Whey protein isolate and glycomacropeptide decrease weight gain and alter body composition in male Wistar rats

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The effect of feed protein type on body composition and growth has been examined. Evidence exists that whey protein concentrate is effective at limiting body fat expansion. The presence of caseinomacropeptide, a mixture of glycosylated and non-glycosylated carbohydrate residues, in particular glycomacropeptide (GMP) in whey protein concentrate may be important for this effect. The influence of whey protein isolate (WPI) and GMP on weight gain and body composition was examined by feeding Wistar rats ad libitum for 7 weeks with five semi-purified American Institute of Nutrition-based diets differing in protein type: (1) casein; (2) barbequed beef; (3) control WPI (no GMP); (4) WPI + GMP at 100 g/kg; (5) WPI + GMP at 200 g/kg. Body composition was assessed, and plasma samples were assayed for TAG, insulin and glucose. Body-weight gain was lower (−21%) on the control WPI diet relative to casein, with a non-significant influence associated with GMP inclusion (−30%), the effect being equivalent at both levels of GMP addition. Renal and carcass fat mass were reduced in the highest GMP diet when compared with WPI (P<0.05). Plasma insulin was lowered by GMP at the highest addition compared with WPI alone (−53%; P<0.01). Plasma TAG in the WPI + GMP (200 g/kg) group were lower (−27%; P<0.05) than the casein and beef groups. In conclusion, GMP appears to have a significant additional influence when combined with WPI on fat accumulation. WPI alone appears to have the predominant influence accounting for 70% of the overall effect on body-weight gain. Mechanisms for this effect have not been identified but food intake was not responsible.

Glycomacropeptide: Whey protein isolate: Weight gain: Insulin

Diets that are high in protein at the expense of carbohydrate have been shown to be beneficial in weight and body fat loss in human subjects. Human studies have shown that on an energy basis, protein is more satiating than other macronutrients. The nature of the protein source may also be relevant, although this has not been well studied. Research has shown in laboratory rats fed a high-protein diet (300 g/kg) that whey protein concentrate was significantly more effective than red meat in reducing body-weight gain and body fat content, when energy intakes were comparable. In human short-term studies whey protein has been shown to enhance satiety and decrease food intake relative to casein.

Glycomacropeptide (GMP), the glycosylated fraction of caseinomacropeptide is a C-terminal fragment of κ casein released by endopeptidase chymosin (rennin) and is present in whey from cheese manufacture at concentrations in the order of 600 mg/l and this may have been responsible for the whey protein concentrate effect reported in rats by Belobrajdic et al. GMP has been shown to stimulate cholecystokinin (CCK) release, thereby potentially having an influence on satiety and food intake. GMP has been detected in the plasma of volunteers after milk or yoghurt ingestion, suggesting that GMP may be produced in the gut before being absorbed into the circulation via intestinal cells.

The aim of the present study was to determine if a GMP-enriched whey protein isolate (WPI) has a significant effect on weight gain, growth rate and body composition, when compared with WPI without GMP, casein and barbequed (BBQ) beef. It was hypothesised that feeding NatraPep, a commercial source of GMP-rich WPI, would decrease both food intake and gain of abdominal and subcutaneous fat.

Experimental methods adopted

Animals

Fifty male Wistar rats, age 12 weeks, were obtained from the Animal Resource Centre (Murdoch University, Western Australia, Australia).

Upon arrival the animals were housed in wire cages to minimise coprophagy and were maintained in an air-conditioned environment of 23 ± 2°C with a 12 h light–12 h dark cycle. Rats were given standard rat chow and water ad libitum for 48 h to acclimatise to their new environment. Once established, all animals were randomised and sorted into five (n 10) treatment groups of equal body weight. Each group was then placed onto one of the five American Institute of Nutrition (AIN)-93-based experimental diets (see Table 1) for the duration of the 7-week study. All animals were weighed weekly and food intakes measured twice weekly.

