

## Review Article

# The nutraceutical role of the *Phaseolus vulgaris* $\alpha$ -amylase inhibitor

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(Received 14 May 2007 – Revised 28 September 2007 – Accepted 27 October 2007 – First published online 11 March 2008)

The present review assesses the potential of the *Phaseolus vulgaris*  $\alpha$ -amylase inhibitor isoform 1 ( $\alpha$ -AI1) starch blockers as a widely used remedy against obesity and diabetes. Consumption of the  $\alpha$ -amylase inhibitor causes marginal intraluminal  $\alpha$ -amylase activity facilitated by the inhibitor's appropriate structural, physico-chemical and functional properties. As a result there is decreased postprandial plasma hyperglycaemia and insulin levels, increased resistance of starch to digestion and increased activity of colorectal bacteria. The efficacy and safety of the amylase inhibitor extracts, however, depend on the processing and extraction techniques used. The extracts are potential ingredients in foods for increased carbohydrate tolerance in diabetics, decreased energy intake for reducing obesity and for increased resistant starch. Research developments in the distribution and biosynthesis of the  $\alpha$ -amylase inhibitor, relevant physico-chemical properties, the molecular starch-blocking mechanism, anti-obesity and anti-diabetes effects, safety of extracts and the need for research into their potential anti-colorectal cancer effect are discussed.

**$\alpha$ -Amylase inhibitor: Common beans (*Phaseolus vulgaris*): Diabetes: Hyperglycaemia: Obesity: Toxicity**

Common beans (*Phaseolus vulgaris* L.) are among the world's grain legumes most used for direct human consumption<sup>(1)</sup>. The common bean  $\alpha$ -amylase inhibitor isoform 1 ( $\alpha$ -AI1), one of their non-nutritive bioactive factors<sup>(2)</sup>, discovered in 1945 by Bowman<sup>(3)</sup>, has been extracted and used in several commercial anti-obesity and anti-diabetes products referred to as starch blockers. A starch blocker is a substance that interferes with the breakdown of complex carbohydrate leading to a reduced digestibility or prolonged digestion such that energy derived from the carbohydrate is reduced or the rate of body absorption of the energy in form of glucose is reduced<sup>(4)</sup>.

In the 1980s, use of the starch blockers from common beans to control obesity and diabetes was a research issue, but it has presently re-emerged with efforts being taken for its consideration as 'generally regarded as safe'<sup>(5)</sup>. Detailed investigations revealed that many of the commercially available amylase inhibitor extracts (starch blockers) failed to influence starch digestion due to low  $\alpha$ -amylase inhibition activity in humans<sup>(6,7)</sup>. Recent developments, however, with improved extraction methods such as supercritical carbon dioxide extraction, fractionation and heat treatment<sup>(8)</sup> have led to demonstrable efficacy of the starch blockers in humans. Despite some contrary reports, the starch blockers from common

beans have been demonstrated to at least cause subtle weight loss, which has been shown to have advantages relative to drastic weight loss<sup>(9)</sup>. On the other hand, extensive research has shown that obesity is on the increase worldwide and predisposes individuals directly or indirectly to diabetes mellitus and various forms of cancer<sup>(10–13)</sup>.

The common bean  $\alpha$ -amylase inhibitor extracts are legally more acceptable based on the *de minimis* concept<sup>(14)</sup> than new synthetic pharmaceutical products and recently some patents have been documented on their effective extraction<sup>(8)</sup>. Safety and efficacy of such dietary supplements, however, are of critical importance since regulatory authorities such as the United States Food and Drug Administration consider them as conventional foods and manufacturers do not need to register and get product approval<sup>(15)</sup>. Although there have been advancements in the several aspects of the  $\alpha$ -amylase inhibitor from common beans, few attempts have been made to summarise and integrate them from a nutritional point of view. In response, the present paper assesses the potential of the *P. vulgaris*  $\alpha$ -amylase inhibitor as an extensive remedy against obesity and diabetes based on research developments in its distribution, relevant physico-chemical properties, starch-blocking mechanism, evidence of beneficial effects and its safety.

**Abbreviations:**  $\alpha$ -AI1,  $\alpha$ -amylase inhibitor isoform 1;  $\alpha$ -AI2,  $\alpha$ -amylase inhibitor isoform 2;  $\alpha$ -AIL,  $\alpha$ -amylase inhibitor like; PHA, phytohaemagglutinin; PPA, porcine pancreatic amylase.

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### Distribution and biosynthesis of the *Phaseolus vulgaris* $\alpha$ -amylase inhibitor

Natural  $\alpha$ -amylase inhibitors have been extracted from various sources. The *P. vulgaris*  $\alpha$ -amylase inhibitor, however, has relatively wide potential as an extensive anti-obesity and anti-diabetes remedy because common beans are grown widely in the world<sup>(16)</sup>; the pure form has not been associated with deleterious effects such as asthma and dermatitis which have been associated with some cereal amylase inhibitors<sup>(17–19)</sup>, and it has unfunctionality relative to other potential inhibitors which are bifunctional<sup>(20)</sup>.

Although common beans have three isoforms of  $\alpha$ -amylase inhibitor (isoform 1 ( $\alpha$ -AI1); isoform 2 ( $\alpha$ -AI2);  $\alpha$ -amylase inhibitor like ( $\alpha$ -AIL)), the  $\alpha$ -AI1 isoform with anti-amylase activity in humans is the most widely distributed of the isoforms and is found in most of the common bean accessions grown worldwide<sup>(21–24)</sup>. This makes efforts of extraction from any part of the world possible and, in addition, common beans are adapted to different ecological environments<sup>(1)</sup>.

In the bean plant,  $\alpha$ -AI1 is only found in the seeds and is concentrated in the axis<sup>(25)</sup>. It is three times more concentrated in the axis than in the cotyledon. Apparently this is because there is more efficient glycosylation in the axis relative to the cotyledon. There is no  $\alpha$ -AI1 in other organs of the plant<sup>(25)</sup>. According to Moreno & Chrispeels<sup>(26)</sup>,  $\alpha$ -AI1 accumulates in seeds to make up about 9–11% of the total seed protein. This percentage can provide a substantial yield of the inhibitor from a given amount of common beans although the extraction method may limit the yield.

