

**THE EFFECTS OF NOVEL RESISTANT STARCH BEADS ON APPETITE CONTROL
& ENERGY INTAKE IN INDIVIDUALS LIVING WITH OVERWEIGHT OR OBESITY
– A PILOT TRIAL**

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Abstract

Background: Resistant starch (RS) has been suggested to play a role in appetite control, energy intake, and metabolic health. The novel resistant starch beads used in this study aim to investigate their effects on these parameters in individuals living with overweight or obesity. The objectives of this study were to evaluate the impact of RS on appetite sensations, energy intake, and food palatability, as well as its potential effects energy expenditure.

Methods: In a randomized, double-blind study, participants were assigned to receive either placebo, 130kcal maltodextrin (MDS) or 130kcal Resistant Starch (RS) supplement dispersed in a 290kcal smoothie at random over the course of three sessions. Appetite and palatability (Visual Analog Scale (VAS)), energy intake (ad libitum buffet), energy expenditure (TEE, VO₂, VCO₂, RER) and metabolic markers (Insulin, PYY, GLP-1) were assessed at each session. Statistical analyses were conducted using repeated measures ANOVA to determine within- and between-group differences.

Results: RS supplementation did not result in significant differences in total energy intake compared to the MD or control placebo groups ($p = 0.874$). VAS scores for appetite sensations showed no significant effect of RS on desire to eat ($p = 0.515$), hunger ($p = 0.581$), fullness ($p = 0.680$), or prospective food consumption (PFC) ($p = 0.681$) compared to the MD or control placebo groups. Palatability ratings did not differ significantly between the RS, MD, and control groups ($p = 0.282$). Additionally, RS did not significantly affect total energy expenditure ($p = 0.160$), VO₂ ($p = 0.108$), VCO₂ ($p = 0.379$), or respiratory exchange ratio (RER) ($p = 0.666$)

Conclusions: RS supplementation over the course of three sessions did not significantly alter appetite sensations, energy intake, metabolic markers, or palatability compared to both MD and control placebo groups in individuals with overweight or obesity. These findings suggest that RS may not have a substantial impact on these parameters within the context of this study, although further research is needed to explore its potential benefits in different populations and with varying dosages.

List of Abbreviations

AUC	Area Under Curve
BMI	Body Mass Index
BMRU	Behavioural and Metabolic Research Unit
CCK	Cholecystokinin
EI	Energy Intake
GI	Gastrointestinal
GLP-1	Glucagon-like Peptide 1
NPY	Neuropeptide Y
POMC	Pro-Opiomelanocortin
PYY	Peptide YY
RDS	Rapidly Digested Starch
REE	Resting Energy Expenditure
RS	Resistant Starch
SCFA	Short Chain Fatty Acid
SDS	Slowly Digested Starch
SI	Small Intestine
TEE	Total Energy Expenditure
VAS	Visual Analogue Scale

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Literature Review

1. Introduction

When it comes to understanding obesity and its causes, from a physiological standpoint, energy balance, appetite regulation, and glycemic control are common topics of interest (Alfenas & Paiva, 2007). With obesity being one of the leading risk factors of all-cause mortality and affecting over 1.9 billion individuals worldwide, treatments catered towards reducing the incidence of obesity are pivotal (Haththotuwa et al., 2020). However, it seems that as of late, a stalemate has been reached, such that aside from caloric deficits induced by diet or exercise, hormonal changes seem to play a role in weight regain; specifically, though increases in hunger, appetite, and energy intake (EI) (Busetto et al., 2021). Recently, there has been a growing interest in the use of resistant starch (RS) whose use has provided preliminary findings to help reduce appetite and EI (DeMartino & Cockburn, 2020). These starches have shown potential in regulating appetite, enhancing satiety, and regulating overall energy intake, thereby providing a promising strategy for weight management. RS refers to starch molecules that resist digestion in the small intestine, making their way to the large intestine where they are fermented by the gut microbiota, yielding byproducts such as short chain fatty acids, capable of exerting physiological effects on anorectic hormones such as peptide YY (PYY) and glucagon-like peptide 1 (GLP-1) (Shi & Maningat, 2017). However, due to the instability of many RSs during the food preparation process and the different forms of RSs available, the most stable and effective form of RS supplementation is yet to be discovered (Wolever et al., 2016). The current literature describes various types of RS capable of exerting physiological benefits, however, one in particular (RS3) has begun to show the most promising effects. Through a retrogradation process to be described in further detail later, these starches have an increased resistance to enzymatic breakdown in the small intestine, allowing for

an enhanced signaling for gut hormone secretion and overall satiety signals once in the large intestine (Asp & Björck, 1992). As such, understanding the effects of these RS on appetite control and energy intake is critical as it could provide insights into the development of novel strategies catered to combating the rising prevalence of obesity. By examining the current literature on the topic, this review will aim to summarize the current evidence supporting the use of RS as a dietary supplement as well as identify gaps and offer suggestions for future investigations.

