RESEARCH ARTICLE

PHYSIOLOGICAL EFFECTS OF RESISTANT STARCH

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ABSTRACT

In a rapidly changing world, with altered food habits and stressful life styles, consumers are demanding nutraceutical foods that contain basic nutritional properties with additional health benefits. Resistant starch is defined as the total amount of starch and the products of starch degradation that resists digestion in the small intestine. Resistant starch (RS) is one of the most abundant dietary sources of non-digestible carbohydrates and has a number of physiological effects beneficial for health. Starches that were able to resist the digestion will arrive at the colon where they will be fermented by the gut microbiota, producing a variety of products which include short chain fatty acids that can provide a range of physiological benefits. Resistant starch positively influences the functioning of the digestive tract, microbial flora, the blood cholesterol level and assists in the control of diabetes. This review analyzes the physiological effects of resistant starch consumption.

INTRODUCTION

Before the early 1980s, starch was assumed to be fully digestible in human intestine. Englyst *et al*. (1982) during their research on measurement of non-starch polysaccharides recognised the presence of a small fraction of starch that was resistant to hydrolysis by exhaustive enzymes like α-amylase and pullulanase *in vitro* and coined the term "resistant starch" for this fraction. This fact has led to the classification of starch into two groups: 'available' starch (digestible) and 'resistant starch' (indigestible).

Definition and types of resistant starch

The term 'resistant starch' was used by Asp and Bjorck (1992) to designate a starch fraction that resisted pancreatic amylase/pullulanase degradation *in vitro* after dispersion in boiling water, following solubilisation with potassium hydroxide or dimethylsulphoxide. According to Asp (1992) resistant starch is the sum of starch and the products of starch degradation not absorbed in the small intestine of healthy individuals. Englyst *et al*. (1992) and Perera *et al*. (2010) defined resistant starch as the fraction of starch, which escapes digestion in the small intestine and digested in the large intestine. Englyst *et al*. (1996) indicated that RS is the sum of starch and starch-degradation products that reach the human large intestine.

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Englyst *et al*. (1992) classified starches on the basis of their digestibility into five groups namely RDS, RS1, RS2, RS3$_a$ and RS3$_b$. Readily digestible starch (RDS) are completely digested in small intestine, RS1 is physically inaccessible starch and are partially digested in small intestine, RS2 is resistant starch granules which have little digestion, RS3$_a$ is retrograded starch and are partially digested in small intestine and RS3$_b$ is retrograded starch, not digested in small intestine.

Depending on the resistance of starch to enzymes, Haralampu (2000), Nugent (2005), Sajilata *et al*. (2006) and Lunn and Buttriss (2007) subdivided resistant fractions into four: RS1, RS2, RS3, and RS4 which are also called as type I, II, III, and IV starches respectively. Ratnayake and Jackson (2008) and Sanz *et al*. (2009) classified RS in foods as RS1, RS2, RS3 and RS4. According to the authors, RS1 is physically inaccessible starch; RS2 is found in raw starch granules; RS3 is present in retrograded starch and RS4 is the starch that is chemically modified to obtain resistance to enzymatic digestion. According to Bird *et al*. (2000) RS1 is the starch granules that are physically inaccessible to the digestive enzymes, as these are enclosed in the intact cell walls. According to Sajilata *et al*. (2006) RS1 is heat stable in normal cooking operations (Figure 1). Sharma *et al*. (2008) indicated that RS2 is the raw, ungelatinised native starch molecule present in granular form. The authors also indicated that in raw starch granules, starch is tightly packed in a radial pattern and therefore relatively dehydrated which limits the accessibility of enzymes (Figure 2).

