



- 20 **List of abbreviations used:** RS, resistant starch; RDS, readily digestible starch; HAM-RS2,
- 21 high-amylose maize type-2 resistant starch; AUC, area under curve; FFA, free fatty acid; PYY, peptide
- 22 YY; GLP-1, glucagon-like peptide-1; GPR, G-protein coupled receptors
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30 **Abstract**

31 Good glycemic control, which is vital for patients with type 2 diabetes, could be achieved via dietary  
32 intervention. Resistant starch (RS) is a type of carbohydrate that largely resists digestion in the small  
33 intestine. Instead, it is fermented by the gut microbiota that resides in the large intestine into  
34 short-chain fatty acids (SCFAs), which are found to have beneficial effects on human glucose  
35 metabolism. This review first provides an overview of the classification of different types of RS, as  
36 well as the fermentation process of RS by the gut microbiota. The effects of RS consumption that  
37 contribute to glycemic control were then discussed with reference to animal and human studies.  
38 Although beneficial effects of RS consumption were observed, results from animal and human studies  
39 were inconclusive regarding the mechanisms behind. Additional research effort is necessary in order to  
40 have a better understanding of the effects of habitual RS consumption on glycemic control.

41

42 **Keywords:**

43 Resistant starch, blood glucose, insulin resistance, adiposity

44

45 **Introduction**

46 According to the World Health Organization (WHO), chronic high blood glucose is the third largest  
47 reason for premature mortality [1]. It was estimated that in the year of 2015, one in every 11 adults  
48 around the world had diabetes and 12% of the global health expenditure was spent on treating diabetes  
49 [2]. Maintaining glycemic control has been established as the primary treatment goal for diabetes and  
50 pre-diabetes, as it can reduce the chance of complication and mortality [2]. Lifestyle interventions,  
51 including dietary changes, has long been suggested as the primary treatment to enable patients to  
52 manage their blood glucose level [3].

53

54 Blood glucose level is directly affected by the intake of readily digestible carbohydrates, such as  
55 sucrose and starch, which is the polymer formed by glucose molecules linked together by alpha-1,4 and  
56 alpha-1,6 glycosidic bonds. Starch is mostly digestible in the human gastrointestinal tract, except  
57 resistant starch (RS), which are special forms of starch able to resist digestion in the stomach and small  
58 intestine [4]. Instead, it reaches the large intestine mainly undigested and is fermented by the bacteria  
59 that reside there. Research has shown that RS consumption positively affects blood glucose metabolism  
60 in human [5, 6]. With the recent emergence of human and animal trials regarding the breakdown of  
61 indigestible carbohydrate by the gut microbiota, our understanding of the effect of RS consumption on  
62 blood glucose control has been greatly enhanced. This review provides an update on the evidence and  
63 the mechanisms involved.

64

## 65 **Classification of Resistant Starch**

66 Englyst et al. [4] classified different types of RS into four main categories, depending on the cause of  
67 resistance to digestion. One new form of resistant starch was discovered later on and became the fifth  
68 type of RS, resulting in the new classification as shown in **Table 1**. RS1 refers to starch molecules that  
69 are contained in an indigestible outer layer, such as cell wall and protein matrix. RS2 refers to starch  
70 molecules with type B or C polymorph. Molecules in these structures are less susceptible to enzyme  
71 hydrolysis [7]. RS3 refers to starch molecules that have undergone retrogradation i.e. the realignment  
72 of starch molecules upon cooling after gelatinization. Retrograded starch molecules have a higher  
73 gelatinization temperature, and these molecules are unable to fit into the substrate binding site of  
74 amylase [8]. RS4 are starch molecules that have undergone chemical modifications, such as the  
75 addition of cross-linkages or chemical derivatives. These modifications include limiting the ability of  
76 the starch molecule to swell during heating, or changing the structure of the molecule such that it can  
77 no longer fit into the binding site of the digestive enzymes [9]. It was found that the reaction  
78 parameters of RS4, such as the availability of reactant and reaction temperature, could be modified to  
79 control the ability of the molecules produced to resist digestion [10]. RS5 refers to the complex that  
80 consists of a fatty acid molecule and an amylose chain, which are straight chains of glucose molecules  
81 linked together by alpha-1, 4 glycosidic bonds. The complexes then aggregated to form a superstructure,  
82 which were found to be resistant to enzymatic hydrolysis [11, 12]. **Figure 1** illustrates the structures of

83 different types of RS.

84

### 85 **Fermentation of Resistant Starch by Gut Bacteria**

86 The fermentation of RS in the large intestine is a stepwise process, involving different bacteria. The  
87 outer protective layer (if there is any) is first degraded, then the starch polymers are broken down into  
88 oligosaccharides and the glycolytic processes follow, with short-chain fatty acids (SCFAs) being the  
89 major end products.