2. Overview of Appetite Control

Feeding behaviour is based on several factors in the body. Specifically, certain hormones and neural circuits regulate energy intake and expenditure which further lead to changes in energy stores and consequently weight changes. Certain hormones and peptides involved in the control of feeding include glucose, insulin, ghrelin, glucagon-like peptide 1 (GLP-1), and peptide YY (PYY). Collectively and independently, these factors play critical roles in the regulation of appetite, satiety signaling, and energy balance. Therefore, diving deeper into their specific mechanisms can help provide insights into the physiological mechanisms surrounding feeding behaviour and potential interventional strategies in the management of overweight and obesity. A schematic of this process is also shown below (see figure 1).

2.1 Glucose

As the primary energy source of the body, glucose plays a central role in the control of feeding. Fluctuations in blood glucose levels have effects on appetite and satiety through numerous pathways. Specifically, hypoglycemia (low blood glucose) stimulates hunger and promotes increased food intake, while hyperglycemia (high blood glucose) suppresses appetite. The mechanisms underlying these effects involve the integration of both peripheral and central

pathways (Vella & Camilleri, 2017). Centrally, hypothalamic regions in the brain are involved in appetite control and regulations. The hypothalamus receives information from the periphery and integrates glucose sensing and neuronal signaling to regulate feeding. Conversely, peripheral signaling occurs in specialized glucose sensing cells located in both the gastrointestinal (GI) tract, and pancreas. These cells are responsible for the release of blood hormones PYY and GLP-1 that act on the central pathway to regulate appetite and satiety through negative feedback loops (Suzuki et al., 2010; Vella & Camilleri, 2017).

2.2 Insulin

In addition to glucose, insulin also plays a critical role in the regulation of appetite and energy intake. Insulin facilitates the uptake and use of glucose by cells in the body and is also a hormone that promotes satiety through actions on both the central nervous system and peripheral tissues of the body (Schwartz et al., 2000). Specifically, insulin has been found to suppresses appetite through decreasing the activity of neuropeptide Y (NPY) and increasing the activity of pro-opiomelanocortin (POMC) neurons(Könner & Brüning, 2012). NPY is an appetite enhancer that stimulates food intake, whereas POMC neurons are a group of neurons in the hypothalamus that promote satiety. Additionally, insulin is also involved in peripheral signaling through the production and release of leptin and ghrelin (discussed below) from adipose tissue, and the stomach, respectively (Schwartz et al., 2000). Insulin has been found to promote both the production and release of leptin, while also inhibiting the release of ghrelin, collectively contributing to its overall satiety promoting benefits(Schwartz et al., 2000).

2.3 Ghrelin

Ghrelin is a peptide hormone primarily produced by the endocrine cells that line the inside of the stomach. Ghrelin plays a significant role in the overall regulation of appetite and energy intake,

with its levels varying before and after meals (Kojima et al., 1999). Specifically, ghrelin acts on the hypothalamus where it binds to growth hormone secretagogue receptors (GHSRs) to trigger orexigenic (appetite-stimulating) signaling pathways which leads to increased hunger and the initiation of food-seeking behaviours (Cowley et al., 2003). Additionally, in a similar fashion to insulin, ghrelin has also been shown to be involved in the activation of NPY neurons, known to stimulate appetite and overall energy intake, however, in contrast to insulin, ghrelin is involved in the activation of NPY rather than suppression, in order to enhance overall food-seeking behaviours (Müller et al., 2015)

2.4 GLP-1 & PYY

GLP-1 and PYY are both gut-derived hormones that regulate appetite and energy intake. GLP-1, released from endocrine L-cells, exerts its effects through the activation of GLP-1 receptors located in the hypothalamus and brainstem. Specifically, GLP-1 reduces appetite through slowing down gastric emptying, delaying the absorption of nutrients, and promoting a feeling of fullness (Batterham et al., 2003). Additionally, GLP-1 is also involved in promoting the release of other satiety hormones such as cholecystokinin (CCK), and by reducing the release of appetite stimulants such as ghrelin (Flint et al., 1998). PYY, released in response to food intake, is secreted by specialized cells in the GI tract; primarily in the colon and ileum (Batterham et al., 2003). It acts on Y2 receptors also located in the hypothalamus and brainstem to reduce food intake and appetite. Similar to GLP-1, it also slows gastric emptying, delays nutrient absorption, and promotes satiety (Batterham et al., 2003).