At the conclusion of the study the rats were fasted overnight (16 h) and euthanased using 4% fluothane in oxygen

Abbreviations: AIN, American Institute of Nutrition; BBQ, barbequed; CCK, cholecystokinin; GMP, glycomacropeptide; WPI, whey protein isolate.

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The three dairy proteins used were produced by Murray Goulburn Nutritional (Brunswick, Victoria, Australia). WPI was prepared from skimmed milk by membrane separation and dialysis providing a caseinomacropeptide- and GMP-free product, NatraPep, protein source, NatroPro dialysis providing a caseinomacropeptide- and GMP-free product prepared from skimmed milk by membrane separation and dialysis providing a caseinomacropeptide- and GMP-free product, NatraPep, provided by either NatraPro™ (WPI), NatraPep™ (GMP-rich WPI), casein or BBQ beef. The varied additions of the final diets can be seen in Table 1.

Table 1. Detailed diet compositions of the five treatment groups

<table>
<thead>
<tr>
<th>Ingredient (g/kg diet)</th>
<th>Casein*</th>
<th>BBQ beef†</th>
<th>Control WPI‡</th>
<th>WPI + GMP at 100 g/kg§</th>
<th>WPI + GMP at 200 g/kg$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein</td>
<td>300</td>
<td>300</td>
<td>300</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>Sucrose</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>Maize starch</td>
<td>253</td>
<td>253</td>
<td>253</td>
<td>253</td>
<td>253</td>
</tr>
<tr>
<td>α-Cellulose</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Sunflower-seed oil</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>AIN-93 mineral mix</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>AIN-93 vitamin mix</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Energy content (kJ/g)</td>
<td>18-1</td>
<td>16-0</td>
<td>18-0</td>
<td>17-7</td>
<td>18-3</td>
</tr>
</tbody>
</table>

BBQ, barbecue; WPI, whey protein isolate; GMP, glycomacropeptide.

* Casein: 850 g protein/kg, 12 g fat/kg, 0.13 g Ca/kg, 67 g moisture/kg, 0.2 g K/kg, 0.005 g Na/kg and 30 g ash/kg.
† BBQ beef: 700 g protein/kg, 250 g fat/kg, 0.12 g Ca/kg, 30 g moisture/kg, 9.2 g K/kg, 1.4 g Na/kg, 0.7 g Mg/kg and 10.2 g ash/kg.
‡ WPI: 924 g protein/kg, 11 g fat/kg, 1.3 g Ca/kg, 32 g moisture/kg, 8.1 g K/kg, 5.4 g Na/kg, 0.12 g Mg/kg and 35 g ash/kg.
§ NatraPro™ (WPI): 924 g protein/kg, 11 g fat/kg, 1.3 g Ca/kg, 32 g moisture/kg, 8.1 g K/kg, 5.4 g Na/kg, 0.12 g Mg/kg and 35 g ash/kg.
$ NatraPep™ (GMP-rich WPI): 765 g protein/kg, 676 g GMP/kg, 3 g fat/kg, 0.02 g Ca/kg, 43 g moisture/kg, 0.003 g K/kg, 41.2 g Na/kg, 0.0002 g Mg/kg and 100 g ash/kg.

The BBQ beef endproduct was derived by browning the meat on a barbecue hotplate before being dried at 40°C to a powdered consistency, containing 700 g protein/kg, 250 g fat/kg, 0.12 g Ca/kg, 9.2 g K/kg, 1.4 g Na/kg, 0.7 g Mg/kg and 10.2 g ash/kg.

Protein sources

The three dairy proteins used were produced by Murray Goulburn Nutritional (Brunswick, Victoria, Australia). WPI was prepared from skimmed milk by membrane separation and dialysis providing a caseinomacropeptide- and GMP-free protein source, NatraPro™ (WPI), that contained 924 g protein/kg, 0 g GMP/kg, 11 g fat/kg, 1.3 g Ca/kg, 8.1 g K/kg, 5.4 g Na/kg, 0.12 g Mg/kg and 35 g ash/kg. The BBQ beef endproduct was derived by browning the meat on a barbecue hotplate before being dried at 40°C to a powdered consistency, containing 700 g protein/kg, 250 g fat/kg, 0.12 g Ca/kg, 9.2 g K/kg, 1.4 g Na/kg, 0.7 g Mg/kg and 10.2 g ash/kg.

