Short communication

Evaluation of role of concentration and molecular weight of oat \(\beta\)-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 \(\beta\)-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.

\(\beta\)-Glucan: Molecular weight: Glycaemic response

The (1→3)(1→4)-\(\beta\)-D-glucan (oat \(\beta\)-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 \(\beta\)-glucan isolate was shown to lower serum cholesterol levels in hypercholesterolaemic subjects by 9% (Braaten et al. 1994). Viscous soluble fibres like oat \(\beta\)-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 \(\beta\)-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 \(\beta\)-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 \(\beta\)-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 \(\beta\)-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 \(\beta\)-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 ($\eta$) 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 polysaccaride, 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 18 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 evaluation 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:

$$\Delta G = 7.93 - 0.68 \log \frac{c}{M_w} - 1.01 \log \frac{c}{M_w},$$

where $\Delta G$ 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 ($\Delta G$) and molecular weights* and concentrations of β-glucans |
|---|---|---|---|---|
| Sample | $\Delta G$ (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 | 0.88 | 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.
To express the relationship on a two-dimensional plot (Fig. 1), equation 1 may be approximated to the form:

$$\Delta G = 7.30 - 0.90 \log_{10}(c \cdot M_w),$$

(2)

when \(R^2 = 0.83\) \((P = 0.0112)\). 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 \((P = 0.36)\). 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 \(\Delta G\) values did not indicate a significant lack of fit for either model \((P > 0.50)\). 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:

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

(3)

where \(\eta\) 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 \(\log_{10}(c \cdot M_w)\):

$$\text{AUC} = 289 - 32 \log_{10}(c \cdot M_w),$$

(4)

with \(R^2 = 0.77\) \((P = 0.0215)\). 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:

$$\text{GI} = 358 - 48 \log_{10}(c \cdot M_w),$$

(5)

with \(R^2 = 0.83\) \((P = 0.0113)\). 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, \(\eta_{sp0}\)), like molecular weight, is a more fundamental variable for evaluation, and was used by Ellis et al. (1995) in pig studies. Specific viscosity, \(\eta_{sp}\), is \((\eta - \eta_s)/\eta_s)\) where \(\eta\) is viscosity of sample and \(\eta_s\) is viscosity of solvent.

For oat \(\beta\)-glucan (Doublier & Wood, 1995), for \(c > 0.3\%\) (w/v) and \(\eta_{sp0} > 10\text{ mPa.s}\):

$$\eta_{sp0} \propto (c[\eta])^{3.9},$$

(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 \(\beta\)-glucan was found to be (M. U. Beer, unpublished results):

$$[\eta] = 7.3 \times 10^{-4} (M_w)^{0.72}.$$  

(7)

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

$$\Delta G = A' + B' \log_{10}(cM_w^{0.72}).$$

(8)

The values found empirically for \(A'\) and \(B'\), by fitting \(\Delta G\)
in terms of $\log_{10}(c \times M_w^{0.72})$, are $6.27$ and $-0.99$ respectively ($R^2 = 0.973, P = 0.0293$). However, viscosity was determined, not at zero shear rate but at $30 \text{ s}^{-1}$, 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:

$$\Delta G = A' + B' \log_{10}(c) + 0.72 B' \log_{10} M_w,$$

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 $30 \text{ s}^{-1}$ 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


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