2.5 Flowchart of Feeding Behaviour and Control

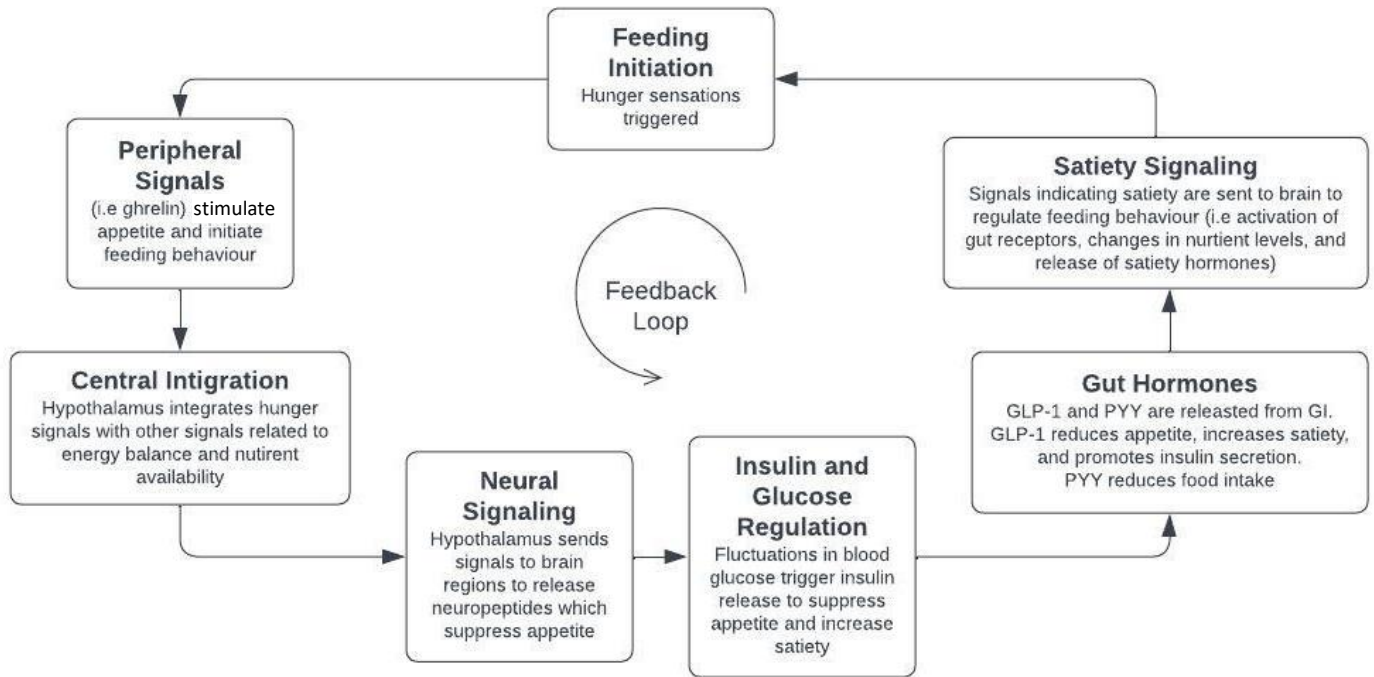


Figure 1. Schematic of Feeding Behaviour and Control (Myers & Olson, 2012; Schwartz et al., 2000)

3. Overview of Starches

With a general overview of feeding behaviour discussed, one typical source of food involved in this process includes starch. Starches are a form of complex carbohydrate found in abundance in a variety of foods. They serve primarily as a source of energy for humans and are also a key component of grains, legumes, and some root vegetables. Structurally, starch molecules consist of chains of amylose and amylopectin; two long glucose chains that are involved in different digestion patterns. Typically, when starches are consumed, they undergo digestion in the GI tract. This involves the enzymatic action of salivary and pancreatic amylase, as well as other intestinal brush border enzymes (Raigond et al., 2015). The roles of these enzymes are to break down large

starch molecules into smaller glucose subunits, capable of entering the vascular system (Shi & Maningat, 2017). Like many sorts of foods, starches undergo the same aforementioned feedback loop where peripheral signals in the digestive tract communicate with the brain to regulate appetite and overall levels of satiety (Shi & Maningat, 2017).

4. Resistant Starches

Depending on a starch's supramolecular structure (i.e. arrangement of amylose and amylopectin chains), its rate of enzymatic hydrolysis, glucose release, and absorption into the gastrointestinal tract, it can be classified as either rapidly digestible (RDS), slowly digestible (SDS) or resistant (RS) (Birt et al., 2013). Unlike RDS and SDS, which have total glucose release times of approximately 20 and 100 minutes respectively, any starch not digested after 120 minutes escapes the small intestine (SI) and is referred to as a RS (Birt et al., 2013; Shi & Maningat, 2017). These RSs are unique in structure such that rather than hydrolyzing in a similar manner to RDS and SDSs, RSs, like dietary fibres, are undigested polysaccharides resistant to digestive enzymes in the SI. As a result, they enter the colon where they are fermented by the gut microbiota yielding the production of short chain fatty acids capable of upregulating various anorectic hormones responsible for increasing levels of satiety (Shi & Maningat, 2017). The proposed mechanisms of this process will be discussed later on (DeMartino & Cockburn, 2020; Shi & Maningat, 2017).

4.1 Resistant Starch Variations

RSs have been categorized in five different ways based on structure and function (H. N. Englyst et al., 1992). RS1 is considered a physically inaccessible starch due to its similarity with plant cell walls. It is typically found in whole grains and seeds, and its intact wall restricts digestive enzymes from accessing it, causing it to be considered resistant to digestion (H. N. Englyst et al., 1992). RS2 is a starch resistant due to its granular structure. Specifically, RS2 contains a compact and

crystalline structure of granules that make it less accessible to digestive enzymes. These starches are typically found in foods such as raw potatoes and high-amylose cornstarch(H. N. Englyst et al., 1992). RS3, known as a retrograded starch, is formed when cooked foods, such potatoes, rice, or pasta, are cooled. The cooling process of RS3, retrogradation, causes the starch to resist digestion in the small intestine due to the formation of a resistant crystalline structure, and reach the large intestine intact(H. N. Englyst et al., 1992). RS4 is a chemically modified starch which is used in various food manufacturing processes to provide commercial properties such as improved texture, stability, or shelf-life, and RS5 is a high amylose content starch which can be formed during food processing and is also able to be prepared under certain controlled conditions. However, the current body of literature surrounding this starch variation is limited (Raigond et al., 2015)