90

91 Studies have shown that the ability of bacteria to adhere to the resistant starch molecules is an  
92 important first step for fermentation, and it was found that this process involves multiple binding  
93 proteins. For example, the starch-utilization gene (*sus*) cluster, that was identified in the genome of the  
94 bacteria *Bacteriodes thetaiotaomicron*, coded for a variety of proteins which were responsible for the  
95 transportation of carbohydrate molecules into the periplasm of the bacterial cell and the breakdown of  
96 the molecule [13]. Cellulosome is another complex which was found to be involved in the digestion of  
97 cellulose, the protective layer which prevents the digestion of starch in RS1. In this complex, different  
98 protein components were found to be responsible for attaching cellulose molecules to the bacterial cell  
99 surface, as well as for the subsequent breakdown of them [14]. Multiple strains of gut bacteria have  
100 been found to utilize this mechanism in starch degradation [14]. In addition, starch binding of some  
101 gram-positive bacteria was found to be accomplished via cell-bound  $\alpha$ -amylase [5]. After the adhesion

102 of bacterial cells to the starch molecules, enzymes were responsible in cleaving different bonds within  
103 the molecules, such as  $\alpha$ -amylase and  $\alpha$ -glucosidase for cleaving  $\alpha$ -1,4 linkages, and type 1 pullulanase  
104 for cleaving  $\alpha$ -1,6 linkages [5, 15].

105

106 The main products of RS fermentation are SCFAs, which include acetate, propionate and butyrate [16,  
107 17]. It was found that SCFA production mainly happens in the cecum and proximal colon, where the  
108 pH was found to be the lowest [18]. The SCFAs produced were mainly absorbed by colonocytes or  
109 metabolized by other gut bacteria, with only 5-10% of the SCFAs excreted with feces [19].

110

### 111 **Effects of Resistant Starch Consumption leading to Improved Glycemic Control**

112 RS consumption has been shown to improve glycemic control in both animal and human studies, yet  
113 the mechanisms behind remain poorly understood. Several possible mechanisms are discussed below,  
114 as outlined in **Figure 2**.

115

### 116 **Reduction in Glycemic Load**

117 The rate of digestion of RS-containing food in the small intestine is much lower when compared with  
118 food containing only readily digestible starch (RDS). As a result, consumption of such food leads to a  
119 sustained and lower level of glucose release [20]. This effect is reflected by the glycemic index (GI), a  
120 ranking system which organizes different food items according to the change of glycemic response

121 upon food consumption [21]. Upon inducing retrogradation in the test foods, researchers observed a  
122 decrease in starch digestibility of the treated food when compared with the untreated food [22]. They  
123 also observed a slower rise in blood glucose level in human subjects upon consuming the treated food,  
124 when compared with those consuming the untreated food [22, 23].

125

126 It should be noted that the beneficial effects on postprandial glucose metabolism upon RS consumption  
127 were observed only when RS replaced RDS, but not when RS was added to RDS (the concept was  
128 shown in **figure 3**). In a study conducted by MacNeil *et al.* [24] different test foods were produced by  
129 mixing normal wheat flour and RS2-containing flour at different ratios and were consumed by subjects  
130 with type 2 diabetes. The researchers observed lower incremental area under curves (AUC) and lower  
131 peak levels of postprandial glucose and insulin in subjects who consumed the RS2-containing test food,  
132 which had the same amount of carbohydrate with the control food. This difference was not observed in  
133 subjects who consumed the other type of test food, which was made by adding RS2 directly to a  
134 portion of control food. Similar findings were seen in a study conducted by Luhovyy *et al.* [25] whose  
135 team replaced the wheat flour by RS2-containing flour when producing the test food, so that the total  
136 amount of carbohydrate was the same between the treatment food and the control food. Also, they  
137 observed a dose-dependent effect of RS content on postprandial glucose level, such that consuming a  
138 higher dose of RS led to a lower AUC of postprandial glucose curve. On the other hand, in studies  
139 where RS was added as an extra portion to the test foods, the results on postprandial glucose and



140 insulin levels were mixed i.e. both positive and negative results were observed [26-28]. The reason  
141 behind this was that the postprandial glucose level was directly affected by the portion of available  
142 carbohydrate, thus adding RS alone without decreasing the available carbohydrate content of the food  
143 may not efficiently decrease postprandial glucose levels [24]. This view was supported by the European  
144 Food Safety Authority (ESFA), which recommended the replacement of digestible carbohydrate by RS  
145 rather than addition for improvement to be observed in postprandial glycaemic control [29].

