GIP concentrations similar to the maximum values observed in actively feeding ob/ob mice (Flatt et al., 1983a). Plasma GIP concentrations declined quickly indicating a relatively short half-life of the hormone in ob/ob mice.

Fig. 1b shows the plasma insulin responses to endogenous GIP released by oral administration of fat. A substantial increase in plasma GIP concentrations was achieved similar to values in fed ob/ob mice. In mice treated with saline at 30 min, basal plasma glucose concentrations (not illustrated) were maintained within the range 7-10 mmol/l throughout. In these mice there was only a very small increase in plasma insulin concentrations. However, mice receiving an intraperitoneal injection of glucose at 30 min showed a rise in plasma glucose concentrations exceeding 30 mmol/l. These mice exhibited a marked insulin response, which was much greater than the response to glucose alone (Flatt & Bailey, 1981b).

The results demonstrate an insulinotropic effect of both exogenous and endogenous GIP at physiological concentrations in ob/ob mice. This effect is dependent on raised plasma glucose concentrations. Thus it is envisaged that elevated plasma GIP concentrations in the presence of post-prandial hyperglycaemia make an important contribution to the hyperinsulinaemia of ob/ob mice.


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**Short-term effects of wheat bran supplementation on glucose and lipid metabolism in man**

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Extensive studies in man and experimental animals have demonstrated that the addition of certain plant fibres to the diet is accompanied by a significant decrease in plasma cholesterol, triglyceride and glucose concentrations (Kay & Truswell, 1977; Jenkins et al., 1978; Anderson & Chen, 1979). Several water-soluble fibres such as pectin and guar gum have hypocholesterolaemic and hypoglycaemic effects (Jenkins et al., 1977) but the effects of the water-insoluble fibre wheat bran have been variable in this respect (Chen & Anderson, 1979). Muclaginous fibres, although effective modulators of glucose and lipid metabolism, are of limited palatability. The present study was therefore undertaken to clarify the possible short-term effects of dietary wheat bran supplementation on lipid and glucose metabolism in man.

Seven healthy University students (three males and four females, aged 18–22 years) volunteered to take part in this study. None of the subjects smoked or received any form of medication. Each volunteer's normal diet was supplemented with 0.3 g of unprocessed wheat bran/kg body weight per day for 6 weeks. At the onset, and after 5 weeks of wheat bran supplementation, 4-day semi-quantitative records of dietary intake were made to determine if the consumption of wheat bran had altered the diet. Fasting blood samples were taken before and after 6 weeks after the addition of wheat bran to the diet. A 0.5 ml aliquot of whole blood was used for glycosylated haemoglobin determination (Kynoch & Lehmann, 1977). Aliquots of plasma were used for the determination of cholesterol (Rude1 et al., 1972), triglycerides (Soloni, 1971) and glucose concentrations (Stevens, 1971). An increase in dietary fibre consumption by 34% was associated with only minor changes in the intake of protein, fat, carbohydrate, alcohol and cholesterol. As shown in Table 1, there were no significant changes in plasma cholesterol, triglyceride or glucose concentrations after 6 weeks of wheat bran supplementation. Plasma HDL-cholesterol was significantly increased while both LDL-cholesterol concentrations and glycosylated haemoglobin were significantly decreased. The lack of effect of wheat bran supplementation on plasma cholesterol, triglyceride and glucose concentrations is in agreement with previous studies (Jenkins et al., 1975; Truswell & Kay, 1976). However, the increase in plasma HDL-cholesterol after 6 weeks is interesting with regard to the suggestion that this

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**Table 1. Short-term effects of wheat bran supplementation on glucose and lipid metabolism in man**

<table>
<thead>
<tr>
<th>Duration of wheat bran supplementation</th>
<th>0 weeks</th>
<th>6 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma total cholesterol (mg/100ml)</td>
<td>186 ± 17</td>
<td>193 ±11</td>
</tr>
<tr>
<td>Plasma HDL-cholesterol (mg/100ml)</td>
<td>72 ± 5</td>
<td>105 ± 7*</td>
</tr>
<tr>
<td>Plasma LDL-cholesterol (mg/100ml)</td>
<td>85 ± 7</td>
<td>64 ± 6*</td>
</tr>
<tr>
<td>Plasma total triglyceride (mg/100ml)</td>
<td>73 ± 9</td>
<td>53 ± 7</td>
</tr>
<tr>
<td>Plasma glucose (mg/100ml)</td>
<td>89 ± 3</td>
<td>96 ± 4</td>
</tr>
<tr>
<td>Glycosylated haemoglobin (%)</td>
<td>11.0 ± 0.6</td>
<td>6.0 ± 0.2**</td>
</tr>
</tbody>
</table>

Values are the means ± S.E.M. for seven subjects. Statistical significance was evaluated by Student's paired t-test: *P < 0.05, **P < 0.001 compared with the value before wheat bran supplementation.