4.2 Why RS3?

Of all RSs, RS3 has provided the most promising results with regards to its overall stability and resistance to digestion due specifically to its ability to withstand food processing (Shi & Maningat, 2017). This retrograded starch is formed when cooked starchy foods are cooled, where they form resistant crystalline structures. Specifically, in comparison to other starches, RS3 offers advantages that make it a more appealing supplement compared to other forms. For one, RS3 is incorporated in a wider variety of foods, meaning there is familiarity with this starch in typical diets making its supplementation more convenient for consumers (Hans N. Englyst & Hudson, 1996). In contrast, RS1 (although naturally occurring) is limited to specific food sources making it less readily available as well as utilized in typical diets. Moreover, RS3 also contains an enhanced resistance to digestion allowing it to reach the large intestine intact without requiring cooking or additional processing such as in RS2. This has been supported in a review by Sajilata et al (2006) and findings

by Asp & Björck (1992) who suggested that in comparison to other starch variations, RS3 is considered to be the most resilient to food processing, making it capable of being incorporated in food products while also remaining resistant to pancreatic amylases. In addition to its structural resilience, RS3 compensates for the various limitations surrounding the other starch forms. With RS1 already being heavily present in foods such as pasta and unprocessed grains, its use does not diversify one's diet, making it a redundant supplement (Shi & Maningat, 2017). RS2 and RS5 also have a major limitation being that they cannot withstand food processing. A review by Pięłowska and colleagues indicated that heating these sorts of starches, such as when baking or cooking, degrades their structure, causing them to have a lower resistance to digestion (Pięłowska et al., 2020). This in turn causes them to be digested in the earlier portions of the SI and therefore unavailable in sufficient amounts once they reach the colon (Pięłowska et al., 2020). Finally, RS4 is a type of starch that has been modified by chemical methods. Such starches are used as specialty food ingredients (thickeners, emulsifiers, fat replacers, etc) and are not intended to be used as dietary supplements for regulatory purposes (BeMiller, 2018). With many of these limitations being centered around starch stability and utilization as a food supplement, a starch capable of withstanding digestion by pancreatic enzymes, easy to consume in any sort of diet, and a natural food source rather than one altered by chemical methods, could serve as a more effective dietary supplement for the reduction of appetite and EI. Additionally, with RS3 being the most capable at withstanding food processing (Asp & Björck, 1992; Sajilata et al., 2006), the development of a practical RS3 supplement, capable of compensating for the limitations observed with other RSs, could serve as a potent dietary solution to be used as an adjunct in the weight loss industry.

4.3 Retrogradation Process of RS3

As aforementioned all native starches contain two polymers, amylose and amylopectin, which dictate the organizational structure of the starch, built through tightly packed granules containing insoluble monosaccharides linked together to form a chain (Raigond et al., 2015). Upon cooking, this structure is lost, and the starch becomes digestible. However, upon cooling of the cooked starch, the polysaccharide chains reorganize and once again become resistant to digestion, however even more so than in their previous form (Higgins & Brown, 2013; Raigond et al., 2015; Shi & Maningat, 2017). The process in which these amylose and amylopectin polymers reassociate to form a more organized and stable structure is known as retrogradation (Raigond et al., 2015). Specifically, the polysaccharide chains containing varying amounts of amylose and amylopectin are dissociated with heat-moisture treatments, and then gelatinize when cooled; increasing the number of straight chain linkages bound together (Margareta Leeman et al., 2006). Retrograded starches contain an increased crystalline structure, increasing the thermostability of α 1-6 glycosidic linkages in the starch (Raigond et al., 2015). As a result, it becomes more difficult for digestive enzymes to break down the glycosidic bonds within the polymer which causes the starch to become more resistant (Shi & Maningat, 2017). Hence, it is through the continuous heating and cooling processes that starches have been suggested to increase in resistance, permitting them to escape digestion by intestinal enzymes, and make their way to the colon for fermentation (Margareta Leeman et al., 2006). A study by Li & Gao investigated the effects of varying heat moistures on the formation of RS3s (S. Li et al., 2011). Through comparing various moisture percentages at 120°C for 12 hours, it was found that the RS content increased significantly after heat treatment. Specifically, the heat-moisture treatment increased the aggregation of straight chain polymers, making it more difficult for the glycosidic bonds to be

accessed by digestive enzymes. This was suggested to be the greatest contributor to the increased resistance of the starches(S. Li et al., 2011). Additionally, another study by Li & Guo also found that heat-moisture treatments reduced the digestibility and increased the resistance of rice flour starches(Z. Li et al., 2021). In contrast to the first study, this study provided the treatment at 120 °C for only 2h and was also able to identify significant reductions in in-vitro digestibility and enhancements in RS content with rice flour starches(Z. Li et al., 2021). Together, these findings suggest that the retrogradation process has been an effective method of enhancing starch resistance and provides supportive insight towards the use of this process in producing RS food supplements.