According to Asp and Bjorck (1992) RS3 represents the most resistant fraction in the heat-processed foods and is mainly the retrograded amylose formed during cooling of gelatinised starch. Cummings *et al.* (1996), Haralampu (2000) and Nugent (2005) indicated RS4 as the chemically modified form, which cannot be broken down due to formation of new glycosidic linkages by substitution reactions. Bird *et al.* (2000) noticed RS4 type of resistant starch as esterified, cross-bonded starch. Mermelstein (2009) indicated a fifth type of soluble polysaccharide called "resistant maltodextrins" namely Nutriose® and Fibresol®2 derived from processed starch. Fuentes-Zaragoza *et al.* (2010) classified RS into five categories: RS1-RS5. According to the authors, RS1 is the starch that is physically inaccessible to digestion, RS2 is raw or ungelatinised starch, RS3 is retrograded starch, RS4 is chemically modified starch and RS5 is an amylose-lipid complex in starch.
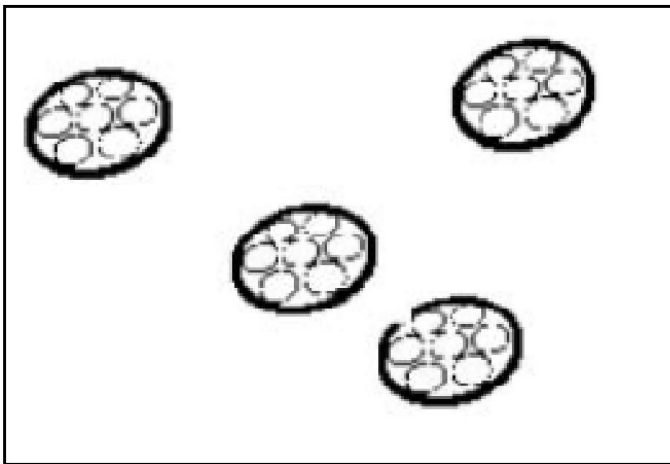
Source: Sajilata *et al.* (2006)

Figure 1. Structure of resistant starch type 1 (RS1)
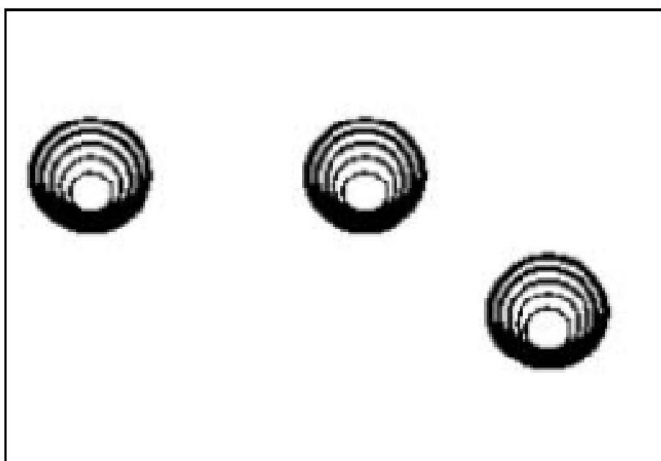
Source: Sajilata *et al.* (2006)

Figure 2. Structure of resistant starch type 2 (RS2)

Food sources of resistant starch

Whole grains are rich sources of fermentable carbohydrates including dietary fibre, resistant starch and oligosaccharides (Slavin, 2004).

Lunn and Buttriss (2007) indicated that fibre provided by the whole grain includes a resistant starch component and varying amounts of soluble and fermentable fibres. Resistant starch is naturally found in cereal grains, seeds and in heated starch or starch containing foods (Charalampopoulos *et al.*, 2002). Tharanathan and Mahadevamma (2003) noticed RS in legumes and indicated that in legumes, starch gets partially modified into resistant starch during processing. The authors also noticed lower digestibility of legume starch when compared to cereal starch due to the high amylose content. Rochfortt and Panozzo (2007) also noticed high RS in pulses which are useful in retaining their functionality even after cooking. Bednar *et al.* (2001) pointed out higher RS concentrations in legumes due to the relationship between starch and protein, which is more resistant to hydrolysis. Giczewska and Borowska (2003) indicated very high diversity of resistant starch content in legumes which vary from a few per cent to about 80 per cent.

Yue and Waring (1998) noticed 0.5 to three per cent RS in native food sources, like peas, bananas and processed cereal foods like bread, pasta and breakfast cereals. Bednar *et al.* (2001) indicated that starch present in spaghetti is more slowly digested due to the presence of densely packed starch. Nugent (2005) and Sajilata *et al.* (2006) reported the occurrance of RS1 in cell or tissue structures of partly milled grains, seeds, and vegetables. The authors also indicated the occurrence of RS2 in raw starch granules like potato, green banana, high amylose corn and RS3 in cooked and cooled potatoes, bread, cornflakes and food products developed with repeated moist heat treatment. Sharma *et al.* (2008) also indicated the presence of RS3 in cooked and cooled potatoes and breads. Nugent (2005) reported the presence of RS4 in breads and cakes in which modified starches have been used. Unripe banana was found to be rich in RS (Tribess *et al.*, 2009; Fuentes-Zaragoza *et al.*, 2010). However, Rodriguez-Ambriz *et al.* (2008) pointed out that although unripe banana is rich in indigestible carbohydrates, mainly RS and dietary fibre, cooking renders the native RS digestible.

