *et al*. (1991) and Wood *et al*. (1989, 1994). β-Glucan was extracted by sodium carbonate at pH 10, and after isoelectric precipitation of protein, isolated by precipitation with ethanol. Depolymerised β-glucan was prepared by mild acid hydrolysis (0·1M-HCl, 70°C, 15 and 60 min) from the original oat gum, and isolated by precipitation with ethanol. Viscosity (η) was determined on a Carri-Med Controlled Stress Rheometer (Carri-Med Ltd, Dorking, Surrey, England. (No longer in manufacture.)) using cone and plate geometry (6 cm 2°, 4 cm 2° and 4 cm 1°) in a range of shear rate up to 100 /s. Data were analysed using the apparent viscosity at 30 /s.

Subjects consumed 50 g glucose in a fixed volume of 500 ml water containing polysaccharide, or control with glucose alone. Three different trials with 9–11 subjects each were analysed. In one study (Braaten *et al*. 1991) 14·5 g doses of oat and guar 'gums' were compared (the gums used contained about 80–90 % polysaccharide). In a second study, 7·2, 3·6 and 1·8 g oat gum were compared and in a third study 7·2 g of the two partially hydrolysed oat gums. After subjects had consumed the glucose 'drinks', blood glucose and insulin levels were determined over 3 h.

The data from each experiment (Braaten *et al*. 1991; Wood *et al*. 1994) were examined (Wood *et al*. 1994) to determine if the groups used in each experiment had significantly different plasma glucose and insulin characteristics. Generally similar mean baseline plasma glucose, and response to the glucose control meal (peak plasma glucose increment about 3·0 mmol/l), were observed in each experiment and analysis of the glucose data (peak increment, area under curve (AUC)) from the control meals showed no statistically significant differences between the different subject groups. Thus dose and viscosity effects were evaluated using all data points from experiments 1–3, leading to significant ($P < 0·0001$) inverse linear relationships between increment, excursion and the area under the 2 h AUC of both blood glucose and insulin, and $\log_{10}$[viscosity] (at 30 /s) (Wood *et al*. 1994).

Determination of molecular mass

Molecular weight distribution of the oat β-glucan was determined by HPSEC essentially as described by Beer *et al*. (1997). Two columns (300 × 7·5 mm) in series (Shodex OHpak KB806M, Waters Ultrahydrogel; Waters, Milford, MA, USA) and a Waters model 590 pump were used for HPSEC. Samples were filtered (0·45 μm) before analysis. The columns were maintained at 40°C and eluted with 0·1M-$NaNO_3$ buffer at 0·6 ml/min. A Perkin-Elmer ISS 100 autosampler and injector was used with an injection volume of 150 μl, with detection by refractive index, viscosity (Model 250, Viscotek, Houston, TX, USA), and right angle laser light-scattering (RALLS, Viscotek). The system was controlled and the data processed by TRISEC V2·7 software (Viscotek). Values are average of duplicates.

Analysis of data

The blood glucose increment data from the three trials were re-analysed on the basis that the viscosity measured was a function of concentration (c) and M_w, using the general linear models procedure of SAS (Statistical Analysis Systems Inc., Cary, NC, USA). Regression analysis was done on the basis of weighted means (subject numbers 9, 10 and 11 in the three trials). Analysis was also done on the individual responses to check for possible lack of fit of the models used.

Results and discussion

The molecular weights and concentrations of the oat β-glucans used in the clinical studies are summarised in Table 1. Accuracy of the M_w values is uncertain (although difference between replicates in the system is < 5 %). We determined a M_w of 214 000 for a standard β-glucan. Another laboratory, similarly using HPSEC and the Viscotek detector system, found a M_w of 268 000, or using a multi-angle light scattering detector a M_w of 278 000 (M. Fishman, personal communication). Many factors, such as the refractive index increment, dn/dc, used in calculations, may influence the absolute value. In the following analyses, these differences would alter the modifying constants of equations, but not the general validity.