5. Proposed Mechanism of Action for Starch Digestion

Aside from structural differences to RDS and SDSs, RSs have been found to elicit specific changes to appetite and overall satiety(Shi & Maningat, 2017). This is apparent through increases in the secretion of anorectic hormones PYY and GLP-1, known to slow down gastric emptying, reduce post-prandial blood glucose levels, increase intestinal transit time (Onaga et al., 2002), and elicit satiety (Willis et al., 2009). However, the specific mechanism behind these effects remains unclear. Thus far, it is known that when food is consumed, it travels along the digestive tract stimulating the release of various pancreatic hormones responsible for digestion(Rose, 2019). Specific to the distal portion of the SI, when food reaches this point in the digestive tract, a hormonal feedback loop signals to the brain that there is sufficient food in the system and that food intake can be reduced. As such, gastric emptying decreases, satiety levels increase, and appetite levels decrease in a process known as the ileal break(Van Citters & Lin, 1999). Throughout this process, the absorption of nutrients is performed by the epithelial cells surrounding the intestinal walls. Along with these epithelial cells are endocrine L cells, responsible for slowing down peristalsis and

increasing the rate of segmentation(Van Avesaat et al., 2015). The concentration of these cells increase when descending the digestive tract to ensure increased absorption with higher food intakes(Van Avesaat et al., 2015).

6. Resistant Starch Digestion

For the resistant aspects of a starch to elicit their proposed appetite suppressing effects in the colon, they must be able to surpass digestive processes within the SI(Shi & Maningat, 2017). When starches are consumed, they travel along the SI in a similar manner to any other food, breaking down exponentially the further they travel. Starch molecules contain multiple components, organized with varying levels of RDSs, SDSs, and RSs (Shi & Maningat, 2017). As starch molecules travel through the SI, the RDS layers are met first, followed by the SDS, and finally the RS portion of the starch. However, due to the varying levels of these components within a starch polymer, a starch supplement organized with specific RDS;SDS:RS ratios, could ensure that the highest concentration of RS reaches the large intestine for the upregulation of anorectic hormones (Shi & Maningat, 2017). Taking this process into account, a method of targeting the delivery of glucose to the distal portion of the colon, and the fermentation of RSs in the colon are possible strategies for eliciting appetite suppressing effects; specifically, using high amounts of SDS and RS within a food supplement.

7. Effects of Resistant Starch on Glycemic Response

Currently, there exists several studies that have investigated the effects of RS supplementation on post-prandial glucose responses. A study done in Arizona State University in 2021 examined the effects of supplementing 30g of RS in the form of a plain muffin, on glycemic response in

individuals living with obesity. The study included 8 participants who were randomly assigned in a 2 by 2 cross-over format. Their results indicated that post-prandial glucose levels were significantly lower than the control groups at the 2-hour mark, but also at the 30, 60, and 90 minute marks as well. Moreover, post-prandial insulin levels also showed significant reductions. Based on their findings, it had been suggested that 30g of RS distributed in a muffin could serve as an effective supplement for the reduction of the glycemic response. In addition to these findings, another paper by Pugh et al (2023) compared the effects of various RS types on glycemic responses in diabetic participants. In this review, researchers assessed 36 different randomized control trials where they found that both RS1 and RS2 lowered acute postprandial glucose, and that RS2 specifically, also improved acute AUC post-prandial insulin levels. However, although data was found to support the use of both RS1 and RS2, the researchers identified a clear need for additional studies on the effects of RS3 in order to elucidate its effect on glucose metabolism and for its use as a dietary supplement (Pugh et al., 2023).

8. Effects of Resistant Starch on Subjective Appetite Ratings

Aside from effects on the glycemic response, reductions in subjective appetite ratings are also a promising benefit of RS supplementation. However, there seems to be more insignificant effects rather than effective ones. One study in 2018 examined the acute effect of RS on food intake, appetite, and satiety in males living with obesity. They supplemented 48g of RS and assessed post-prandial glucose, appetite, insulin, and GLP-1 every 30 mins for 7 hours. These researchers found significant reductions in EI, however, there were no significant effects of feelings of satiety, although they were measured 24h following supplementation (Al-Mana & Robertson, 2018). Moreover, an older study by Emilien and colleagues (2014) also found that when fed RS muffins,

although participants showed significant reductions in EI, no significant reductions in subjective appetite ratings were found. This has led to much confusion surrounding the effects of RS on subjective appetite since there have been consistent reductions in food intake, while appetite changes seem to remain insignificant (Emilien et al., 2017). However, a meta-analysis in 2020 did seem to enhance the current knowledge on appetite ratings. The analysis, completed by Amini et al (2020) assessed various RS supplementation human trials, up to 2019, and found that in studies that utilized >25g of RS, specifically, there were significant reductions in subjective appetite ratings. However, in studies that provided <25g of RS, no significant reductions in subjective appetite were found. As a result, their findings provide promising support for effects on appetite so long as the RS supplement contains greater than 25g of RS (Amini et al., 2021).