Synthesis of  $\alpha$ -AI1 occurs at the same time as that of phaseolin and phytohaemagglutinin (PHA) and also it accumulates in the protein storage vacuoles<sup>(25)</sup>. The  $\alpha$ -AI1 is a typical bean lectin, which is synthesised in the rough endoplasmic reticulum, modified in the Golgi body through removal of a signal peptide and *N*-glycosylation, and transported to the protein storage vacuoles where it is proteolytically processed. SDS-PAGE, used for microsomal fractions, shows that Mr 30 000–35 000 fractions are associated with endoplasmic reticulum, while 14 and 19 kDa are associated with Golgi body and storage vacuoles<sup>(25,27)</sup>. The  $\alpha$ -AI1 is detectable 17 d after pollination in the cotyledons and axis of the plant seed. The amounts increase to a constant maximum after 28 d until maturity, although the amount on a dry basis decreases slightly during drying<sup>(25)</sup>. The  $\alpha$ -amylase inhibitor is therefore suitably obtained from non-dried common beans. However, there is need for research to access maturity indexes for optimum inhibitor levels in beans to be used for extraction of the inhibitor for maximum economy. The distribution and biosynthesis show that the common bean  $\alpha$ -amylase inhibitor is a suitable candidate as a widely used remedy against diabetes, obesity and for other related beneficial effects.

### Favourable physico-chemical properties of the *Phaseolus vulgaris* $\alpha$ -amylase inhibitor

The inhibition efficiency, specificity, absence of deleterious carbohydrate-binding action associated with PHA and the action of the  $\alpha$ -amylase inhibitor relative to similar agents such as acarbose or cyclodextrins have been shown to be

based on its structure and molecular weight. In addition, to enable improvements in the use and application of the inhibitor, an understanding of the starch-blocking activity in terms of functional and biochemical factors is necessary.

### Structural properties of the *Phaseolus vulgaris* $\alpha$ -amylase inhibitor

The three common bean lectin compounds PHA, arcelins and  $\alpha$ -AI ( $\alpha$ -AI1,  $\alpha$ -AI2,  $\alpha$ -AIL) have an amino acid sequence homology of about 50–90%<sup>(28)</sup>. In a study on genes that encode for  $\alpha$ -AI1 in white and black beans, Lee *et al.*<sup>(29)</sup> found similarities of 40 and 43%, 52 and 53%, and 93 and 95% with PHA, arcelins and previously determined  $\alpha$ -AI1 sequences respectively. These observations corresponded to major differences in the number of surface loops in the three-dimensional structures of the lectins. PHA has three loops, arcelin has two of the loops,  $\alpha$ -AIL has one shortened loop, while the loops are completely absent in  $\alpha$ -AI1 and  $\alpha$ -AI2<sup>(30)</sup>. The inhibitor has no carbohydrate-binding activity due to lack of carbohydrate-binding loops that are present in PHA<sup>(27,31,32)</sup>. The inhibitor, therefore, if extracted efficiently, is bound not to possess the deleterious effects associated with PHA. Several researchers using various methods have shown the deletions in the sequences to be an indication of evolutionary relationship between the lectins<sup>(26,29,30,33,34)</sup>. Le Berre-Anton *et al.*<sup>(35)</sup>, using graphical docking methods, concluded that the extra loops, presence of extra glycan moieties and lack of proteolytic processing in PHA, arcelins and  $\alpha$ -AIL were responsible for their lack of inhibitory activity relative to  $\alpha$ -AI1. The extra loops caused steric hindrance that prevented them from entering the active site of mammalian amylases to enable binding<sup>(35)</sup>.

The  $\alpha$ -amylase inhibitors  $\alpha$ -AI1 and  $\alpha$ -AI2 exist in their native form as typical lectin tetramer structures ( $\alpha_2\beta_2$ )<sup>(35)</sup>. The  $\alpha$  and  $\beta$  chains are formed through a two-step proteolytic processing in the protein storage vacuoles which leads to formation of the active form of the inhibitor from a precursor<sup>(27,30,36,37)</sup>. The process involves removal of a short-chain carboxy terminus and proteolytic cleavage at the carboxyl side of Asn77 by action of a carboxypeptidase or an asparagyl-specific endopeptidase leading to the formation of the two chains<sup>(26,27,30,36)</sup>. When compared with the precursor,  $\alpha$ -AIL and with a transgenically produced inhibitor in tobacco which all have the proteolytic processing site, the proteolytic processing is responsible for the removal of a structural constraint in the inhibitor which enables it to acquire the inhibitory activity<sup>(27,34,37)</sup>. Based on structural models resulting from nucleotide sequences of  $\alpha$ -AI1, Lee *et al.*<sup>(29)</sup> showed this structural constraint to consist of a bend in the region next to Asn 77.

Between  $\alpha$ -AI1 and  $\alpha$ -AI2, only the former shows inhibitory activity against mammalian amylases. This has been explained in terms of inhibitor structural properties. There is a 78% homology in amino acid sequence between them and both undergo post-translational cleavage, yet  $\alpha$ -AI2 has no inhibitory effect on mammalian amylases<sup>(34)</sup>. The differences in the sequence between the two therefore have a significant effect on the inhibitory activity<sup>(34)</sup>. Le Berre-Anton *et al.*<sup>(35)</sup> explained the difference in specificity between  $\alpha$ -AI1 and  $\alpha$ -AI2 to result from lower stability of binding interactions

with mammalian amylases by  $\alpha$ -AI2. They explained that two hairpin loops were responsible for the stability of an  $\alpha$ -AI1 – porcine pancreatic amylase (PPA) complex, by the formation of fifteen hydrogen bonds with PPA in the active site cleft. With  $\alpha$ -AI2, however, there were only eight of the hydrogen bonds formed due to deletions and replacements of residues in the loops of  $\alpha$ -AI2 relative to  $\alpha$ -AI1. The deletions and replacements included two residues (Tyr34 and Asn35) present in loop L1 of  $\alpha$ -AI1, which were deleted, and residues Tyr186, Tyr37 and Tyr190, which were replaced by His175, Val35 and Phe179 in  $\alpha$ -AI2. These replacements could not interact with any residue from the PPA active site by hydrogen bonding<sup>(35)</sup>.

According to Santimone *et al.*<sup>(38)</sup> the inhibitor protomers are bound together non-covalently mainly through hydrophobic interactions. Higaki & Yamaguchi<sup>(39)</sup> suggested that glycan moieties played a role in holding the protomers together. The *N*-glycosylation according to Sawada *et al.*<sup>(40)</sup> does not have an effect on the activity of the inhibitor since it occurs in positions that do not interact with mammalian amylases during binding. Removal of the glycan moieties by Gibbs & Alli<sup>(41)</sup> did not also affect the activity of a purified  $\alpha$ -amylase inhibitor from white kidney beans. Bompard-Gilles *et al.*<sup>(42)</sup>, however, noted that although it did not take part directly in amylase binding, the glycan moiety at Asn 12, during inhibitor–enzyme complex formation, lay in a solvent channel that linked the dimers to the enzyme with the two glycan moiety branches forming an extended conformation that was parallel to the surface of the dimer through water-mediated hydrogen bonding that stabilised the dimers. They concluded, however, that the glycan moiety did not take part in the binding action of the inhibitor. Sawada *et al.*<sup>(40)</sup> showed that there is limited variation in glycosylation at this point (Asn 12) between  $\alpha$ -AI1 from different accessions. The role of glycan moieties in the inhibitor binding of the  $\alpha$ -amylase therefore is of limited significance and does not affect relative inhibitory activity between accessions.