The analyses. Amino acid analysis of the samples was undertaken by first hydrolising the protein in the samples with 6 M-hydrochloric acid for 24 h to free the amino acids. After hydrolysis the solution was diluted and filtered and the hydrochloric acid was removed under reduced pressure. All amino acids except methionine, cystine and tryptophan, which are partially destroyed in 6M-hydrochloric acid, were hydrolysed with the above procedure. The levels of the hydrolysed amino acids were then determined by first separating them using cation exchange chromatography followed by post-column derivatisation using ninhydrin before spectrophotometric quantification against known standards.

Sulfur amino acids (methionine and cystine). Methionine and cystine plus cysteine were pre-oxidised to methionine sulfoxide and cystic acid respectively with formic acid at 0°C for 16 h followed by 6M-hydrochloric acid hydrolysis. The levels of the sulfur amino acids were then determined using cation exchange chromatography followed by post-column derivatisation using ninhydrin.

Tryptophan. Since tryptophan is destroyed during acid hydrolysis, alkaline hydrolysis is conducted using barium hydroxide with 5-methyl tryptophan as an internal standard. After hydrolysis, free tryptophan was analysed using a reverse-phase column with UV detection at 280 nm.

Dietary sources

Whey protein isolate and body-weight gain

Briefly, the NatraPro™ (WPI), NatraPep™ (GMP-rich WPI) and casein powdered product samples were sub-sampled for the analyses. Amino acid analysis of the samples was undertaken by first hydrolising the protein in the samples with 6 M-hydrochloric acid for 24 h to free the amino acids. After hydrolysis the solution was diluted and filtered and the hydrochloric acid was removed under reduced pressure. All amino acids except methionine, cystine and tryptophan, which are partially destroyed in 6 M-hydrochloric acid, were hydrolysed with the above procedure. The levels of the hydrolysed amino acids were then determined by first separating them using cation exchange chromatography followed by post-column derivatisation using ninhydrin before spectrophotometric quantification against known standards.

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Diet details

All diets were based on a balanced modification of the AIN-93 diet formulation. The experimental diets are described briefly as follows. All diets were balanced to contain 300 g protein/kg, provided by either NatraPro™ (WPI), NatraPep™ (GMP-rich WPI), casein or BBQ beef. The varied additions of GMP-rich WPI to WPI provided a dose response: 0, 100 and 200 g/kg levels of GMP. All other macronutrients remained identical between experimental groups. The protein density of all diets was adjusted using carbohydrate with a 3:2 ratio of maize starch:sugar. The fat content of all diets was adjusted to be 200 g/kg diet. The majority of the dietary fat was provided by sunflower-seed oil with the balance provided endogenously by the protein source. Minerals provided by the different protein sources were balanced across all diets to provide a constant level, as described in AIN-93. Each diet provided an equivalent amount of Ca (5 g/kg) as for the AIN-93 diet. Similarly, diets were designed to be isonitrogenous. A detailed composition of the final diets can be seen in Table 1.
Carcass analysis

Each animal carcass was freeze-dried over 72 h to remove all moisture before being finely ground to obtain a consistent sample for fat and protein analysis on a DM basis. Carcass and liver fat values were determined gravimetrically, following extraction with chloroform–methanol–water (2:1:1, by vol.) as described by Folch et al.\(^\text{(13)}\). Carcass protein was quantified by the Dumas method of Kirsten & Hesselius\(^\text{(14)}\) using a Carlo Erba NA1500 nitrogen analyser (Milan, Italy).

Blood chemistry and hormone analysis

Overnight fasted plasma was isolated from whole blood by centrifugation at 2000 g for 10 min at 5°C (Beckman GS-6R centrifuge; Backman Coulter, Inc., Fullerton, CA, USA) and frozen at −20°C until analysis.