Physiological effects of resistant starch

Nugent (2005) indicated that resistant starch is one of the most abundant dietary sources of non-digestible carbohydrates and has a number of physiological effects beneficial for health (Table 1). Sajilata *et al.* (2006) reported that RS received much attention due to its potential health benefits and functional properties. Koksel *et al.* (2007) indicated the health benefits of heat- moisture treated starch due to their decreased digestibility. Buttriss and Stokes (2008) pointed out that the physiological properties and the potential health benefits of RS varied widely depending on differences in the source, type and dose of resistant starch consumed. Perera *et al.* (2010) reported that resistant starch improves glycaemic and insulinaemic responses and exhibits special functions in the management of metabolic disorders like diabetes and hyperlipidemia and also in the prevention of cardiovascular and colonic diseases. Alexander (2012) indicated RS as a type of dietary fibre essential for prevention and treatment of obesity and type two diabetes mellitus due to its slow release of glucose postprandially, low energy density and colonic health benefits from fermentation in the colon.

Table 1. Physiological effects of resistant starch

Potential physiological effects	Conditions where there may be a protective effect
Improve glycaemic and insulinaemic responses	Diabetes, impaired glucose and insulin responses, the metabolic syndrome
Improved bowel health	Colorectal cancer, ulcerative colitis, inflammatory bowel disease, diverticulitis, constipation
Improved blood lipid profile	Cardiovascular disease, lipid metabolism, the metabolic syndrome
Prebiotic and culture protagonist	Colonic health
Increased satiety and reduced energy intake	Obesity
Increased micronutrient absorption	Enhanced mineral absorption, osteoporosis
Adjunct to oral rehydration therapies	Treatment of cholera, chronic diarrhoea
Synergistic interactions with other dietary components, e.g. dietary fibres, proteins, lipids	Improved metabolic control and enhanced bowel health
Thermogenesis	Obesity, diabetes

Source: Adapted from Brown (2004) and Champ (2004)

Prebiotic

Brown *et al*. (1996) stated that RS acts as a substrate for the growth of probiotic microorganisms and therefore is called a "prebiotic". The authors also suggested RS for use in probiotic compositions to promote the growth of beneficial microorganisms such as *bifido-bacterium*. Brown *et al*. (1997) noticed proliferation of *bifidobacteria* in the intestinal tract of pigs consuming high amylose starch which contained RS and indicated its potential benefit in acting as a prebiotic in humans. Brown *et al*. (1998) noticed that RS added yoghurt maintained the viable counts of *bifidobacteria* over several weeks, which was lacking in yoghurt prepared without RS. Silvi *et al*. (1999) studied the effect of resistant starch on human gut and observed that resistant starch modifies the human gut microflora by stimulating lactic acid bacteria. Sajilata *et al*. (2006) reported that since RS almost entirely passes the small intestine, it behaves as a substrate for the growth of probiotic microorganisms.

Colon Cancer

Asp and Bjorck (1992) stated that in small intestine RS is fermented by the micro flora of the large intestine and indicated high yield of butyric acid from unabsorbed starch, which inhibits the malignant transformation of large intestinal epithelial cells. Robertson *et al*. (2000) opined the health effect of RS due to its fermentation by the colonic microorganisms. Significant changes in faecal pH, bulking as well as greater production of SCFA in the caecum of rats fed with RS preparations have been noticed by Ferguson *et al*. (2000) and Tharanathan and Mahadevamma (2003), which are associated with the decreased incidence of colon cancer.

Champ *et al*. (2003) demonstrated the role of resistant starch in the stimulation of bacteria able to produce butyric acid. Topping *et al*. (2003) reported that RS promotes large-bowel health by preventing inflammatory bowel diseases and colorectal cancer. The authors also indicated that fermentation of complex carbohydrates (RS) by the large-bowel microflora produced metabolic products, especially short-chain fatty acids which promote normal colonic function. The undigested carbohydrate (RS) that reaches the colon is fermented by the intestinal microflora to short-chain fatty acids (SCFA) which is related to a decreased risk of colon cancer (Slavin, 2004). Dronamraju *et al*. (2007) reported the positive effect of RS on the control and prevention of colon cancer. Liu and Xu (2008)

indicated the usefulness of RS as a preventive agent in individuals who are at high risk for developing colon cancer. Sharma *et al*. (2008) also reported that fermentation of resistant starch increases short-chain fatty acids in the colon and also produce high levels of butyric acid. The authors also indicated that butyrate is one of the main energy substrates for large intestinal epithelial cells and have an inhibitory effect on the growth and proliferation of tumor cells; hence RS fractions are useful for preventing colonic cancer.