146

#### 147 **Improved Glycemic Response of the Subsequent Meal**

148 RS consumption may also diminish the glycaemic response of the subsequent meal when compared with  
149 consuming RDS only (**figure 4**). MacNeil *et al.* [24] found that the consumption of RS2-containing  
150 food led to a lower rise in glucose and insulin after the consumption of a subsequent standard meal  
151 three hours later when compared with consuming RDS only. The researchers attributed the improved  
152 response to the increased insulin secretion, which was found to be in line with the variation of the level  
153 of glucose-dependent insulinotropic polypeptide (GIP). However it was previously shown that this  
154 improved postprandial glycaemic response after the second meal was not due to the acute insulin  
155 secretion. Instead, an increase in postprandial glycogen storage, which was caused by a suppressed free  
156 fatty acid (FFA) level in the circulation, was proposed to be the real cause [30]. In contrast, Luhovyy *et*  
157 *al.* [25] found a higher postprandial AUC of glucose upon consuming the second meal in the treatment  
158 group who consumed RS-containing cookies two hours before. They argued that the release of glucose

159 from the previous meal was still ongoing when consuming the second meal, thus leading to the  
160 elevated postprandial glucose level. Although the fact that the second meal being provided *ad libitum*  
161 affected the results, this view was possible as the digestion time of RS could last for up to seven hours  
162 [20]. More studies are needed to investigate the second meal effect of RS consumption, with the  
163 nutrition profile of the second meal standardized for a valid comparison.

164

### 165 **Improvements in Muscular and Hepatic Glucose Handling**

166 The SCFA produced upon the fermentation of RS by the gut microbiota have profound effects on  
167 glucose homeostasis in liver and muscle tissues. G-protein coupled receptors (GPR) 41 and 43, which  
168 are SCFA receptors, have been found on both muscle and liver cell membranes Activation of GPR  
169 41/43 by SCFAs has been found to lead to an increase in glucose uptake and glycogen storage at  
170 muscle tissues [18].

171

172 Unfortunately, studies investigating the effect of RS consumption and the impact of glucose  
173 homeostasis in muscle tissues were scarce. Robertson *et al.* [31] fed an extra 30g of RS2 on top of an  
174 RDS portion to a group of healthy subjects, while the other group had only the RDS portion in their  
175 diet, for 12 weeks. Their postprandial glucose clearance in the muscle was measured by analyzing the  
176 arterialized venous blood collected at the contralateral forearm. The researchers found that subjects  
177 who consumed RS had improved in muscle glucose clearance and insulin sensitivity, as well as a

178 concomitant increase in SCFA uptake at muscle, when compared with patients consuming only RDS.

179 Nonetheless, the AUCs of glucose levels upon receiving meal challenges were not different between

180 the two groups. On the other hand, Bodinham *et al.* [32] fed an additional 40g of RS2 to subjects with

181 type 2 diabetes (T2DM) when compared with the control group. They observed a higher glucose uptake

182 in the muscles in the RS group, although this was not statistically significant. However, they found that

183 the plasma level of propionate and acetate in the RS-consuming subjects were lower. They argued that

184 the lower plasma level of SCFAs could be the result of an increased uptake by the peripheral tissues,

185 which was observed in a previous study [31]. In contrast to the previous study, they observed lower

186 postprandial glucose AUCs for the RS group, when compared with the group without RS consumption.

187 Owing to the inconclusive results, more studies are needed to further establish the role of SCFA in

188 affecting the glucose handling of muscle, as well as the impact of such changes towards the overall

189 glucose homeostasis.

190

191 In addition, since SCFAs have been shown to positively affect the glucose homeostasis of liver [18], it

192 is possible for such benefits to also be conferred by RS consumption. Unfortunately to date there was

193 no human study that looked at this effect, and animal studies were scarce in this regard. Polakof *et al.*

194 [33] fed a batch of rats with a high-fat diet and replaced the carbohydrate portion of the test diet with

195 RS2 for some of the rats. They found that in rats which consumed both the high-fat diet and RS, hepatic

196 insulin sensitivity was improved, and the liver inflammation statuses were alleviated. This

197 improvements were not observed in rats consuming a high-fat diet without RS replacement.  
198 Furthermore, the activities of hepatic enzymes involved in glycolysis (e.g. glucokinase and pyruvate  
199 kinase) were found to be reduced by consuming the high-fat diet, yet this was partially restored by RS  
200 consumption [33]. Given the central role of liver in maintaining blood glucose level and glucose  
201 homeostasis in human [34], the effect of RS consumption on hepatic glucose handling warrants further  
202 investigation. More trials are needed to confirm the relationship between RS consumption and hepatic  
203 glucose metabolism on human.

204

#### 205 **Increase Insulin Sensitivity by Reducing Adiposity**

206 Overweight and obesity have long been referred to as a risk factor for insulin resistance and T2DM.  
207 The prolonged excess in energy intake leads to ectopic fat storage, i.e. fat deposits around internal  
208 organs in the abdominal area. This condition was found to induce local and systemic insulin resistance  
209 via the induction of abnormal inflammation pathways [18, 35]. Moreover, the hypertrophic growth (i.e.  
210 expansion in size) of adipocytes, which also results from a prolonged oversupply of energy, is related to  
211 the development of insulin resistance as well [36, 37]. This is due to the stress induced by the rapidly  
212 expanded adipose tissues as they are inadequately vascularized. As a consequence, the inflammatory  
213 pathways in those stressed adipocytes become activated, and the secretion of pro-inflammatory  
214 cytokines increases, thus interfering with insulin signaling pathways [38]. RS consumption has been  
215 associated with a lower mass of adipose tissues and the suppression of inflammatory pathways (**figure**

216 5).