There are differences in the primary structures of the  $\alpha$ -AI1 from different accessions that have been determined and deposited in the ExPasy database<sup>(40,43)</sup>. These differences, however, do not affect the specific activity of the  $\alpha$ -amylase inhibitors from different accessions<sup>(42)</sup>. There is a difference in activity of the  $\alpha$ -amylase inhibitor extracts from different accessions, however, due to the existence of varying amounts of particular isoforms and iso-inhibitors between accessions<sup>(22,23)</sup>. An accession to be used to obtain starch blockers therefore should be accessed in terms of its average amylase content in order to get higher extraction and activity yields.

According to Le Berre-Anton *et al.*<sup>(35)</sup> and Kasahara *et al.*<sup>(44)</sup>, the tetrameric ( $\alpha_2\beta_2$ ) nature of the inhibitor explains why there are observations that the  $\alpha$ -AI1 inhibitor inhibits two PPA molecules per molecule. This makes it divalent in its mode of inhibitory action and has thus been reported in various studies to have a stoichiometric ratio of 2:1 relative to the 1:1 ratio of acarbose and cyclodextrins<sup>(38,44–46)</sup>. According to Koukiekolo *et al.*<sup>(46)</sup>  $\alpha$ -AI1 is a much stronger inhibitor of PPA than acarbose based on molar concentration. There is 74% inhibition of amylose digestion by  $\alpha$ -AI1 compared with 71% by acarbose, and a 57% inhibition by  $\alpha$ -AI1 compared with 49% by acarbose for maltopentaose hydrolysis. However, based on weight, due to lower molecular weight,

acarbose is a stronger inhibitor<sup>(46)</sup>. Lee & Whitaker<sup>(47)</sup> showed that the molecular weight of the inhibitor is actually 56.7 kDa, and values in the range 14–20 kDa resulted from chemical modification due to the SDS-PAGE method. The rate of reaction of acarbose with the amylase is, however, faster, since there is no requirement for conformational change during binding<sup>(46)</sup>.

#### *Factors that affect the Phaseolus vulgaris $\alpha$ -amylase inhibitor activity*

Various researchers have shown the dependence of the amylase inhibitor activity on pH, temperature, incubation time and presence of particular ions.

The optimum pH for the inhibitory action has been reported as 4.5<sup>(48,49)</sup>, 5.5<sup>(32,49,50)</sup> and 5.0<sup>(51)</sup>, rather than 6.9 – the optimum for mammalian amylase (PPA). The different pH optima reported were probably due to the different incubation temperatures used in the studies. Lajolo & Finardi Filho<sup>(49)</sup> noted different pH optima for salivary and pancreatic  $\alpha$ -amylase of 4.5 and 5.5 respectively. Le Berre-Anton *et al.*<sup>(48)</sup> demonstrated that there is a narrow range around the optimum in which high activity is observed beyond which activity drops drastically. Kluh *et al.*<sup>(43)</sup> illustrated that for maximum activity, the inhibitor requires pre-incubation at low pH (pH 4) relative to the optimum.

Temperature has been reported to have an effect on the activity of the inhibitor. The effect of temperature, however, is less felt at pH 4.5 which is the optimum pH for inhibitor activity than at pH 6.9, the optimum pH for PPA<sup>(43,50)</sup>. According to Le Berre-Anton *et al.*<sup>(48)</sup>, the  $\alpha$ -amylase inhibitor shows no activity at 0°C, then activity increases to a maximum between 22 and 37°C with little change within this range<sup>(51)</sup>. Although Marshall & Lauda<sup>(32)</sup> also reported no activity at 0°C, they showed a 10-fold increase in activity within this range (22 and 37°C). Le Berre-Anton *et al.*<sup>(48)</sup> attributed this discrepancy to different incubation pH used, with the increase occurring when incubated at pH 6.9, the optimum pH of the enzyme. The inhibitor is completely inactivated at 100°C by boiling for 10 min<sup>(32,52)</sup>. Collins *et al.*<sup>(53)</sup> showed that the inhibitor transgenically expressed in peas was only inactivated after heating at over 90°C for 5 min. There is need to characterise the temperature-inactivation profile of the inhibitor further since many potential products in which it can be incorporated would require heat treatment during processing.

The incubation time required for optimum activity has been reported as 10 min by Le Berre-Anton *et al.*<sup>(48)</sup>, 40 min by Marshall & Lauda<sup>(32)</sup> and 120 min by Powers & Whitaker<sup>(51)</sup>. These differences were suggested to be a result of the different pH conditions used in the experiments, with the latter two being obtained when the optimum for  $\alpha$ -amylase activity (6.9) was used and the first when the optimum for the inhibitor (4.5) was used<sup>(48)</sup>. The longer incubation times at pH 6.9 imply that it would require the inhibitor to be taken before or at least with meals in order to achieve substantial *in vivo* inhibitory activity.

Various ions have also been shown to affect the activity of the inhibitor. Lajolo *et al.*<sup>(49)</sup> reported increases in the activity of the inhibitor against salivary amylase mediated by ions in the order nitrate > chloride > bromide > iodide > thiocyanate. Gibbs

& Alli<sup>(41)</sup> reported that chloride ions are important for maximum activity while Ca ions increase the rate of initial binding of the inhibitor to the amylase. They also reported that K, Mg, sulfate and Na ions did not have any effects on the amylase inhibitor activity and so did increased ionic strength<sup>(41)</sup>.

Generally, there is need to further characterise the effect of various functional and biochemical factors on the activity of the inhibitor in order to enable improvements in the use and application of the inhibitor.