Birkett *et al*. (1996) observed that RS significantly attenuates the accumulation of potentially harmful by products of protein fermentation in the human colon. A study conducted by Muir *et al*. (2004) noticed greater faecal output, lower faecal pH, higher faecal concentration and daily excretion of acetate and a higher faecal ratio of butyrate to total short-chain fatty acids and lower concentrations of total phenols and ammonia by feeding diet containing wheat fibre and RS. Fuentes-Zaragoza *et al*. (2011) reported the beneficial effects of RS in preventing constipation, increasing faecal bulk, decreasing production of mutagenic compounds and lowering the colonic pH and ammonia levels.

Study conducted in pigs by Govers *et al*. (1999) observed improved conditions in the distal colonic regions which help to decrease the incidence of tumour by the combined consumption of RS and insoluble non-starch polysaccharides. Le-Leu *et al*. (2002) also noticed a significant increase in faecal bulk, SCFA and butyrate levels and lowered faecal pH in the faeces of rats leading to protection against colon cancer by the combined consumption of RS with bran.

Hypoglycaemic

Consumption of natural resistant starch by humans is beneficial to glycaemic response in diabetic subjects (Giacco *et al*., 1998 and Vonk *et al*., 2000). Increased insulin sensitivity in healthy individuals with the use of RS was noticed by Robertson *et al*. (2003). Raben *et al*. (1994) and Reader *et al*. (1997) noticed reduction in post-prandial glycaemia and insulinaemia due to the metabolism of RS after five to seven hours of consuming food. *In vivo* studies conducted by Higgins *et al*. (2004) indicated lower glucose and insulin responses within two to eight hours of consuming foods rich in RS. In the small intestine, RS is slowly absorbed resulting in decreased postprandial glucose and insulin responses (Haralampu, 2000). Ou *et al*. (2001) indicated three mechanisms of RS influencing post prandial glucose level. One is by inhibiting alpha amylase

from digesting starch into glucose, second is by increasing the viscosity of chyme in the small intestine which slows the rate of glucose uptake and third is by binding glucose which prevents its diffusion into the mucosal cells. Nugent (2005) opined that RS rich foods release glucose slowly leading to a lowered insulin response. The author also indicated significant reduction in postprandial insulinaemia and a small decrease in postprandial glycaemia by the consumption of RS containing foods. Foods containing RS moderate the rate of digestion and the slow digestion of RS has implications for its use in controlled glucose release application (Sajilata *et al*., 2006). Hoebler *et al*. (1999) and Jenkins *et al*. (2002) indicated reduced glycaemic response due to lack of available digestible starch in individuals consuming foods enriched with RS. The physiological effect of RS was found to be due to lowering the content of digestible starch with the replacement of RS (Nugent, 2005).

The influence of the physico-chemical composition of starchy foods in postprandial glucose and insulin responses was indicated by Heijnen *et al*. (1995). Diet rich in RS was associated with a reduced risk of diabetes (Pawlak *et al*., 2004; So *et al*., 2007). On the basis of animal studies conducted by the authors it was seen that high RS consumption improved insulin sensitivity via changes in ectopic fat storage. A nutrition intervention study by Johnson *et al*. (2010) in 20 insulin resistant subjects also revealed improved insulin sensitivity by the consumption of RS.

In a study conducted by Raben *et al*. (1994) among 10 healthy adult men proved the ability of meals containing high levels of RS in lowering the postprandial concentration of blood glucose levels. In a study conducted by Behall and Howe (1995) among ideal and overweight hyperinsulinaemic and non insulinaemic adults indicated decrease in glucose and insulin responses in both normal and hyperinsulinaemic subjects when amylose starch was given. Lintas *et al*. (1995$_a$ and1995$_b$) reported an improved glucose response in volunteers with type 2 diabetes following the consumption of diets rich in natural RS from durum wheat spaghetti, pearled barley or unripe bananas and a worsened glycaemic response following the consumption of ripened bananas. In a feeding trial conducted on rats, Xue *et al*. (1996) observed low blood glucose levels after giving retrograded high amylose barley containing 18 per cent RS, when compared to a diet containing starch from waxy barley.