217

218 Animal studies were able to demonstrate the beneficial effects of RS consumption on fat metabolism

219 and glucose and insulin tolerance. For instance, Harazaki *et al.* [39] fed obese rats with a diet with 55%

220 (w/w) high-amylose maize type-2 RS (HAM-RS2) for four weeks and observed improvements in

221 insulin sensitivity, when compared with rats fed the control diet (55% cornstarch instead of HAM-RS2).

222 They also found that the size of the mesenteric adipocytes in RS2-fed rats was smaller than those fed

223 the control diet. In addition, the mRNA levels of molecules related to the inflammation of adipose

224 tissues were found to be lower in RS-fed rats. Apart from that, Polakof *et al.* [33] conducted a 9-week

225 feeding trial on three groups of Wistar rats: one group was fed a low-fat diet (5% fat), one consumed

226 the high-fat diet (30.4% fat), and the other group consumed the high-fat diet with HAM-RS2 replacing

227 the carbohydrates (41.6% w/w). They found that the group which consumed the high-fat diet showed

228 the greatest glucose excursion and insulin secretion, while both measurements for the RS group were

229 similar to the low-fat diet group. Moreover, genes coding for important proteins involved in fatty

230 oxidation (e.g. *PPAR1*) were down-regulated, and those coding for proteins involved in lipogenesis (e.g.

231 *SREBP-1c*) were up-regulated in the high-fat diet group when compared with the low-fat diet group.

232 These elevated expressions were not shown in the RS group. Results from these studies showed that RS

233 consumption lowered the abdominal fat mass, alleviated the inflammatory status and improved the

234 insulin resistance caused by the consumption of a high-fat diet.

235

236 Meanwhile, results from human studies have been inconclusive, such that the improvements in glucose  
237 metabolism did not always occur with improvements in adipose tissue weight or release of  
238 pro-inflammatory cytokines. The trial by Robertson *et al.* [31] showed positive results: they observed  
239 improvements in insulin sensitivity using euglycemic-hyperinsulinemic clamp(s) in a group of subjects  
240 adding 30g RS2 into their diet every day for 12 weeks, over those who did not incorporate RS into their  
241 diet. They also found that the postprandial output of triacylglycerol (TAG) from adipose tissues and the  
242 rate of action of hormone-sensitive lipase decreased in the treatment group. Yet in some studies,  
243 changes in anthropometric measurements and adipose tissue content were not detected [28, 40, 41], and  
244 the release of pro-inflammatory cytokines were found to be similar between treatment group and  
245 control group [32, 40, 41]. For example, in the feeding trial conducted by Maki *et al.* [41], participants  
246 (overweight adults) received different treatments: consuming only RDS, an extra 15g or extra 30g/day  
247 of HAM-RS2 (~60% RS2) in a randomized crossover manner. At the end of the study, no difference in  
248 body weight and waist circumference was observed. Moreover, improvement in insulin sensitivity was  
249 only observed in male subjects, while no difference in fasting levels of pro-inflammatory cytokines was  
250 observed between treatment conditions. In another 12-week feeding trial conducted by Johnston *et al.*  
251 [28] on insulin resistant adults, one group consumed an extra 40g of HAM-RS2 while the other group  
252 consumed only RDS. It turned out that the body weight and fat storage on all body locations measured  
253 were not significantly different between the two groups. Also, no variation was seen in fasting levels of

254 inflammatory factors (e.g. IL-6 and hsCRP). However, the insulin sensitivity was improved for the  
255 group consuming HAM-RS2. The results from human studies may imply that the relationship between  
256 improvement in adiposity and the improved insulin sensitivity is more indirect than it is previously  
257 assumed.

258

259 Several explanations were provided for the inconsistent results in terms of the changes in adiposity and  
260 insulin sensitivity upon consuming RS. Some argued that this is because the treatment dosage used in  
261 animal studies were too high for human consumption (up to 50% w/w), thus hindering the translation  
262 of results into human studies [28]. Also in mice studies since RS were fed shortly after the mice were  
263 born, adipose tissue remodeling and a lower ectopic fat storage could take place with growth. On the  
264 other hand, adipose tissues in human were already *in situ* at the beginning of the studies, thus the  
265 changes in adiposity may be less visible [28]. It is also worth to note that while some studies included  
266 the level of free fatty acid (FFA) in circulation as a study outcome, it has been argued that high FFA  
267 levels *per se* do not lead to insulin resistance [38]. It has been found that in obese individuals,  
268 hyperinsulinemia may be a mechanism to suppress FFA release, while the release of FFA decreased  
269 with the expansion in the mass of adipose tissues [42]. Alternative hypotheses for the impaired insulin  
270 sensitivity in the context of overweight or obesity, such as the abnormal adipose fat storage and the  
271 dysfunction in the release of adipokines and cytokines, have been proposed [42].