### The starch-blocking mechanism of the *Phaseolus vulgaris* $\alpha$ -amylase inhibitor

Research into the mechanism of the *P. vulgaris*  $\alpha$ -amylase inhibitor action shows that the inhibitor is effective in preventing starch digestion by completely blocking access to the active site of the enzyme. The molecular-level binding of the action of the amylase inhibitor on human pancreatic amylase and PPA was reviewed in detail by Payan<sup>(54)</sup>. During inhibition, several components of the inhibitor molecule, amylase molecule and the whole system have been reported to play important roles in the mechanism. The main components that participate in the mechanism include two loops of the inhibitor (L1 and L2) made up of residues 29–46 and 171–189 respectively<sup>(35,38,42)</sup>, the amylase domains A and B plus the active site surface loop (residues 303–312)<sup>(32,40,41)</sup>, the active site non-loop residues (Cl binding site and Asp197, Glu233; Asp300 and Arg74 in human pancreatic amylase only<sup>(42,55)</sup>), the active site lining and gate aromatic residues<sup>(42)</sup>, the chlorine ion of the amylase<sup>(56)</sup> and system aspects such as the inhibitor:enzyme ratio<sup>(38)</sup> and pH<sup>(55)</sup>. Based on the effects of chemical modifications on activity of the inhibitor, Ho & Whitaker<sup>(57)</sup> proposed that His, Trp, Tyr and Arg residues were important in the mechanism of the inhibitor. Mirkov *et al.*<sup>(58)</sup> suggested the active site of  $\alpha$ -AII to be made up of Arg in the  $\alpha$ -subunit, and Trp and Tyr in the  $\beta$ -subunit, which are located in a TrpSerTyr motif. Takahashi *et al.*<sup>(59)</sup> who, however, postulated that the arginine residues were not essential in the mechanism, supported these results. Bompard-Gilles *et al.*<sup>(42)</sup> attributed these observations to the participation of the residues in hydrophobic interactions. On the other hand, Da Silva *et al.*<sup>(60)</sup> showed that no particular structure in the amylase inhibitor–amylase complex was solely responsible for the inhibitory action.

In the course of the binding action, the inhibitor approaches the enzyme active site cleft by way of the loops, which leads to the formation of an extensive network of bonds between the loop residues and parts of the active site<sup>(42)</sup>. The network of bonds involves mainly hydrogen bonds which may be direct or water mediated, hydrophobic bonds and protein–protein bonds, especially in areas outside the active site<sup>(42)</sup>. The bond network formation is accompanied by conformational changes in parts of the amylase in adjustment to docking of the inhibitor, which occurs in the active site surface loop (residues 303–312)<sup>(41,42,55,61,62)</sup>, the domains of the amylase (domains A and B) and in the areas near the surface loop in the active site<sup>(42)</sup>. Although several researchers have elucidated the inhibitor binding reactions, there is need for more work to establish and confirm the actual sequence of events during the inhibitory mechanism. This would provide more insight into the binding reactions and provide more knowledge

that would help in developing similar synthetic inhibitors. It is, however, clear from the research in its mechanism that the inhibitor is effective in preventing starch digestion by completely blocking access to the active site of the enzyme<sup>(42)</sup>.

### *Efficiency of $\alpha$ -amylase inhibitor isoform 1 extracts in reducing activity of amylases in man*

An effective reduction in activity of intraluminal amylases is the underlying source of all the beneficial effects obtained from the inhibitor. Several researchers have shown a decrease of intraluminal amylase activity *in vivo*, in all parts of the gastrointestinal tract, hence reducing the rate of evolution and absorption of glucose in the lumen (Table 1). In human subjects Layer *et al.*<sup>(6)</sup> reported a decrease in duodenal amylase activity and length of inhibition time, which were dependent on the dose of application of the inhibitor. In another human study, decreased duodenal, ileal and jejunal amylase activity, with no apparent effect on trypsin levels, was observed<sup>(7)</sup>. Brugge & Rosenfeld<sup>(63)</sup> showed a 96% decrease in duodenal amylase activity in human subjects after taking starch-containing meals with an incorporated laboratory-purified amylase inhibitor.

Studies have shown marginal middle and proximal gastrointestinal tract amylase activity a few hours after feeding with meals containing the inhibitor and a complete abolition of activity after 4 h of feeding<sup>(6)</sup>. Inhibition results in malabsorption of starch and passage into distal parts of the ileum<sup>(6,7)</sup>. Various levels of the resultant malabsorption have been reported. Layer *et al.*<sup>(6)</sup> reported a malabsorption level of 20% of ingested starch, while other workers have reported lower levels. Brugge & Rossenfeld<sup>(63)</sup> reported a level of 7.0 (SD 1.4)% and Boivin *et al.*<sup>(64)</sup> documented a concentration-dependent level of up to 18% with 2.9 mg of inhibitor. The different levels reported could have been due to differences in activity and amounts of  $\alpha$ -AII used. Some changes occur in response to the presence of excess starch in the duodenum and the passage of excess starch into the distal parts of the ileum in order to increase the rate of digestion<sup>(65)</sup>. They include reduced rate of gastric emptying<sup>(6)</sup> and increased secretion of amylase by the pancreas, in addition to general changes in pancreaticobiliary secretions<sup>(65,66)</sup>. The onset of reduced gastric emptying occurs after the first 2 postprandial hours<sup>(65,66)</sup>. The mechanism that initiates these changes was postulated to involve carbohydrate-mediated hormonal and non-vagal neural responses, since changes in plasma hormonal levels (peptide YY, neurotensin and gastric inhibitory peptide) were associated with changes in gastric emptying<sup>(66)</sup>. These changes, however, were associated with subtle increase in glycaemia relative to controls without the inhibitor<sup>(66)</sup>. The anti-amylase activity of the inhibitor *in vivo* is also decreased by the amount and type of starch in the duodenum, with liquid starch being more potent than solid starch in the reduction<sup>(6)</sup>.

According to Brugge & Rosenfeld<sup>(63)</sup>, the form in which the inhibitor is applied, whether powder or tablet form, has no effect on the inhibitory activity when incorporated in meals. This implies that various forms of extract products can be developed depending on a particular targeted functionality and still have the desirable inhibitory activity.

**Table 1.** Human studies on the efficacy of *Phaseolus vulgaris*  $\alpha$ -amylase inhibitor isoform 1 extracts on starch digestion and resultant effects