Plasma glucose and TAG concentrations were determined using enzymic kits (Roche Diagnostics, Basel, Switzerland) on a Hitachi 902 auto-analyser (Roche Diagnostics, Corp., Indianapolis, IN, USA).

Plasma insulin concentrations were determined using high-sensitivity rat-specific ELISA kits (ALPCO Diagnostics, Windham, NH, USA).

Statistical analysis

Statistical analysis was carried out with Statistical Package for the Social Sciences for Windows version 11.5.0 standard statistical software (SPSS, Inc., Chicago, IL, USA). Data are mean values and standard deviations, unless otherwise specified. Procedures used included one-way ANOVA with Tukey’s post hoc testing and correlation analysis between the variables GMP and plasma insulin. Differences were considered to be significant at \(P<0.05\).

Results

Protein source: amino acid analysis

The two WPI products, NatroPro\(^\text{TM}\) (WPI) and NatraPep\(^\text{TM}\) (GMP-rich WPI) plus casein were analysed for their amino acid profile as shown in Table 2. The GMP-rich WPI product was found to have noticeably lower concentrations of amino acids relative to WPI: leucine (down 66%), tyrosine (down 72%), phenylalanine (down 47%), lysine (down 49%), arginine (down 66%), tryptophan (down 72%), and histidine (down 57%).

There was no significant difference between groups in food intake and final body weight. There was a significant reduction in body-weight gain (\(P<0.05\)) with GMP-fed animals compared with casein- and BBQ beef-fed animals. There was also a significant difference between the WPI-fed animals when compared with casein-fed animals (\(P<0.01\)). There was no significant difference between WPI- and GMP-rich WPI.

Body composition

There were significant decreases in the perirenal fat deposition (Fig. 2) in the animals fed both levels of GMP compared with the casein-fed animals (\(P<0.05\)). Similarly, there were significant decreases in the testicular and abdominal fat mass when the WPI + GMP (200 g/kg)-fed animals were compared with the casein-fed animals (\(P<0.05\)). Perirenal fat weight and carcass fat was significantly lower (\(P<0.05\)) in the WPI + GMP (200 g/kg)-fed animals relative to the control WPI rats.

<table>
<thead>
<tr>
<th>Amino acid (g/100 g product)</th>
<th>NatroPro(^\text{TM}) (WPI)</th>
<th>NatraPep(^\text{TM}) (GMP-rich WPI)</th>
<th>Casein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threonine†</td>
<td>4.93</td>
<td>11.09</td>
<td>4.07</td>
</tr>
<tr>
<td>Valine†</td>
<td>5.31</td>
<td>6.28</td>
<td>6.38</td>
</tr>
<tr>
<td>Isoleucine†</td>
<td>5.61</td>
<td>7.71</td>
<td>4.81</td>
</tr>
<tr>
<td>Leucine†</td>
<td>12.79</td>
<td>4.21</td>
<td>8.67</td>
</tr>
<tr>
<td>Valine†</td>
<td>5.31</td>
<td>6.28</td>
<td>6.38</td>
</tr>
<tr>
<td>Isoleucine†</td>
<td>5.61</td>
<td>7.71</td>
<td>4.81</td>
</tr>
<tr>
<td>Leucine†</td>
<td>12.79</td>
<td>4.21</td>
<td>8.67</td>
</tr>
<tr>
<td>Phenylalanine†</td>
<td>3.42</td>
<td>1.82</td>
<td>4.69</td>
</tr>
<tr>
<td>Lysine†</td>
<td>10.23</td>
<td>6.12</td>
<td>7.32</td>
</tr>
<tr>
<td>Histidine†</td>
<td>2.01</td>
<td>0.86</td>
<td>2.98</td>
</tr>
<tr>
<td>Tryptophan†</td>
<td>1.65</td>
<td>0.44</td>
<td>1.07</td>
</tr>
<tr>
<td>Methionine†</td>
<td>2.35</td>
<td>1.20</td>
<td>2.62</td>
</tr>
<tr>
<td>Serine</td>
<td>4.16</td>
<td>6.13</td>
<td>5.74</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>17.59</td>
<td>15.96</td>
<td>19.83</td>
</tr>
<tr>
<td>Proline</td>
<td>4.91</td>
<td>8.22</td>
<td>9.96</td>
</tr>
<tr>
<td>Glycine</td>
<td>1.48</td>
<td>1.04</td>
<td>1.72</td>
</tr>
<tr>
<td>Alanine</td>
<td>5.50</td>
<td>4.43</td>
<td>2.74</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>10.43</td>
<td>7.82</td>
<td>6.42</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>3.43</td>
<td>0.96</td>
<td>5.05</td>
</tr>
<tr>
<td>Arginine</td>
<td>2.75</td>
<td>0.94</td>
<td>3.62</td>
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<tr>
<td>Cysteine</td>
<td>2.5</td>
<td>0.65</td>
<td>0.29</td>
</tr>
<tr>
<td>Ammonia</td>
<td>1.99</td>
<td>1.84</td>
<td>2.42</td>
</tr>
</tbody>
</table>