The weighted multiple regression revealed a significant ($P = 0·042$) relationship (R^2 0·88) of the form:

$$\Delta G = 7·93 - 0·68 \log_{10}(c) - 1·01 \log_{10}(M_w), \qquad (1)$$

where ΔG is the peak blood glucose increment above fasting level, c is concentration, and M_w is the weight average molecular weight of the β-glucan. The standard errors for the intercept, and log c and log M_w modifiers were 1·3, 0·28 and 0·22 respectively, based on six means ($c > 0$).

Table 1. Plasma glucose increments (ΔG) and molecular weights* and concentrations of β-glucans

Sample	ΔG (mmol/ml)	Concentration (%) of β-glucan	M_p	M_w
Oat gum	1·9	2·35	886 850	804 950
	1·8	1·17	886 850	804 950
	2·1	0·58	886 850	804 950
	2·5	0·29	886 850	804 950
15 min†	2·3	1·29	221 000	250 800
60 min†	2·9	1·29	96 350	101 850

* M_p, peak molecular weight; M_w, weight average molecular weight.
† 15 min and 60 min hydrolysed oat gums.

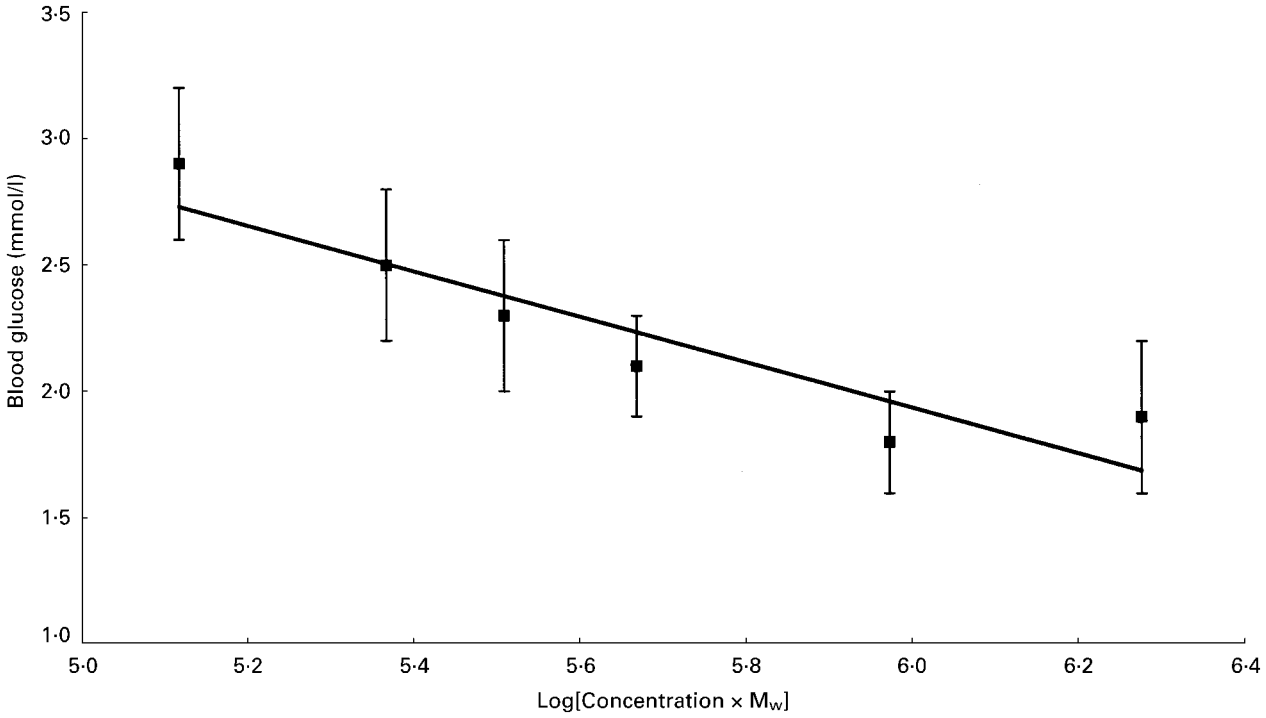

Fig. 1. Plot showing the relationship of peak blood glucose increment (ΔG) (mean peak level above starting value) to $\log_{10}$ of concentration (g/100 ml) and weight average molecular weight (M_w) of β-glucan consumed. Values are means with between subject standard errors represented by vertical bars.