Dose/duration	Main results*	Reference
Acute, two commercial starch-blocker tablets, 16 666 units of activity with six healthy subjects	No difference in postprandial plasma glucose, insulin and breath hydrogen	Carlson <i>et al.</i> <sup>(68)</sup>
Acute, 500 mg commercial starch blocker with two healthy subjects	Starch blocker ineffective in reducing energy intake	Bo-Linn <i>et al.</i> <sup>(69)</sup>
Acute, 500 mg commercial starch blocker with eight healthy subjects	Commercial starch blocker ineffective <i>in vitro</i> and <i>in vivo</i>	Hollenbeck <sup>(67)</sup>
Acute, three healthy subjects; 2–5 mg/ml for 90 min	Purified extract effective but commercial blocker ineffective	Layer <i>et al.</i> <sup>(6)</sup>
Acute, 5 and 10 g with four healthy subjects	Purified inhibitor orally taken blocks starch digestion; no abdominal problems; decreased postprandial hyperglycaemia and insulin levels	Layer <i>et al.</i> <sup>(7)</sup>
Acute, 3–8 g with thirteen healthy subjects	Physical form has no effects on inhibitory activity of orally taken inhibitor	Brugge & Rosenfeld <sup>(63)</sup>
Acute, perfused over 7 h at 9.9 mg/min with eighteen healthy subjects	Orally taken inhibitor starch digestion blocking effect; changes in GIT motility and GIT-related hormones	Jain <i>et al.</i> <sup>(65)</sup>
Acute, perfused at 3.3 mg in 570 ml, with eighteen healthy subjects	Orally taken starch-blocking effect; GIT motility accompanied; changes in pancreaticobiliary secretions	Jain <i>et al.</i> <sup>(66)</sup>
Acute, dose 2.0 and 2.9 g with eight healthy subjects	Starch digestion blocked; no abdominal problems; changes in plasma insulin and glucose	Boivin <i>et al.</i> <sup>(64)</sup>
445 mg Phase 2 <sup>®</sup> tablet before meals with thirty slightly obese subjects	Highly significant combined change in anthropometric parameters ( $P < 0.001$ )	Celleno <i>et al.</i> <sup>(4)</sup>
1500 mg Phase 2 <sup>®</sup> before each meal with sixty healthy subjects	Subtle weight loss with body-fat loss and no observed deleterious effects	Meiss & Ballerini <sup>(85)</sup>
1500 mg Phase 2 <sup>®</sup> , twice daily before meals for 8 weeks, with twenty-seven healthy subjects	Subtle loss of body weight and three-fold decrease in plasma TAG levels relative to controls	Udani <i>et al.</i> <sup>(86)</sup>
Two and eight tablets of commercial starch blocker for 3 and 4–5 months respectively, with twenty-two obese subjects	Starch blocker has no synergistic effect on weight reduction under reduced energy conditions	Diaz <i>et al.</i> <sup>(87)</sup>

GIT, gastrointestinal tract.

\* Statistical significance at  $P < 0.05$  unless mentioned.

The inefficiency of the amylase inhibitor reported by researchers in the early 1980s was mainly due to low activity and purity of the commercial starch blockers<sup>(67–69)</sup>. The manufacturers employed methods based on extraction of  $\alpha$ -AII by Marshall & Lauda<sup>(32)</sup>. A simple partial extraction of the inhibitor by Layer *et al.* led to a 30–40-fold increase in inhibitor concentration by dry weight<sup>(6)</sup>. The resultant *in vivo* inhibitory activity and length of inhibitory time were dose dependent compared with commercial inhibitor and crude extracts that were only effective at high doses. This showed that low activity was the cause of apparent inefficiency and hence the highest possible  $\alpha$ -amylase activity should be a target for extraction processes.

Impurities were also reported in the starch blockers which were found ineffective<sup>(70,71)</sup>. The trypsin inhibitor, one of the potential inhibitor extract impurities<sup>(70)</sup>, would lead to increased trypsin secretion which has been associated with decreased  $\alpha$ -AII activity due to non-specific secretion of excess amylase by the pancreas<sup>(66,72)</sup>, while the pure amylase inhibitor is not associated with changes in chymotrypsin activity in rats<sup>(73)</sup>. According to Yoshikawa *et al.* <sup>(74)</sup>, chymotrypsin reduces inhibitor activity *in vitro* rapidly within 2 h, pepsin slightly and the inhibitor is highly resistant to trypsin digestion. The amylase inhibitor had been earlier hypothesised ineffective in reduction in energy intake due to proteolysis by gastric enzyme, high amylase activity and unfavourable pH conditions in the duodenum<sup>(68)</sup>. Gibbs & Alli<sup>(41)</sup>, on the other hand, showed that the inhibitor was resistant to proteolysis *in vitro* by physiological amounts of chymotrypsin and pronase. It has also been shown that the amylase inhibitor is

stable in gastric and duodenal juices<sup>(65,75)</sup> and reduces *in vivo* amylase activity<sup>(63,65,72)</sup>. The activity, however, is slightly reduced (15%) by the unfavourable pH in the duodenum<sup>(6,64)</sup>.

In summary, despite several factors that may reduce the amylase inhibitor activity *in vivo*, the activity has been shown to be sufficient and hence the *P. vulgaris* inhibitor is applicable as an intraluminal  $\alpha$ -amylases inhibitor.

### The beneficial effects of the *Phaseolus vulgaris* $\alpha$ -amylase inhibitor

#### Decreased obesity due to *Phaseolus vulgaris* $\alpha$ -amylase extracts

Currently there is a shift from synthetic anti-obesity prescribed medications to natural ones, due to undesirable long-term side effects of synthetic prescribed medications<sup>(76,77)</sup>. Though acarbose and voglibose, which are approved by the Food and Drug Administration, reduce blood glucose levels, they also induce abnormalities in hepatic enzyme levels, yet natural anti-glycosidase extracts do not exhibit such effects<sup>(78)</sup>. The *P. vulgaris*  $\alpha$ -amylase inhibitor extracts have an anti-obesity effect as shown by the various researches although there are some uncertainties (Table 1). The effect is derived from the mobilisation of body fat reserves due to energy restriction as a result of the  $\alpha$ -amylase inhibitory action.

In studies by Puztai *et al.* <sup>(79)</sup>, there was a reduction in body fat in rats due to the consumption of raw kidney beans. They, however, attributed the effect to the presence

of PHA through some unknown mechanism. The effect could also have arisen due to the presence of amylase inhibitors in the common beans since the lean body content of the obese rats was not affected. Hangen & Bennink<sup>(80)</sup> showed that rats fed diets containing black and navy beans were able to achieve a reduction in body weight and the fat percentage directly associated with anorexia and starch escape of digestion in the ileum. In their studies the amount of starch that escaped digestion was higher than the amount of resistant starch originally in the diet.

Incorporation of the inhibitor in diets leads to a reduced integrated postprandial plasma glucose area by 85% and a lower than fasting level of late postprandial plasma glucose according to Layer *et al.*<sup>(7)</sup>. The total energy in form of glucose obtained from the diet is therefore reduced leading to mobilisation of fat in the body.

Several reports have shown increases in breath hydrogen on ingestion of food with an active amylase inhibitor. This is as a result of action of distal ileum enterocytes on undigested starch that passes digestion sites<sup>(63–65,81,82)</sup>. Although action of the enterocytes releases energy to the body, 50 to 20% of the total energy in the by-passed starch is not released<sup>(4)</sup>. The total energy therefore is still bound to be reduced resulting in mobilisation of fat reserves.