Reader *et al*. (1997) reported a decrease in postprandial blood glucose in persons suffering from type II diabetes mellitus by the consumption of food bar containing commercial RS3 ingredient. Achour *et al*. (1997) studied the effect of a meal containing 50g of RS3 in comparison with 50g fully digestible corn starch and indicated a decrease in blood glucose during absorptive state in subjects fed with retrograded amylose meal. Animal studies conducted by Murray *et al*. (1998) also indicated a reduction in the postprandial area under the curve for glucose and insulin by the intake of commercial RS3.Hoebler *et al*. (1999) in a study conducted among eight healthy subjects indicated that breakfast meals based on bread prepared by substituting high amylose maize starch for a part of wheat flour had a low glycaemic index compared to bread rich in amylose and spaghetti.

In subjects fed with bread containing increased levels of RS2, Behall and Hallfrisch (2002) noticed low blood glucose responses. The authors suggested that more than 50 per cent amylose is needed for a significant change in postprandial glucose. Reader *et al*. (2002) studied the effect of RS bar, traditional bar and candy bar on insulin and glucose response of type II diabetes mellitus subjects and indicated 50 per cent decrease in the glucose area among subjects who consumed RS bar. Robertson *et al*. (2003) examined the effect of RS consumption on insulin sensitivity and indicated that high RS diet had a significant effect in reducing blood glucose levels. Robertson *et al*. (2005) also noticed increased insulin sensitivity due to high RS supplementation. The authors opined that high doses of RS2 and long term consumption are essential to enhance insulin sensitivity.

Behall and Scholfield (2005) noticed low glucose response in subjects fed with high amylose chips and muffins. The authors also noticed higher glucose and insulin response in hyperinsulinaemic subjects compared to normal individuals. Behall *et al*. (2006) studied the effect of muffins containing low, medium and high RS on post-prandial glucose response and indicated that high RS treatment decreased glucose area under the curve compared to low RS treatment. Mitra *et al*. (2007) also indicated a decrease in fasting blood glucose in type II diabetes mellitus subjects by giving 150g of rice containing 8-10 per cent RS for 12 weeks. In a study conducted by Kendall *et al*. (2010), a declining trend in glucose in subjects consuming cereal bars and beverage containing varying levels of RS3 was observed. The authors also indicated that higher doses of RS3 are needed to produce significant decline in postprandial glucose. Johnson *et al*. (2010) noticed an improvement in insulin sensitivity by giving 40g of 60 per cent RS2 for 12 weeks in Type 2 diabetic subjects.

Hasjim *et al*. (2010) noticed reduced glucose and insulin area under the curve in adults when meal consisting of RS bread was given compared to white bread. Li *et al*. (2010) compared the postprandial glucose response in healthy subjects by giving RS2 rice, white rice and glucose and indicated the significant effect of RS2 rice and white rice in lowering glucose when compared to glucose load. Alexander (2012) studied the effect of RS derived from corn by giving corn porridges containing 3.1 per cent, 8.4 per cent and 28.9 per cent RS to overweight and obese subjects and indicated that RS substitution improved acute and peak postprandial glucose response and observed significantly lower mean plasma glucose in subjects who consumed 28.9 per cent RS. Thus, the author noticed an improvement in acute and peak postprandial glucose response by RS supplementation. Kwak *et al*. (2012) noticed significant decrease in postprandial glucose concentrations in diabetic and pre-diabetic subjects by consuming a dose of six gram RS daily for four weeks.

Kimura (2013) analysed the effects of resistant starch on postprandial glycaemic response in obese animals and indicated the usefulness of RS in controlling glucose concentrations. Chiu and Stewart (2013) studied the effect of high and low RS rice on glycaemic response among healthy adults and the effect of RS was found to be evident with long term intake.

Behall *et al*. (1989) did not notice any significant response among healthy subjects who consumed a diet containing 70 per cent high amylose and 70 per cent high amylopectin separately for five weeks. Kim *et al*. (2003) also did not observe any improvement in blood glucose or insulin concentrations in streptozotocin-induced diabetic rats when RS rich diet was fed. Nugent (2005) pointed out that there is a lack of consensus regarding the precise effects of RS on insulin and glucose responses. According to the author though various studies have reported an improvement in these measures following the consumption of RS rich test-meal, few studies did not show any effect or the effect was found to be physiologically irrelevant.