To express the relationship on a two-dimensional plot (Fig. 1), equation 1 may be approximated to the form:

$$\Delta G = 7{\cdot}30 - 0{\cdot}90 \log_{10}(c.M_w), \quad (2)$$

when R^2 0·83 ($P = 0{\cdot}0112$). Estimating an additional modifier, separately for each of $\log_{10}(c)$ and $\log_{10}(M_w)$ in equation 1, does not give a significantly better fit ($P = 0{\cdot}36$). Thus, although equations 1 and 2 are not algebraically equivalent, the difference between the two is not statistically significant. Furthermore regression analysis based on all fifty-nine individual ΔG values did not indicate a significant lack of fit for either model ($P > 0{\cdot}50$). An eventual plateau is to be expected, and exclusion of the high c data point in equation 2 slightly improves R^2 (0·97) and P (0·0018), but this cannot be taken to mean that a plateau has been reached at this point, although the relationship of the point to the trend line might suggest this.

Above a critical concentration, apparent viscosity may be empirically related to both concentration and M_w in a power law function (Fig. 2). Since:

$$\Delta G = a + b \log_{10}(\eta), \quad (3)$$

where η was viscosity at 30 /s, and a and b constants (Wood *et al*. 1994), a relationship such as equation 2 was to be expected.

The 2 h AUC for glucose also shows an inverse linear relationship with $\log_{10}(c.M_w)$:

$$AUC = 289 - 32 \log_{10}(c.M_w), \quad (4)$$

with R^2 0·77 ($P = 0{\cdot}0215$). As commented previously (Wood *et al*. 1994), when sufficient measurements are taken (i.e. every 10 min) the time independent peak values appear to better reflect group response than values averaged at a fixed point in time (as in the group averaged glycaemic response curves) or AUC values.

Similarly, the relationship with glycaemic index (GI) was:

$$GI = 358 - 48 \log_{10}(c.M_w), \quad (5)$$

with R^2 0·83 ($P = 0{\cdot}0113$). Thus there is little difference whether peak glucose increment, AUC or glycaemic index is used.

'Zero shear' mass specific viscosity (specific viscosity measured at zero shear rate, η_{sp0}), like molecular weight, is a more fundamental variable for evaluation, and was used by Ellis *et al*. (1995) in pig studies. Specific viscosity, η_{sp}, is $(\eta - \eta_s)/\eta_s$) where η is viscosity of sample and η_s is viscosity of solvent.

For oat β-glucan (Doublier & Wood, 1995), for c > about 0·3 % (w/v) and η_{sp0} > about 10 mPa.s:

$$\eta_{sp0} \propto (c[\eta])^{3{\cdot}9}, \quad (6)$$

where c is concentration and $[\eta]$ intrinsic viscosity. The exponent of 3·9 is typical of random coil polysaccharides.

The Mark-Houwink-Sakurada (MHS) equation relating $[\eta]$ to M_w of oat β-glucan was found to be (M. U. Beer, unpublished results):

$$[\eta] = 7{\cdot}3 \times 10^{-4} (M_w)^{0{\cdot}72}. \quad (7)$$

If the relationship of equation 3 is held to zero shear rate, then combining equations 3, 6 and 7:

$$\Delta G = A' + B' \log_{10}(cM_w^{0{\cdot}72}). \quad (8)$$

The values found empirically for A′ and B′, by fitting ΔG

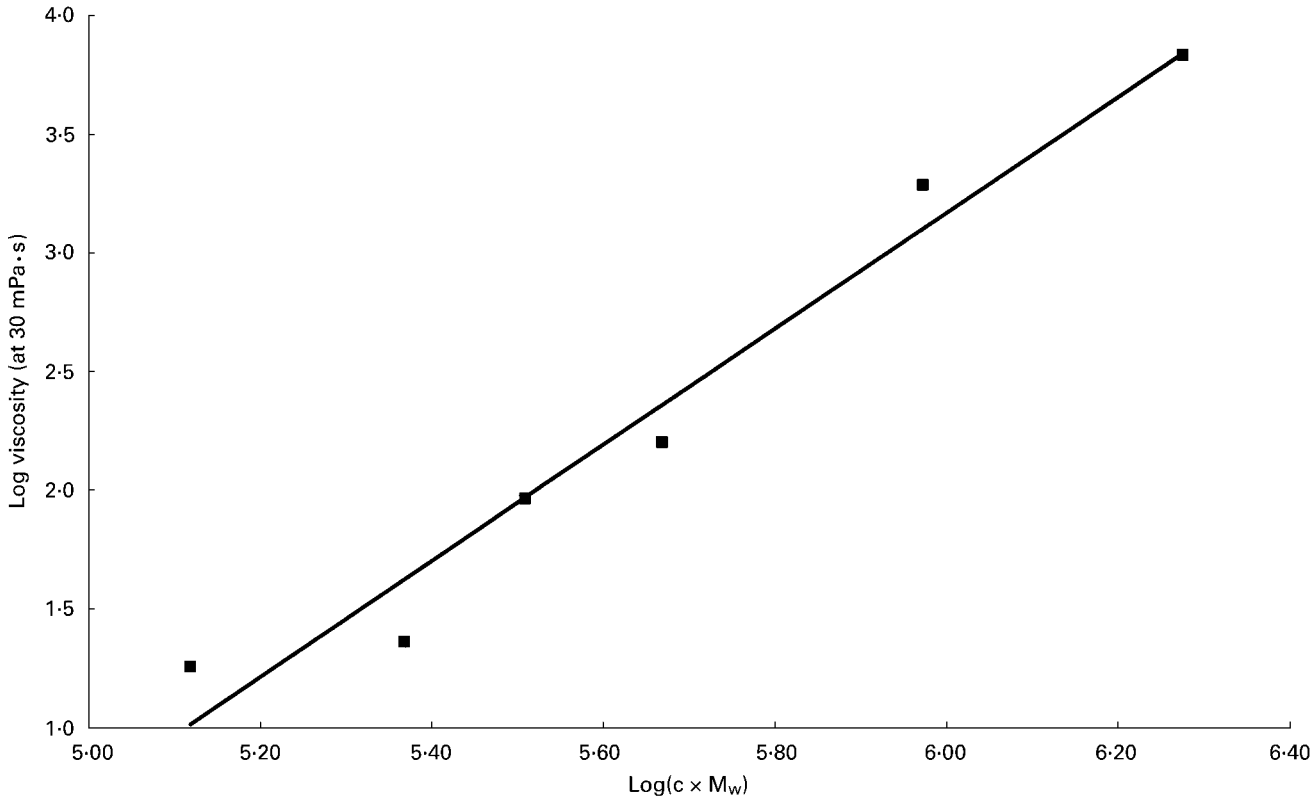

Fig. 2. Plot showing the relationship between $\log_{10}$ viscosity at 30/s and $\log_{10}$ of concentration (g/100 ml) and weight average molecular weight (M_w) of β-glucan (R^2 0·97, $P = 0{\cdot}0004$).

in terms of $\log_{10}(c \,.\, M_w^{0{\cdot}72})$, are 6·27 and –0·99 respectively (R^2 0·73, $P = 0{\cdot}0293$). However, viscosity was determined, not at zero shear rate but at 30 s^{-1}, so concentration and M_w values from equations 6 and 7 cannot be substituted in equation 3, which might have a different form, and would have different constants, if zero shear viscosity had been used.

Equation 8 expands to:

$$\Delta G = A' + B' \log_{10}(c) + 0{\cdot}72\ B' \log_{10} M_w, \qquad (9)$$

which has the same general form as equation 1, where the exponents of M_w and c were estimated. Clearly, however, the relative magnitudes of the modifiers of $\log_{10}$c and $\log_{10}$M are different in these two equations, as would be expected because viscosity was the value at a shear rate of 30 s^{-1} and not specific viscosity at zero shear rate.