The amylase inhibitor was found to induce reduced growth in weaned young male rats by Maranesi *et al.*<sup>(83)</sup>, which they attributed to reduced energy intake due to the inhibitor. The reduced energy intake was accompanied by increase in levels of plasma NEFA. There have been several positive results indicating reduced obesity by researchers using a commercial  $\alpha$ -AI extract referred to as Phase 2<sup>®</sup> (Pharmachem Laboratories, Inc., Kearny, NJ, USA). According to Chokshi<sup>(84)</sup>, Phase 2<sup>®</sup> is prepared using thermoprocessing conditions to substantially inactivate haemagglutinating activity and trypsin inhibitory activity while preserving substantial  $\alpha$ -amylase inhibition activity. The product is also tested for the presence of other antinutritional factors or potentially toxic substances with standard levels of >3400 haemagglutinating units/g and >40 trypsin inhibitor units/g<sup>(84)</sup>. Celleno *et al.*<sup>(4)</sup> reported a highly significant difference ( $P < 0.001$ ) in combined obesity anthropometric measures between subjects taking a dietary supplement containing 445 mg Phase 2<sup>®</sup> in a 30 d study with controls on microcrystalline cellulose–maltodextrin. In their study, changes relative to controls were observed in body weight, adipose tissue thickness, waist circumference, hip circumference, right thigh circumference and fat mass. Although these were accompanied by a just significant lean mass loss, the total weight loss was more due to fat mass loss than lean mass loss<sup>(4)</sup>. It was shown in a double-blind placebo-controlled clinical trial by Meiss & Ballerini<sup>(85)</sup> that feeding Phase 2<sup>®</sup> for 30 d resulted in a 4% decrease in body weight, accompanied by a 10.45% reduction in body fat, and a skin echography revealed an 11.63% reduction in adipose membrane. This study also showed that Phase 2<sup>®</sup> caused a change in hip, thigh and waistline circumferences. In a similar study, Udani *et al.*<sup>(86)</sup> also reported an average weight loss of 95 g (0.21 lb)/week and an average of 263 mg/l reduction in TAG for individuals taking Phase 2<sup>®</sup>. These results were, however, not statistically significant due to the low sample size used.

On the other hand, Bo-Linn *et al.*<sup>(69)</sup>, in a study of commercial starch blockers, found no changes in faecal energy output when the inhibitor was taken compared with inhibitor-less controls. In a controlled double-blind placebo study, a commercial starch blocker was found to be ineffective relative to controls in reducing the weight of obese women on a BMR-equivalent diet<sup>(87)</sup>. More recently in toxicity studies of amylase inhibitor in rats, no effects of plasma lipoproteins<sup>(77)</sup> and weight gain have been observed<sup>(5)</sup>.

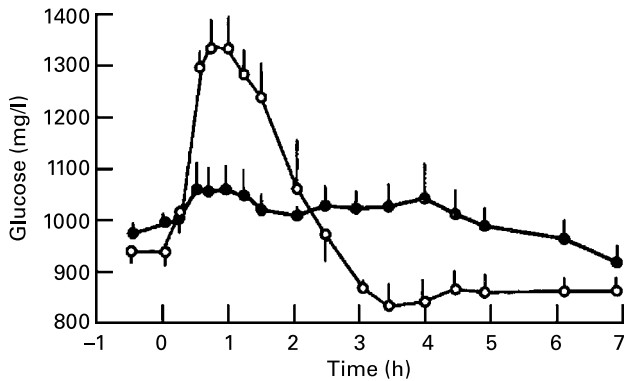
Given the exhibited starch-blocking ability of the amylase inhibitor by Phase 2<sup>®</sup> relative to earlier forms of commercial extracts<sup>(4,85,86)</sup>, the amylase inhibitor has anti-obesity effects, although the effect of the extracts that results from reduced energy intake depends on a given manufacturer's methods of manufacture and extraction as regards the maintenance of high anti-amylase activity and purity.

#### *The anorexigenic effect of Phaseolus vulgaris $\alpha$ -amylase inhibitor isoform 1 extracts*

Some works have suggested an anorexigenic effect as an underlying cause of obesity reduction. The mechanism of the anorexigenic effect of the amylase inhibitor is, however, not clearly understood<sup>(88)</sup>. It has been reported that the amylase inhibitor fed chronically to rats reduces feed intake<sup>(82)</sup>. The inhibitor in further studies also reduced water intake in diabetic rats in addition to reduced food intake<sup>(81)</sup>. However, the  $\alpha$ -amylase inhibitor in a study on the toxicity of a commercial starch blocker was found to have no anorexigenic effect after 28 d<sup>(5)</sup>. A similar study showed that the anorexigenic effect in Sprague–Dawley rats was felt only after 77 d of feeding<sup>(77)</sup>. The anorexigenic effect may therefore be only achieved with prolonged exposure to the inhibitor. More research is, however, needed in human subjects to assess the anorexigenic effect of the inhibitor further.

#### *Reduced postprandial plasma hyperglycaemia and insulin due to $\alpha$ -amylase inhibitor isoform 1 extracts*

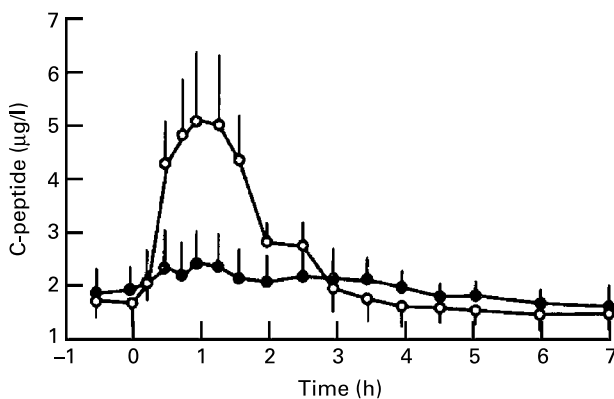
Changes in postprandial plasma glucose levels have been reported when the amylase inhibitor is taken with a starch-containing meal or before the meal (Fig. 1). Earlier reports, using commercial starch blockers with low activity, could not show changes in postprandial plasma glucose<sup>(67,68)</sup>. Kotaru *et al.*<sup>(73)</sup> and Menezes & Lajolo<sup>(72)</sup> showed smoothed and retarded hyperglycaemia in rats fed rations containing the purified  $\alpha$ -amylase inhibitor. A reduction of 85% in postprandial plasma glucose integrated area accompanied by lower than fasting late post-prandial plasma glucose were shown on acute consumption with meals of the inhibitor in human subjects<sup>(7)</sup>. Boivin *et al.*<sup>(64)</sup> also reported decreased integrated area and lower peak plasma postprandial glucose in human subjects on acute application. According to Tormo *et al.*<sup>(82)</sup>, a reduction of hyperglycaemia due to the inhibitor in rats starts 50 min after the consumption of a starch-containing meal. Chronic consumption of the amylase in meals in rats led to reduced mean glycaemia over the period of application. There was variation of significance of the reduced mean hyperglycaemia from day to day, ranging from  $P < 0.01$  to  $P < 0.05$ <sup>(81,82)</sup>.