Yamada *et al*. (2005) reported that a single ingestion of bread containing 6 g RS significantly inhibited postprandial glucose and insulin responses in subjects with fasting blood glucose level of above 110 mg/dl. However, the authors did not notice such effects among subjects with fasting blood glucose level below 110 mg/dl. Shimada *et al*. (2008) indicated reduced levels of glucose-dependent insulinotropic polypeptide m-RNA in normal and type 2 diabetes rats fed with RS. A clinical trial conducted by Bodinham *et al*. (2010) also did not notice significant effect on the appetite and postprandial glycaemic response in healthy adults by supplementation 48g of RS.

Hypocholesterolemic

Mathe *et al*. (1993) indicated the beneficial effect of RS in lowering plasma cholesterol levels in genetically obese and lean rats. Younes *et al*. (1995) also indicated the effectiveness of RS in lowering plasma cholesterol and triglyceride levels when compared to drugs.

Animal experiments conducted by Han *et al*. (2003) indicated the effect of RS from bean starch in reducing serum cholesterol. Kim *et al*. (2003) studied the effect of RS from corn or rice in reducing cholesterol levels in diabetic rats and indicated its significant effect in lowering plasma total cholesterol. Martinez-Flores *et al*. (2004) also reported the effect of diets containing cassava starch blended with RS or oat fibre in lowering total cholesterol levels in the serum and liver when compared to the diet of cassava starch without added fibre. Nugent (2005) reported the effect of RS in lowering lipid metabolism and noticed a decrease in total lipids, total cholesterol, low density lipoproteins (LDL), very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), triglycerides and triglyceride-rich lipoproteins. Hypocholesterolemic effect of RS was demonstrated by Sajilata *et al*. (2006) in experiments conducted using RS diet containing 25 per cent potato. Mitra *et al*. (2007) indicated a decrease in total cholesterol and LDL cholesterol when 150g of RS3 rice containing 8-10 per cent RS was given daily to DM2 subjects for 12 weeks. Ha *et al*. (2012) reported the effect of the retrograded rice in lowering plasma cholesterol, liver cholesterol and triacylglycerol contents in adipose tissue when compared to those in the common rice group.

Weight Reduction

Higgins *et al*. (2004) noticed increased mobilisation and use of fat stores by consuming a diet rich in RS. The authors also indicated

the significant effect of RS in increasing postprandial lipid oxidation and thus reducing the fat accumulation by replacing total dietary carbohydrate with RS. Nugent (2005) indicated that RS-rich foods lead to a muted generation of hunger signals and reported the role of RS rich foods in the treatment of obesity and weight management. The effect of retrograded rice powder which had higher RS levels in lowering body weight gain was indicated by Ha *et al*. (2012). Kimura (2013) also reported the beneficial effect of RS for dietetic treatment of obesity.

Use of resistant starch in the diet as a bioactive functional food component to increase gut hormones and thus reducing energy intake was indicated by Keenan *et al*. (2006). Resistant starch consumption to reduce adiposity and weight gain in obesity-prone and obesity-resistant rats, due to a reduction in energy intake and changes in gut hormones was indicated by Belobrajdic *et al*. (2012). In a study conducted by Raben *et al*. (1994) among healthy adults of ideal body weight indicated that fully digestible starch supplementation increased satiation upto six hours postprandial compared to RS supplementation. Willis *et al*. (2009) noticed fullness even after three hours of feeding muffin containing RS. Bodinham *et al*. (2010) noticed low food intake after RS supplementation in healthy adults over the entire 24 hour period. Anderson *et al*. (2010) indicated a decrease in post meal intake after RS supplementation in tomato soup. Amount of RS in soup treatment had correlated with reduced food intake at 120 minutes. Kendall *et al*. (2010) observed opposite results in appetite ratings of subjects fed with 25g RS in cereal bar and found a decreasing trend in average satiety during the entire two hour post meal time period.

Conclusion

By definition, resistant starch does not release glucose within the small intestine, but rather reaches the large intestine, where it is consumed or fermented by colonic bacteria (gut microbiota). The fermentation of resistant starch produces short-chain fatty acids, including acetate, propionate and butyrate and increased bacterial cell mass. The fermentation of resistant starch produces more butyrate than other types of dietary fibers. Resistant starch may confer considerable benefits to human colonic health. There is also a need for properly designed, controlled studies to determine the effects of RS on human lipid and glucose metabolism, spanning over longer time periods.

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