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Abbreviations used: HDL, high-density lipoprotein; LDL, low-density lipoprotein.
fraction may protect against atherosclerosis. The concurrent decrease in plasma LDL-cholesterol might also be beneficial in relation to its possible atherogenic action (Kannel et al., 1979). The mechanism by which wheat bran affects HDL- and LDL-cholesterol is unknown. The decrease in glycosylated haemoglobin after 6 weeks of wheat bran supplementation might be related to changes in the rate of gastric emptying and intestinal transit leading to retardation of absorption, and the lowering of postprandial glucose peaks. The present study has demonstrated that wheat bran supplementation of the normal diet has beneficial short-term effects on various aspects of lipid and glucose metabolism. This illustrates the possible role of wheat bran supplementation in the promotion of health and treatment of certain metabolic diseases in man.


Studies of chick kidney alkaline phosphatase

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The proximal tubule of the kidney is the major site at which phosphatase is reabsorbed from the glomerular filtrate. We are studying phosphate transport into chick-kidney tubules and it became important for us to characterize the alkaline phosphatase that is associated with the brush-border membrane of these cells. Although mammalian phosphatases have received wide study, the properties of avian enzymes are less well documented (see Chang & Moog, 1972, however).

Kidney tissue (approx. 5g) from 3-week-old chicks was homogenized in 35cm³ of 0.01m-Tris/HCl buffer, pH 7.6, containing 1mM-Mg²⁺ and 10μM-Zn²⁺ (buffer A). The homogenate was stirred with 20cm³ of n-butanol for 30min to release alkaline phosphatase. Chromatography was performed on DEAE-cellulose equilibrated with buffer A, and the proteins were eluted with a stepped gradient of 0.1–1m-NaCl dissolved in the same buffer. Fractions containing high alkaline phosphatase activity were concentrated and subjected to gel filtration on Sephadex G200. Pooled fractions from the filtration were subjected to triazine-dye affinity chromatography on an agarose-red column. The concentration of the elution buffer was increased stepwise from 0 to 1m-KCl dissolved in buffer A. A total purification of 83-fold was obtained to give a specific activity of 3.4nmol/min per mg of protein.

The M₀ and subunit nature of the enzyme was investigated by electrophoresis in SDS/polyacrylamide gel (Saini & Done, 1970). Pretreatment of the alkaline phosphatase with dodecyl sulphate plus β-mercaptoethanol at 100°C results in dissociation into monomers accompanied by a total loss of activity. The catalytic activity is unimpaired, however, after treatment with the detergent alone at 30°C. Enzymic activity was detected on gels by the method of Ramasamy & Butterworth (1974) and protein was stained by an ultraviolet-sensitive method employing silver nitrate (Oakley et al., 1980). Inactive subunits were detected by prelabeling the enzyme with [³²P]P, before denaturation and dissociation (Malik & Butterworth, 1976). The molecular weight of the oligomeric enzyme was also determined by gel-permeation chromatography and sucrose-density-gradient centrifugation (Malik & Butterworth, 1976).

The results obtained in these studies indicate that the enzyme is composed of identical subunits of 68000-M₀, and that catalytic activity resides in both dimeric and tetrameric forms of the phosphatase (Table 1). The tetrameric form probably represents the native state of the alkaline phosphatase since the active dimer only appears after detergent treatment.

Chick-kidney alkaline phosphatase resembles the mammalian enzyme in that its activity is greatly stimulated by Mg⁺⁺ ions (Ahlers, 1974). Zn⁺⁺ ions are also essential for catalytic activity, and in its sensitivity to inhibition by levamisole, it again resembles mammalian kidney phosphatase (Van Belle, 1972).

In an investigation of the catalytic importance of histidine residues, the enzyme was incubated at 20°C with

<table>
<thead>
<tr>
<th>Method of study</th>
<th>Size of main component</th>
<th>Catalytic status</th>
<th>Presumed state of aggregation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose-density-gradient centrifugation</td>
<td>260000</td>
<td>Active</td>
<td>Tetrameric</td>
</tr>
<tr>
<td>Gel-permeation chromatography</td>
<td>&gt; 200000</td>
<td>Active</td>
<td>Tetrameric</td>
</tr>
<tr>
<td>SDS/polyacrylamide electrophoresis</td>
<td>150000</td>
<td>Active</td>
<td>Dimeric</td>
</tr>
<tr>
<td>SDS/polyacrylamide electrophoresis after treatment with SDS and β-mercaptoethanol</td>
<td>68000</td>
<td>Inactive</td>
<td>Monomeric</td>
</tr>
</tbody>
</table>

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