272

273 **Effects on Gut Hormone Release**

274 Another possible mechanism where RS consumption may impact on blood glucose control is via the  
275 induction of gut hormone release, mainly glucagon-like peptide-1 (GLP-1) and peptide YY (PYY).  
276 GLP-1, secreted by intestinal L-cells, is a type of incretin hormone able to stimulate insulin secretion  
277 and inhibit glucagon secretion [43]. GLP-1 is also related to pancreatic beta-cell proliferation and the  
278 enhancement of peripheral insulin sensitivity [43]. PYY, which has been found to be expressed both in  
279 the GI tract and in the pancreas, is initially found to inhibit appetite thus lowering energy intake [44].  
280 Nonetheless, in recent studies it has also been found to exhibit paracrine and exocrine effects on  
281 pancreatic islet cells, leading to enhanced insulin secretion [44]. The releases of both hormones are  
282 triggered by the presence of nutrients in the intestinal lumen, which is detected by membrane-bound  
283 transporters found on enterocytes along the intestinal lining [45]. In recent studies, SCFA receptors  
284 were found to be present in the distal gut and were associated with enhanced GLP-1 and PYY secretion  
285 [18] (**figure 5**).

286

287 In animal studies, both increase [46-48] and decrease [49] in serum level of GLP-1 and PYY had been  
288 found when comparing between animals consuming diets with RS and those with digestible cornstarch  
289 as a control, while the effect on blood glucose homeostasis and insulin sensitivity varied. For example,  
290 Zhou *et al.* [46] fed healthy rats with either RS2 (30% of diet) or normal cornstarch for 10 days and  
291 found that the serum levels of GLP-1 and PYY in the RS group were elevated throughout the day of



292 data collection when compared with the group consuming cornstarch as a control. In the same study, a  
293 separate group of rats received the same dietary treatments, followed by streptozocin injections in order  
294 to induce diabetes. The RS group showed improved glucose tolerance when compared with the  
295 cornstarch group. In contrast, da Silva *et al.* [49] showed that after feeding pigs with retrograded starch  
296 (RS3, 35% of diet) for 14 days, the postprandial level of GLP-1 decreased while that of PYY did not  
297 change, when compared with pigs fed the similar amount of readily digestible cornstarch. Nonetheless,  
298 they found a lower postprandial insulin and glucose response in the RS group. They argued that the  
299 lowered bioavailability of nutrients in the food, as a result of RS replacing the readily-digestible  
300 carbohydrates, caused a diminished release of gut hormones [49]. This view was supported by another  
301 RS consumption study carried out on pigs [16]. In that study when comparing the pigs that were fed an  
302 RS diet (RS2, 11.3% w/w) for six days with those that were fed a low fibre diet (0.7% diet) for the  
303 same period of time, they found improvements on postprandial blood glucose and insulin level, despite  
304 no difference in GLP-1 level between treatment groups [16].

305

306 The mixed results of animal studies may partly be due to the physiological differences between the  
307 animal models and the different types and doses of the RS used. Nonetheless, the inconsistent results  
308 between RS consumption and the effect of GLP-1 and PYY may imply a more complicated association  
309 between gut hormone release stimulated by RS consumption and glucose homeostasis. The  
310 mechanisms in how RS consumption changes the release of gut hormones, as well as its subsequent

311 metabolic effects on animal models warrant further investigation.

312

313 Findings from acute feeding studies on human have been inconsistent. In a study run by Bodinham *et al.*

314 [27] a group of healthy adults was fed a test meal with 48g of RS2, while the other group consumed

315 cornstarch instead of RS2 in the meal. The level of postprandial GLP-1 in the RS group is lower than

316 those who consumed the control meal, yet the level of postprandial glucose and insulin did not differ

317 between treatments. In another study, Klosterbuer *et al.* [26] showed that healthy subjects who

318 consumed a standard breakfast with 25g RS3 added had a lower postprandial GLP-1 level, as well as a

319 lower postprandial glucose and insulin level when compared with subjects consuming only the standard

320 breakfast. Edwards *et al.* [50] provided two dishes for two groups of ileostomy patients, one being a

321 wheat porridge made of coarse durum wheat flour (test meal) and the other made with fine durum

322 wheat flour (control meal, the test meal had 33% lower digestible starch content than the control meal).

323 They found that patients consuming the test meal had a lower postprandial glucose level and a lower

324 GLP-1 and PYY level when compared with patients consuming the control meal, although that was not

325 statistically significant. They argued that the lowered digestibility of the test meal decreased the amount

326 of available nutrients, thereby reducing the release of GLP-1 and PYY [50]. However as SCFAs

327 resulted from fermentation by the gut microbiota have also been linked to gut hormone release [18], it

328 is possible that gut hormones produced in this pathway compensated for the suppressed secretion of gut

329 hormones due to a lower bioavailability of nutrients.