9. Effects of Resistant Starch on Anorectic Hormone Responses

In the literature, various researchers have found upregulations of anorectic hormones following RS supplementation. A study by Zhou et al proposed that dietary forms of RS lead to increased satiety in rodents through increased daily plasma levels of PYY and GLP-1 (Zhou et al., 2008). To test their hypothesis, total PYY and GLP-1 plasma levels were measured in RS fed rats at various time points in a 24h period. The results indicated that total plasma levels of PYY and GLP-1 were increased over the 24h period, with body fat percentages being significantly lower than control fed rats after 10 days when food intakes remained similar. However, these findings failed to indicate how these anorectic hormones were upregulated. Although RS supplementation increased levels of PYY and GLP-1, it was later found in a follow-up study that this was due to an increase in short chain fatty acid (SCFA) production following RS fermentation. In their follow-up study, Zhou et al assessed the fermentation of RSs resulting in SCFA production in the gut which have been

suggested to increase proglucagon (a precursor for glucagon), and total PYY gene expression (Zhou et al., 2008). To do so, they sampled levels of proglucagon and PYY gene expression patterns in mice following exposure to certain SCFAs in vivo. When tested, exposure to SCFAs butyrate, propionate, and acetate all significantly increased proglucagon gene expression in an enteroendocrine cell line of RS-fed mice. Additionally, PYY promoter activity in the RS-fed mice was also significantly increased compared to control groups (Zhou et al., 2008). These findings are also supported by the findings of Bonnema et al (2015), who found increased blood levels of butyrate and other SCFAs following dietary supplementation of RS3 in human subjects (Bonnema et al., 2015). Although the findings in these studies were considered significant, the proposed mechanism behind these effects remained unclear (Zhou et al., 2008). However, a 2015 review by Byrne et al was conducted to understand the role of SCFAs on appetite regulation and energy homeostasis (Byrne et al., 2015). Their discussions coincided with the aforementioned information regarding the upregulation of PYY and GLP-1 following supplementation of fermentable carbohydrates. The authors suggested that supplementing fermentable carbohydrates have led to increased stimulation of endocrine L-cell, responsible for increasing circulating concentrations of anorectic gut hormones GLP-1, and PYY, via the production and metabolism of SCFAs (Byrne et al., 2015). This theory was further supported when acetate and propionate precursor knockout mice had reduced PYY and GLP-1 expressions in vivo following fermentable carbohydrate supplementation (Samuel et al., 2008; Tolhurst et al., 2012). However, it was also suggested that there are many different mechanisms responsible for the effects of RS supplementation on EI and appetite, and rather than there being a single mechanism in play, multiple pathways collectively affect appetite regulation and energy homeostasis (Byrne et al., 2015).

10. Effects of Resistant Starch on Energy Intake and Satiety

A commonly sought question in the literature surrounding RS supplementation is centred around changes in EI and satiety. One study by Willis et al (2009) observed increases in levels of satiety and overall levels of fullness in human subjects following ingestion of a non-specified RS compared to other fibers such as oat brans and polydextrose. The study involved 20 healthy men and women who were provided five different fibre types to assess effects on satiety. The researchers utilized a visual analogue scale (VAS) to rate satiety levels, where increases in satiety were found mostly with increases in RS fibres (Willis et al., 2009). These findings were also further supported in a paper by Anderson et al who assessed glycemic response, subjective appetite and food intake in young men (Anderson et al., 2010). The study involved four different treatment interventions with increasing RS content by mass. These included, maltodextrin, regular cornstarch, high-amylose starch, and whole grains, respectfully. When comparing the four different starch treatments, it was found that all treatments aside from the maltodextrin resulted in statistically significant reductions in energy intake compared to the control group (Anderson et al., 2010). However, whole grains (1242.8 +/- 74.6 kcal), which contained the highest RS content (66% by mass through increases in amylose) caused the greatest reduction in food intake (-267.8 kcal) compared to the control group (1510.6 +/- 61.1). Anderson et al also collected data at two different time periods (30mins and 120 mins) to assess at which time points the various treatments could produce anorectic effects(Anderson et al., 2010). Based on the results, it was found that RDS content was correlated with lower food intake at 30 minutes, however, higher RS content was correlated with reduced food intake at the 120-minute mark (Anderson et al., 2010). These findings suggested that higher RS content was positively associated with overall reduced food intake, and that 120 mins is required for RSs to exert effects on overall satiety.