Thus empirical analysis, from limited data but with some support from theoretical relationships, establishes that the glycaemic response, in this model, may be related logarithmically to concentration and molecular weight. If either is kept constant, then equation 1 reduces to a simple linear relationship with log c or log M. In this study, substitution of peak molecular weight (which may be more simply determined) for M_w gave similar relationships, but this would depend on the molecular weight distribution. The treatment described here deals with neutral random coil polysaccharides above the critical concentration.

It follows that in real foods treatments that either lower solubility or bring about depolymerisation of the polysaccharides might reduce physiological effectiveness. It must be recognised, however, that real foods will have various differences from the drink model described here. Firstly, liquid volume of intake is not 'fixed'. Secondly, other food interactions and microstructure will play a role (Brennan *et al*. 1996). It must also be remembered that our measurements were of material as consumed, whereas the site of physiological activity is the gastrointestinal tract. For example, the presence of particulate matter will significantly modify rheological behaviour. Despite these caveats, however, appropriate measurements of real foods based on the above treatment might help clarify the relative contributions of viscosity and other factors to the physiological response. A key step towards achieving this objective would be to relate meal or drink measurements to measurements in the gastrointestinal tract.

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References

Anon (1997) Food labeling: Health claims; Oats and coronary heart disease. *Federal Register* **62**, no. 15, 3584–3601.

Beer MU, Wood PJ & Weisz J (1997) Molecular weight distribution and (1→3)(1→4)-β-D-glucan content of consecutive extracts of various oat and barley cultivars. *Cereal Chemistry* **74**, 476–480.

Braaten JT, Wood PJ, Scott FW, Riedel KD, Poste LM & Collins MW (1991) Oat gum, a soluble fiber which lowers glucose and insulin in normal individuals after an oral glucose load: comparison with guar gum. *American Journal of Clinical Nutrition* **53**, 1425–1430.

Braaten JT, Wood PJ, Scott FW, Wolynetz MS, Lowe MK, Bradley-White P & Collins MW (1994) Oat β-glucan reduces serum cholesterol concentration in hypercholesterolemic subjects. *European Journal of Clinical Nutrition* **48**, 465–474.

Brennan CS, Blake DE, Ellis PR & Schofield JD (1996) Effects of guar galactomannan on wheat bread microstructure and on the *in vitro* and *in vivo* digestibility of starch in bread. *Journal of Cereal Science* **24**, 151–160.

Doublier J-L & Wood PJ (1995) Rheological properties of aqueous solutions of (1→3)(1→4)-β-D-glucan from oats (*Avena sativa* L.). *Cereal Chemistry* **72**, 335–340.

Ellis PR, Roberts FG, Low AG & Morgan LM (1995) The effect of high molecular-weight guar gum on net absorption of glucose and net apparent insulin and gastric inhibitory polypeptide production in the portal blood of the pig: relationship to rheological changes in the jejunal digesta. *British Journal of Nutrition* **74**, 539–556.

Jenkins DJA, Wolever TMS, Leeds AR, Gassull MA, Dilawari JB, Goff DV, Metz GL & Alberti KGM (1978) Dietary fibres, fibre analogues and glucose tolerance: importance of viscosity. *British Medical Journal* **1**, 1392–1394.

Wood PJ, Braaten JT, Scott FW, Riedel D & Poste LM (1990) Comparisons of viscous properties of oat and guar gum and the effects of these and oat bran on glycemic index. *Journal of Agriculture and Food Chemistry* **38**, 753–757.

Wood PJ, Braaten JT, Scott FW, Riedel KD, Wolynetz MS & Collins MW (1994) Effect of the dose and modification of viscous properties of oat gum on plasma glucose and insulin following an oral glucose load. *British Journal of Nutrition* **72**, 731–743.

Wood PJ, Weisz J, Fedec P & Burrows VB (1989) Large-scale preparation and properties of oat fractions enriched in (1→3)(1→4)-β-D-glucan. *Cereal Chemistry* **66**, 97–103.