330

331 Similarly, studies about long-term RS consumption and its effect on GLP-1 release and glucose level  
332 on human yielded inconsistent results. Robertson *et al.* [31] found no effect upon including RS in meals  
333 for 12 weeks on GLP-1 level, yet improvement in glucose clearance and insulin sensitivity was  
334 observed. Another 12-week study ran by Bodinham *et al.* [32] on subjects with type 2 diabetes found  
335 that subjects receiving the treatment food had elevated fasting GLP-1 level but lowered GLP-1 level  
336 after a meal, while a smaller postprandial glycemic variation was also observed.

337

338 In light of the inconsistent findings from acute feeding studies, it has been proposed that a longer study  
339 duration is needed for a better exhibition of the beneficial effects of RS consumption and to determine  
340 the effective dose [27, 28, 51]. Since the human gut microflora takes time to adapt to the continuous  
341 addition of RS in diet [52], the mixed results may not be truly reflecting the effects of RS consumption.  
342 Long term consumption studies would hopefully be able to add on to the body of evidence regarding  
343 the effect of RS consumption towards gut hormone secretion, as well as the subsequent effects on  
344 human glucose homeostasis.

345

#### 346 **Conclusion**

347 The beneficial effects of RS consumption on glycemic control have been widely observed in animal  
348 and human studies, yet the mechanisms behind were still poorly understood. Several mechanisms

349 behind the impact that RS consumption might have on glycemic control were assessed in this review,  
350 yet the evidence was inconclusive – some effects of RS consumption were being shown only in animal  
351 studies but not in human studies. Several reasons could be possible, including the difficulty in  
352 controlling the baseline parameters in human subjects, such as adiposity and gut microbiota profile, as  
353 well as the fact that the amount of RS used in animal studies may not be suitable or effective for human  
354 consumption. Nonetheless, it should be noted that glucose level is influenced by several factors at the  
355 same time, including absorption, clearance, and release from internal organs, thus carefully planned  
356 studies with suitable controls are vital for reliable and valid results. Additional research efforts are  
357 required to further establish the mechanisms behind the beneficial effects of RS consumption towards  
358 glycemic control.

359

360 **Conflict of Interest**

361 The authors have no conflict of interest to declare.

362

363

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517

518

519 Figure legends

520 Figure 1 – different types of RS. (a) the structure of RS1: the starch molecules were encapsulated by a  
521 physical barrier; (b) B-polymorph of starch molecules. The helical amylose chains, as depicted by  
522 circles, are closely and orderly aligned, enabling the structure to resist enzyme degradation; (c) the  
523 process of retrogradation, thereby forming RS3; (d) cross-linkages in RS4 and (e) starch molecules  
524 linked by a new functional group, e.g. acetyl group or phosphate group, forming another type of RS4;  
525 (f) the structure of RS5. The complex is formed by an amylose chain wrapping around a fatty acid  
526 molecule. Multiple complexes aggregate into forming a superstructure, which is resistant to enzyme  
527 degradation.

528 Figure 2 – concept map of the effects of RS consumption. RS, resistant starch; CHO, carbohydrate;  
529 SCFAs, short chain fatty acids; PYY, peptide YY; GLP-1, glucagon-like peptide-1  
530

531 Figure 3 – the difference in effects between (a) consuming RDS only, (b) replacing RDS with RS,  
532 keeping the same amount of total carbohydrate as control, and (c) addition of RS as an extra portion to  
533 RDS. RS, resistant starch; RDS, readily digestible starch.

534

535 Figure 4 – inclusion of RS in the first meal leads to a lower rise of postprandial glucose after  
536 consuming a standardized second meal. RDS, readily digestible starch; RS, resistant starch.

537

538 Figure 5 – the benefits conferred by RS consumption via SCFA production. Broken lines depicts  
539 progression, while solid lines depicts enhancement and inhibition. RS, resistant starch; SCFA,  
540 short-chain fatty acid; PYY, peptide YY, GLP-1, glucagon-like peptide-1, GPR, G-protein coupled  
541 receptors.