WPI, whey protein isolate; GMP, glycomacropeptide.

* For details of diets, see Table 1.
† Essential amino acids.

\begin{figure}[ht]
\centering
\includegraphics[width=\textwidth]{growth曲线.png}
\caption{Growth rate of animals over the 7-week feeding period. Values are means for ten animals per treatment group. (■), Control whey protein isolate (WPI); (×), WPI + glycomacropeptide (GMP) at 100 g/kg; (●), WPI + GMP at 200 g/kg; (▲), casein; (○), barbequed beef.}
\end{figure}
Plasma biochemistry

Biochemical and compositional data are shown in Table 3. Plasma TAG concentrations of the animals fed WPI + GMP at 200 g/kg were significantly lower (P, 0.05) than the casein- and BBQ beef-fed animals. Plasma insulin concentrations of the animals fed both levels of GMP were significantly lower (P, 0.01) than the control WPI-fed animals although WPI elevated plasma insulin compared with casein. A significant inverse relationship (P, 0.001) between plasma insulin and dietary levels of GMP with the WPI groups is shown in Fig. 3.

**Plasma biochemistry**

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**Discussion**

The overall body-weight gain of WPI plus GMP-fed animals was 30% lower than the casein-fed animals, but WPI alone also showed a significant (P<0.01) reduction in body-weight gain when compared with casein-fed animals. This finding supports the work of Belobrajdic et al. (4) where a diet high in whey protein decreased weight gain in comparison with meat. The present study showed nevertheless that GMP offers a small additional (non-significant) benefit in weight control when compared directly with WPI alone. GMP offered an advantage in terms of decreased abdominal fat, particularly renal fat, and carcass fat compared with WPI alone. It was hypothesised that feeding GMP-rich WPI would, through increased CCK production by the endocrine cells of the small intestine(15), have an effect on satiety and reduce food intake(16–18), thereby decreasing weight gain and reducing abdominal and subcutaneous fat deposition. This satiety hypothesis was not supported, however, as there was no difference between the food...
intakes of the rats between the dietary treatments. Plasma CCK was not measured in the course of the experiment but would possibly provide further information as to the efficacy of GMP to stimulate CCK production in this rat model.

The fact that casein, which is potentially a low level source of endogenously generated GMP\(^{(11)}\), provided the highest weight gain in the casein-fed animals suggests that GMP is probably not being released to any significant degree during casein digestion, or has no effect on food intake\(^{(19)}\). Any effect of high dietary Ca on body-weight gain or composition\(^{(20,21)}\) can be excluded as all diets were balanced for Ca.