**Fig. 1.** Effect of  $\alpha$ -amylase inhibition by *Phaseolus vulgaris*  $\alpha$ -amylase inhibitor isoform 1 on postprandial plasma concentration of glucose in response to a starch meal. (○), Placebo ( $n$  4); (●), 5 or 10 g inhibitor ( $n$  4). Values are means, with standard deviations represented by vertical bars. (Adapted from Layer *et al.* (7).)

The ingestion of the amylase inhibitor with meals has also been shown to alter postprandial plasma insulin levels. Boivin *et al.* (64) reported in human subjects a decrease in the integrated areas of plasma insulin secretion-related hormones of gastric inhibitor peptide and C-peptide over baseline values when the inhibitor was part of a composite meal. An abolition of postprandial plasma insulin, C-peptide and gastric inhibitory peptide in human subjects was also documented by Layer *et al.* (7) (see Fig. 2). Lowering of plasma insulin levels was shown to occur 30–40 min after the consumption of a composite ration containing a purified cranberry bean (*P. vulgaris* L.) amylase inhibitor in rats. In another study in rats Menezes & Lajolo (72) showed decreased serum insulin levels in both diabetic and normal rats fed meals containing the amylase inhibitor.

Earlier reports on tests using commercial starch blockers that were found to lack *in vivo* amylase inhibitory activity found the inhibitor ineffective in reducing plasma insulin levels (67,68). It was also found that plasma insulin levels in Wistar rats are not affected by both chronic and acute administration of  $\alpha$ -AI1 (82). The levels were lower than in the fasting



**Fig. 2.** Effect of  $\alpha$ -amylase inhibition by *Phaseolus vulgaris*  $\alpha$ -amylase inhibitor isoform 1 on postprandial plasma concentration of C-peptide in response to a starch meal. (○), Placebo ( $n$  4); (●), 5 or 10 g inhibitor ( $n$  4). Values are means, with standard deviations represented by vertical bars. (Adapted from Layer *et al.* (7).)

state but not statistically significant. Despite these findings, the reduction in plasma insulin and related hormonal levels can increase the carbohydrate tolerance of diabetics. This has been shown to occur on consumption of the  $\alpha$ -amylase inhibitor. There is a need therefore for more research to confirm the effect of the inhibitor on postprandial insulin levels in man and its incorporation in starch-containing foods.

A few studies have been reported on the application of the  $\alpha$ -amylase inhibitor in food products. Udani (89,90) reported successful incorporation of the amylase inhibitor in the form of a proprietary fractionated white bean extract powder (FWBE®) ( $\geq 3000$   $\alpha$ -amylase inhibitor units/mg) into six commercial baked products at levels deemed sufficient for inhibitory activity (750 mg/serving) without significant changes in the acceptability of the products. The main factors that influenced the incorporation were the order of ingredient incorporation and the time–temperature requirements for dough development and baking. Combinations of these factors through trials and iterations were obtained that did not affect consumer acceptability of products with the required amounts of extracts per serving. These results, however, did not report the effect of the incorporation on the glycaemic index of the products. In a similar study (J Udani, unpublished results), using an open label six-arm cross-over design with thirteen randomised subjects, the glycaemic index of white bread was reported to have been significantly reduced ( $P=0.0228$ ) by the addition of 3000 mg StarchLite® powder – a commercial  $\alpha$ -amylase bean extract. There is a need for more research into the application of the amylase inhibitor in these and other products to enable wide application.

### Safety and toxicity of the *Phaseolus vulgaris* $\alpha$ -amylase inhibitor extracts

#### Toxic effects associated with common beans

Haemagglutinin poisoning due to the consumption of raw common beans by animals and humans has been documented in several reports (91–98). In man, acute consumption in all documented cases led to severe symptoms requiring hospitalisation (97,98). In addition, slimming pills consisting of extracts from common beans were found by Kilpatrick *et al.* (71) to cause a skin rash after ingestion. The rash was linked to haemagglutinating activity in the pills at levels of up to 150 mg protein and agglutinated human A, B, or O erythrocytes; the specific lectin activity was 2000 lectin units/mg protein (71). The haemagglutinating activity of common beans varies between accessions in terms of amount and specificity of activity (99–102). Varieties low in PHA such as pinto beans (99) are therefore more suitable candidates as raw material for  $\alpha$ -AI1 extracts. Some acute and subchronic studies have been conducted on the toxicity of  $\alpha$ -AI1 extracts in man and rats.

#### Acute toxicity studies

Acute toxicity is a toxicity response that often occurs immediately after ingestion and is induced by a single exposure. It is measured by the lethal dose 50 (LD<sub>50</sub>) value, which is the amount of a given substance under test that causes death of 50% of the test animals after consuming the substance only once (14). There were no significant signs of acute toxicity or

mortality when 3 g/kg of Blockal<sup>®</sup> (a dietary supplement containing Phase 2<sup>®</sup> at a rate of 1668 mg/kg body weight) was fed to rats<sup>(5)</sup>. The symptoms observed at the acute experimental levels of feeding (1668 mg/kg body weight of Phase 2<sup>®</sup>) were not similar to those caused by PHA, indicating that the Phase 2<sup>®</sup> component used did not contain adequate PHA to cause deleterious effects<sup>(5)</sup>. Variations from normal were not observed in liver function markers, kidney function markers, plasma levels of electrolytes, cholesterol and TAG. The acute toxicity level was established at >5 g Phase 2<sup>®</sup>/kg body weight in another acute oral administration study in adult male and female Wistar rats<sup>(77)</sup> and there was no observed toxicity based on clinical evaluation, biochemical and histopathological analyses at this level of single-dose feeding<sup>(77)</sup>.

#### *Chronic toxicity studies*

Chronic measurement requires a longer time of study, usually about 20–24 months of continuous feeding to rodents. The maximum tolerance dose is the level at which a substance can be fed to an animal without inducing any obvious sign of toxicity<sup>(14)</sup>. In chronic studies, the maximum tolerance dose is typically used with two or more lower levels below<sup>(14)</sup>. Studies have been done on the effect of chronic feeding of the amylase inhibitor. In a subchronic study on the oral toxicity of a standardised white kidney bean extract Phase 2<sup>®</sup> in rats, it was found that there were no mortalities and clinical signs considered of toxicological significance on rats fed doses up to 2500 mg/kg (7 d/week) for a period of 31 d (males) or 32 d (females)<sup>(84)</sup>. No gross abnormalities were observed apart from some isolated cases, which were considered unrelated to the treatments. The microscopic findings in body organs observed which apparently deviated from normal were similar to those commonly observed in the studied rat strain<sup>(84)</sup>. In addition, on the basis of lack of correlation of these findings to microscopic and clinical pathological data, they were considered to have no toxicological relevance<sup>(84)</sup>. The no observed adverse effect level was found to be at least 2500 mg/kg per d for rats, which corresponds to 175 g Phase 2<sup>®</sup>/d in a 70 kg person. It was proposed that the upper limit level of aggregate intake of Phase 2<sup>®</sup>/d from dietary supplement and qualified food use be 6 g/kg per d for a 70 kg person based on the fact that a 30-fold safety factor was used in the experiment<sup>(84)</sup>.