542

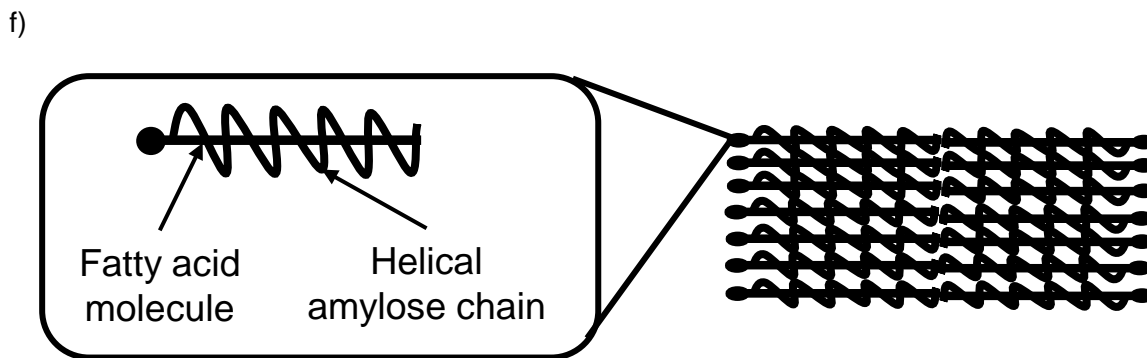
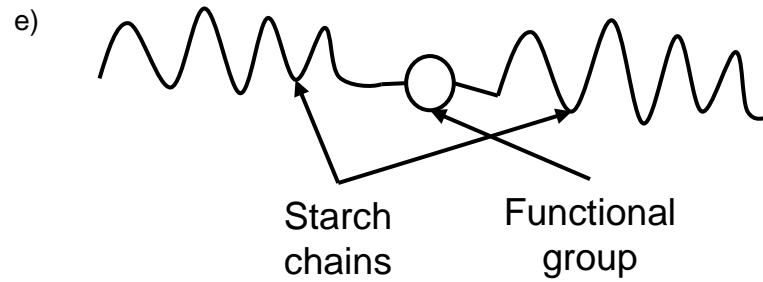
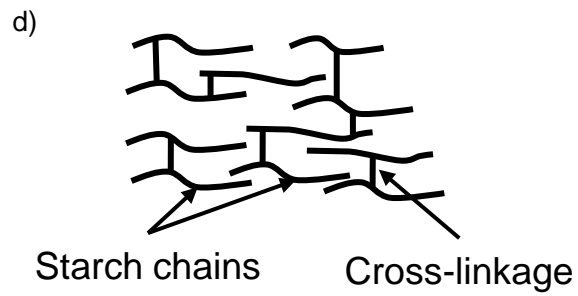
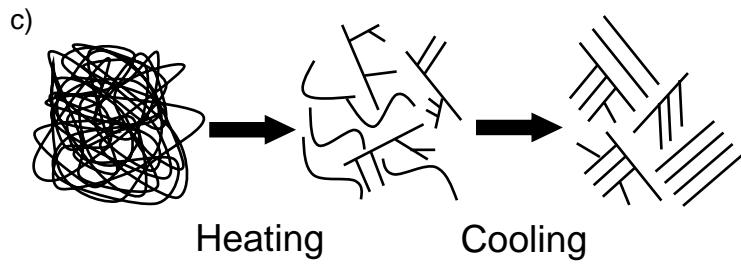
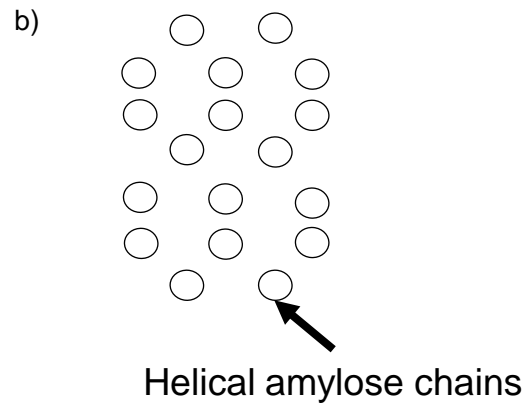
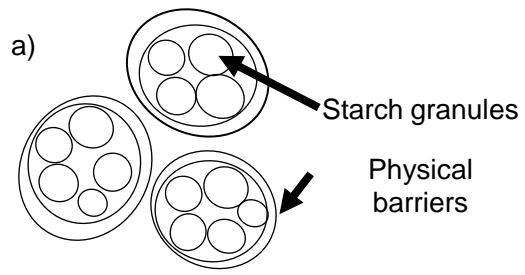
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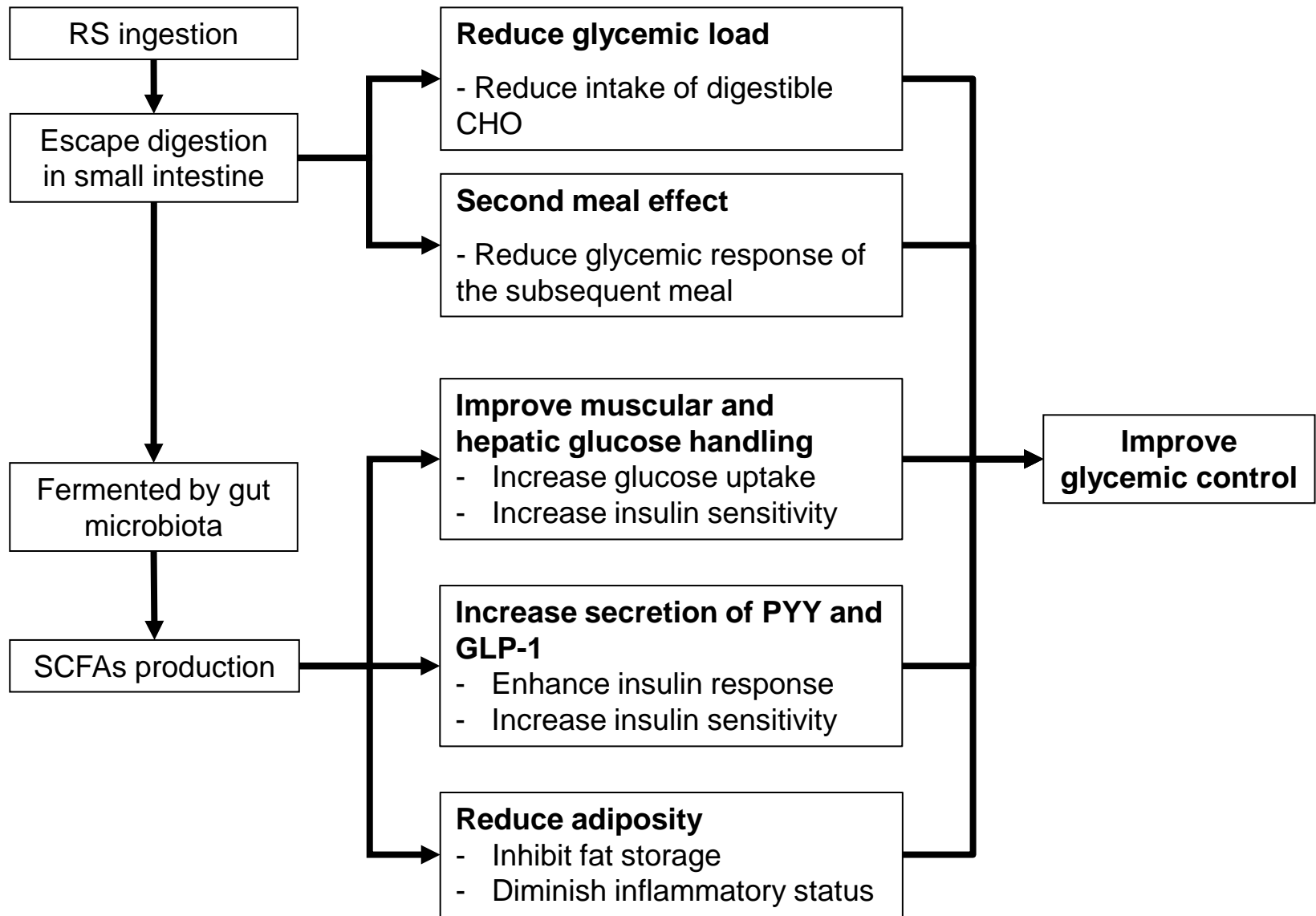
544 Table 1. Classification of RS and examples [4, 9, 53]