11. Effects of Resistant Starch on Energy Expenditure and RER

Currently, there is a gap in the literature surrounding the overarching effects of RS on total EE as well as respiratory exchange ratio (RER). It had been proposed that through the supplementation of low glycemic index foods, there would be a compensatory decrease in postprandial substrate oxidation, further resulting in a lower RER shown through slower glucose release times (Shi & Maningat, 2017). An early study by Heijnen *et al* (1995) suggested that 27g of RS per day were sufficient to lower dietary-induced thermogenesis by an average of 90 KJ/5h. This was also found earlier by Raben *et al.* (1994), who demonstrated that RS supplementation resulted in lower postprandial glucose and insulin responses, correlating with a reduction in thermogenesis. In addition to these earlier findings, Higgins *et al.* (2004) further proposed that RS might improve insulin sensitivity which leads to decreased insulin levels postprandially, and subsequent reductions in the thermic effect of food by lowering the demand for insulin-mediated glucose uptake, suggesting a possible mechanism for the role of RS in reducing EE. Moreover, Robertson *et al.* (2005) observed ten participants who consumed a 30g of RS and found decreases in fat oxidation and increased carbohydrate oxidation following RS supplementation, suggesting a shift in substrate utilization that may contribute to a reduction in EE. Overall, the impact of these mechanisms on total EE and RER remains uncertain however, as compensatory effects may vary depending on the duration of RS supplementation, dosage, and individual metabolic responses (Shi & Maningat, 2017). Therefore, further research is crucial to better understand the relationships between RS, EE, and RER, and to explore whether different doses, forms of RS, and longer supplementation periods could yield more consistent and significant effects on energy metabolism.

12. Conclusion

Based on the current literature, a more resilient and effective form of RS supplement is required. Previous studies have noted RS3 to be a recognized source of RS and resilient to food processing and transformations, making its use in a supplement a promising strategy. Through utilization of the retrogradation process, an RS3 supplement can be manufactured to ensure that high levels of RS reach the colon for fermentation. Further, with only few purposed mechanisms available, this study aims to provide further insight into the method for which RS can elicit changes in the glycemic response, EI, appetite, and satiety, specifically though corroborating with the increased levels of anorectic hormones PYY and GLP-1 found in previous studies.

Study Objectives and Hypotheses

1. Study Objectives

The purpose of this pilot trial is to investigate the effects of a novel source of RS on appetite and EI, in the context of a dietary intervention aimed at reducing RI. In this research, participants were provided with three variations of a breakfast smoothie in a randomized, double-blind placebo-controlled within-subject design trial. The study included individuals living with overweight and obesity aged 20-24 years old. A first objective was to measure energy intake and appetite profiles before and after ad libitum consumption of a meal following a breakfast smoothie high in RS vs high in RDS. A second objective was to evaluate sensory properties and acceptability of the breakfast smoothie offered.

2. Hypotheses

1. The RS pre-meal snack will induce the lowest EI and enhanced satiety during ad libitum meal consumption.
2. The RS pre-snack meal will induce a lower post-prandial RER
3. Consumption of RS snack will be palatable and liked by participants.

Methods and Techniques

1. Setting

This pilot study was conducted in the Behaviour and Metabolic Research Unit (BMRU), located at the Lees campus of the University of Ottawa. Data collection occurred between April 2023 and July of 2024. Ethics certificate is provided in the appendix.

2. Study Population

Six individuals (2 males; 4 females) aged 20-24 years were included in the final study. These six participants were then assigned codes which contained a randomized order of breakfast smoothie conditions. Participant characteristics are presented in **Table 1**.

Participants were recruited through:

- Posting flyers on university campus
- Sending emails through the school of human kinetics student newsletter
- Instagram posts
- Referrals from participants

The following were the inclusion and exclusion criteria:

2.1 Inclusion Criteria:

- Males and females 18 to 55 years old.
- BMI in the overweight to obese category ($> 25.0 \text{ kg/m}^2$).

- Willing to comply with procedures and sign an informed consent form.

2.2 Exclusion Criteria

- Smoker (smoking is known to affect appetite).
- Known serious food allergies.
- Pre-existing cardiovascular disorders, including uncontrolled hypertension, heart failure, and myocardial infarction.
- Current use of thyroid medication, or any medication that could affect appetite.
- Excessive use of alcohol or alcoholism, or current addictions to opiates, cocaine or stimulants as measured by the drug abuse screening test.
- Attempts to lose weight within the last six months.
- A restrained eater based on cut-off score (11 or higher) on the Three-Factor Eating Questionnaire (TFEQ) (Stunkard & Messick, 1985).
- Pregnancy

3. Study Design

An outline of the study is shown below. The study was a randomized, double blind, placebo-controlled within participants design trial. The initial contact with participants was through email or text message to explain a general overview of the study and to get a rough idea of height and weight to ensure they were eligible for a screening visit. After consenting to the study and the initial screening visit, the participants were assigned a pre-randomized code that contained the order in which they would receive each of the 3 condition breakfast smoothies. The randomization process was computerized by the website researchrandomizer.org.

4. Study Procedure

Following an initial screening visit, participants came in on three separate occasions, receiving one of the three conditions in a randomized fashion per visit. The three experimental sessions were as follows: (Condition 1) a negative control condition containing 290 calories of a breakfast smoothie; (Condition 2) a positive control session, containing 130 calories of RDS (maltodextrin) dispersed in the breakfast smoothie accounting 420 calories total, and (Condition 3) an experimental session containing 130 calories of RS (in the form of starch beads) dispersed in the breakfast smoothie accounting to 420 calories total. Following each visit, participants incurred a 2–4-week washout period prior to returning for their next session.