In another study a lower no observed adverse effect level of at least 1112 mg Phase 2<sup>®</sup>/kg body weight was observed in a 4-week toxicity study involving feeding Blockal<sup>®</sup> at 2 g/kg body weight to rats<sup>(5)</sup>. Variations were observed in different parameters during the study but were also considered irrelevant because they were not associated with any histopathological changes, did not vary with sex and were within the range of the historical results obtained in the laboratory. These variations occurred in weight, micro and macro appearance of organs, and some haematological, clinical and urine analyses<sup>(5)</sup>.

Subchronic feeding testing has also been carried out in adult human subjects. In one randomised double-blind placebo-controlled study, tablets of a commercial blocker were given to individuals before carbohydrate-rich meals. An 800 mg tablet containing 445 mg Phase 2<sup>®</sup> was given once per d in an

8370–9200 kJ (2000–2200 kcal) diet with a microcrystalline cellulose and maltodextrin placebo as the control for 30 d. There were no significant deleterious effects reported<sup>(4)</sup>. The average weight of individuals in the study was 74.1 (SD 2.1) kg, hence the level corresponded to a rate of 6 mg Phase 2<sup>®</sup>/kg body weight per d. Udani<sup>(86)</sup>, in a randomised double-blind placebo-controlled subchronic study on human subjects, showed that there were no observed deleterious effects on safety markers of kidney and liver function. The level of Phase 2<sup>®</sup> used in this test was 1500 mg/d with the average weight of the individuals being 87.6 (SD 12.22) kg<sup>(86)</sup>. When subchronically applied to rats at two to twenty times the human subchronic levels recommended by Udani<sup>(86)</sup> the commercial extract Phase 2<sup>®</sup> did not produce signs of toxicity<sup>(77)</sup>. It was concluded that feeding Phase 2<sup>®</sup> to rats at the rate of over 350 g/kg for a 70 kg individual did not produce any adverse effects<sup>(77)</sup>. It was, however, noted in a study on the efficacy of the amylase inhibitor by Tormo *et al.*<sup>(82)</sup> and Pusztai *et al.*<sup>(88)</sup> that chronic administration of the  $\alpha$ -amylase inhibitor in rats leads to changes in organ weights. There is need therefore for more research to completely ensure safety of the amylase inhibitor extracts. However, the use of a starch blocker with at least 3000  $\alpha$ -amylase inhibitor units/g, <3400 haemagglutinating units/g and <40 trypsin inhibitor units/g at the subchronic level of 6.0 g/kg body weight per d for a 70 kg individual has so far been shown to be safe by studies using Phase 2<sup>®</sup>.

#### *Future research on beneficial effects: the potential of $\alpha$ -amylase inhibitor isoform 1 extracts against colorectal cancer*

Several studies have pointed to increased microbial activity in the hindgut on consumption of  $\alpha$ -AI extracts although there are no reports on its effect on butyrate production, which is necessary for anti-colorectal cancer functionality. Based on the definition of resistant starch as the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals<sup>(103,104)</sup>, the presence of the amylase inhibitor in the gut causes an action similar to that of resistant starch or rather increases the amount of resistant starch. Resistant starch has been shown by many workers to have a prebiotic effect and several reviews have been written documenting the effect<sup>(103–107)</sup>. Human and animal studies have shown that butyrate leads to a reduced incidence of colon cancer. Le Leu *et al.*<sup>(108,109)</sup> found that butyrate had an apoptotic response to DNA damage by genotoxic carcinogens in the distal colon of rats, leading to the removal of mutated clones that would progress to malignancy. Distinct patterns of SCFA production are associated with particular polysaccharides and substantial butyrate formation was found to be associated mainly with starch<sup>(110)</sup>.

The amylase inhibitor has been shown to increase the amount of breath hydrogen after the consumption of starch-containing meals as a result of passage of starch into the proximal parts of the colon that is accompanied by microbial activity<sup>(7,63,64,67,68,87)</sup>. This was reported in studies with *in vivo* active inhibitor extracts while studies with extracts that showed no activity did not show increases in breath hydrogen. Collins *et al.*<sup>(53)</sup>, in a study on transgenic pea  $\alpha$ -AI1 in pigs, showed a significant difference in energy content between terminal ileum and faecal matter which they

attributed to energy recovery by hindgut micro-organisms from ileum by-passed starch. No reports on butyrate production were given from these studies. On the other hand, several reports have shown that acarbose, a synthetic pharmaceutical starch blocker that functions in a similar manner to the common bean  $\alpha$ -amylase inhibitor ( $\alpha$ -AII), leads to alteration of colon microbe pathways. The alterations lead to an increase in the overall SCFA production with an increase in the butyrate:total SCFA ratio<sup>(111–114)</sup>. The total faecal SCFA and butyrate output on prolonged acarbose use correlates inversely with proliferation in the rectal upper crypt – a biomarker of risk for colonic neoplasia<sup>(114)</sup>. Future research on the beneficial effects of the  $\alpha$ -amylase inhibitor therefore should also be focused on checking its potential in colorectal cancer prevention as a result of increased butyrate production due to starch in the colon after consumption of reasonable amounts of the inhibitor.

### Conclusion

Although obesity and diabetes are on the increase worldwide, based on the research developments discussed, the common bean (*P. vulgaris*)  $\alpha$ -amylase inhibitor ( $\alpha$ -AII) has potential to serve as a widely used remedy against these conditions while there is need for research on its probable anti-colorectal cancer effect. The potential lies in the fact that the amylase inhibitor is present in most *P. vulgaris* accessions which are widely grown in the world, it has a significant *in vivo* inhibitory capacity based on appropriate structural, physico-chemical and functional properties, and has mediating effects on these conditions although there are some uncertainties. In studies carried out more recently the  $\alpha$ -amylase inhibitor has been found to be safe. There are several aspects of the inhibitor that require further research. These include wider clinical trials over longer times to confirm the efficacy and safety of the inhibitor, ingredient functionality of the inhibitor in various food systems and further elucidation of molecular-level binding interactions to enable synthetic blockers based on the inhibitor to be designed.

### Acknowledgements

This review was sponsored by the National Natural Science Foundation of China (no. 20436020). All contributing authors participated actively in writing and preparation of the manuscript. We are thankful to Dr Wanmeng Mu for his moral support. There are no conflicts of interest to report.

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