In the present study the addition of GMP led to a lowering of fasting plasma insulin concentrations relative to WPI alone. A significantly lower plasma insulin concentration (down 64%; \(P<0.01\)) was observed between the GMP (200 g/kg) and the WPI-alone-fed animals. The relationship of insulin to weight gain in the present study is not clear, as the insulin level in the casein-fed group was not significantly different from that of the GMP (200 g/kg) group, despite considerable differences in some fat depots in the animals. This result differs from previous studies\(^{(4)}\), where lowered fasting plasma insulin concentrations were observed with whey protein concentrate relative to red meat at high protein intakes. This probably reflects the lower fat mass in the whey protein concentrate-fed animals although it cannot be excluded that a minor protein in the WPI fraction (but not in the GMP-enriched protein) elevated insulin levels, even in the fasting state. The elevated insulin with WPI may not necessarily reflect increased insulin resistance as although glucose is higher in all WPI groups than the casein group (non-significant) TAG levels are not elevated.

The amino acid values for the WPI (control), GMP-rich WPI products and casein used in the present study are provided in Table 2. These show low values for GMP-rich WPI in a number of amino acids compared with WPI and casein. As a consequence, dietary methionine could be considered marginal at 0.54 g/kg diet offered to the animals when the highest level (200 g/kg) of GMP was fed. This may have had some consequences for the rats on this diet, potentially diminishing methylating status and influencing liver fat accumulation\(^{(22)}\), although there were no differences shown in the liver weights, the amount of fat in the liver or the final body weights of the animals between the control WPI- and both GMP-fed groups. The calculated level of methionine in the 200 g GMP/kg group was adequate for maintenance of laboratory rats (0.23 g/kg)\(^{(23)}\). Fundamental research done by Archer et al.\(^{(24)}\) has shown that large differences in the energy concentration of diets can alter weight-gain outcomes in male Sprague–Dawley rats. In the present study a small difference was seen in the energy concentrations of the study diets, particularly between the WPI + 200 g GMP/kg and BBQ beef groups (18.3 and 16.0 kJ/g respectively). The difference in energy concentration on weight gain can been ruled out as the significant reduction seen in body-weight gain was between the GMP-fed animals (average 18.0 kJ/g) and the casein- (18.1 kJ/g) and BBQ-beef-fed animals (16.0 kJ/g), suggesting no influence of energy concentration on weight-gain outcomes in the present study.

The ability of some dairy foods to impact beneficially on weight control in both animal and human studies has been noted in some but not all studies\(^{(25,26)}\). Similarly the effect of CCK on satiety and food intake has been confirmed in some but not all human studies\(^{(9,10,27)}\). Until recently the research focus has been on the relationship between dairy Ca\(^{(28,29)}\) and satiety and weight loss and dairy fat\(^{(30)}\) and CCK levels. The nutritionally functional properties of dairy products are now being more closely examined and it has been reported that Ca associated with dairy foods has a greater effect on adiposity than Ca alone\(^{(28,29)}\). There have been few well-designed studies examining the ability of dairy peptides to suppress appetite. A recent study by Gustafsson et al.\(^{(31)}\) failed to show any effect on satiety when the milk protein caseinomacropeptide was fed as a solution to human subjects. The highest level fed was ten-fold lower than the maximum offered in the present study and was not offered as part of a dairy- or whey-based meal. A number of other functions relating to health are attributable to caseinomacropeptide and have been reviewed by Thomä–Worringer et al.\(^{(32)}\). These deserve more investigation with regard to their relevance in a dietary context.

It has been suggested that whey proteins and dairy Ca contribute to limiting body fat accumulation and offer advantages in maintaining optimal body composition\(^{(33)}\). Further work in human subjects is needed to elucidate the influence of GMP-rich WPI and WPI on body weight and body fat. In conclusion, GMP-rich WPI appears to have a significant additional influence on the effect of WPI alone on fat accumulation. However, in the present study WPI appears to be the predominant influence, accounting for 70% of the effect on body-weight gain observed.

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and interpretation and draft manuscript editing. P. M. C. contributed to the data analysis and interpretation, draft manuscript editing and liaison with the commercial partner. This research was funded by CSIRO Human Nutrition and in part by Murray Goulburn Co-operative Co. Ltd. None of the authors had a personal or financial conflict of interest.

References