<b>Classification</b>	<b>Description</b>	<b>Example</b>
RS1	Physically inaccessible starch	Whole grains
RS2	Starch with B- or C-polymorph	Uncooked potato, high-amylose maize (HAM) starch
RS3	Retrograded starch	Cooked and cooled potato starch
RS4	Chemically modified starch	Cross-linked starch in thickeners
RS5	Amylose-lipid complex	Palmitic acid-amylose complex

545





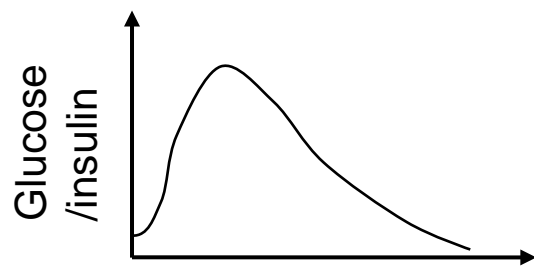




a)



Control



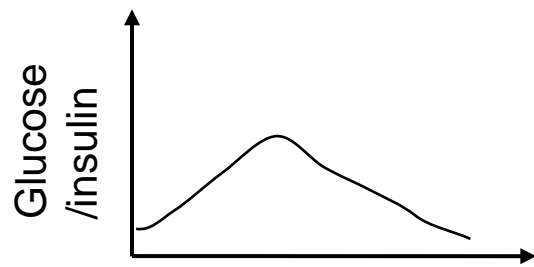
Time

b)



Part of RDS  
replaced by

RS

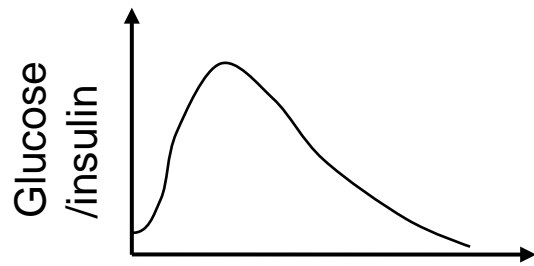


Time

c)

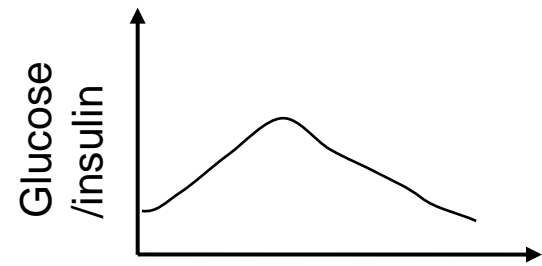


RS added as an  
extra portion



Time

**OR**



Time

Inconsistent  
results