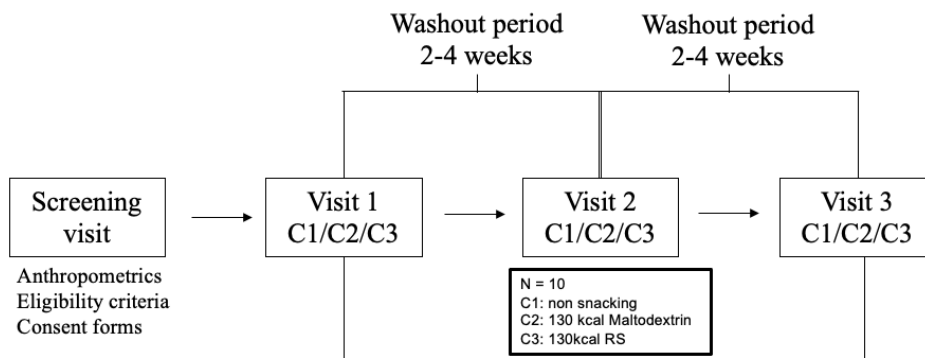


Figure 2. General Overview of Study Procedure with C1,2,3 indicating condition number. Non snacking = smoothie alone without RDS or RS.

5. Initial Screening Visit

After emailing participants and conducting a brief screening, interested participants were brought into the lab for a 30 minute in-person screening assessment. As soon as they arrived, potential participants were introduced to the study goals and procedures and were asked to sign an informed consent form. Then, weight and height were measured to ensure that their BMI was > 25.0 kg/m², and the participant completed a Three Factor Eating Questionnaire. Following these steps and

based on whether the participant met all inclusion criteria, the participant's first session was booked. Any participant who exhibited one or more characteristics of the exclusion criteria mentioned above was excluded from the study.

6. Experimental Sessions

Each of the three laboratory visits occurred as depicted in the figure below. Participants arrived at the laboratory after an overnight fast and fasting glycemia, energy expenditure, and appetite profiles were measured. At 8h30 participants were given 5 minutes to consume their refrigerated breakfast smoothie containing a total of 290 calories (condition 1) or 420 calories (condition 2 or 3). For all conditions: fasting blood was sampled at 7h30, followed by draws every 15 minutes within the first hour post-smoothie consumption. After the first hour, blood was drawn every 30 mins with the last draw taking place at 12h30. A fasted appetite profile (Visual Analogue Scale) was measured at 7h30. Following smoothie consumption, appetite profiles were measured every 30 minutes until 12h30. At 12h30, participants self-selected what they want to eat from a validated food-menu and energy and macronutrient contents of the food eaten were assessed. A final appetite profile was also done at 12h50 following ad libitum food consumption. During the session, the participants were asked to refrain from moderate to strenuous physical activity. They were allowed to read or use personal electronics during the waiting periods.

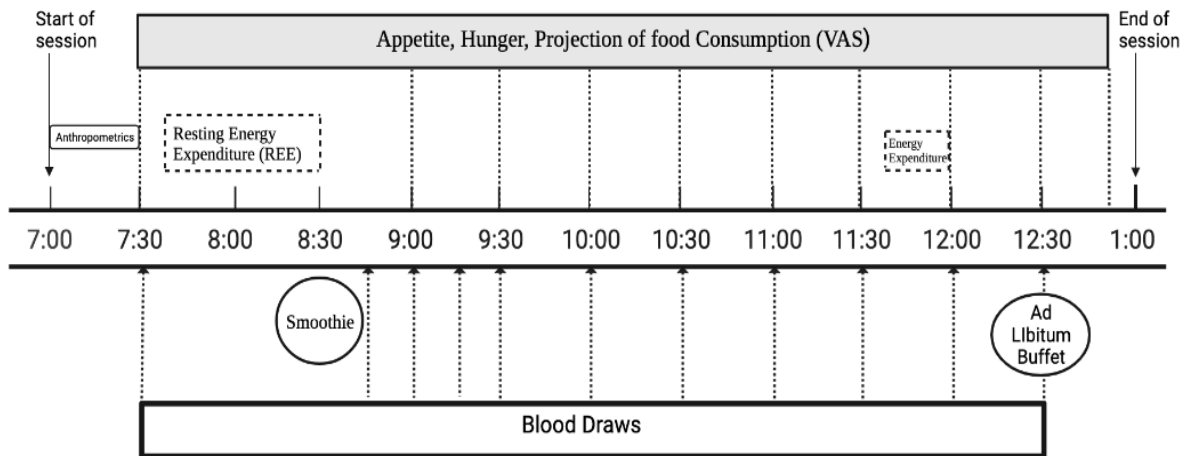


Figure 3. Flowchart of a typical day of collection.

7. Method of Supplementation

For this study, RS beads that contain high amounts of SDS and RS, with 90% RS content by weight, were created to be provided as an appetite suppressing supplement. They were created based on the retrogradation process, making them stable enough to withstand any food preparation processes, allowing for preservation of health benefits. The intended form of these beads made them suitable for inclusion in beverages such as fruit smoothies for a final product similar to a bubble-tea drink. They are organized in the spherical manner to protect the RS portions; utilizing a large volume of SDS as a protective coat to allow the highest level of RS to reach the large intestine. However, following mock runs of the beads, participants were found to struggle with their consumption, causing a re-evaluation of the supplement. As such, the beads were crushed to decrease their size, allowing for easier consumption. A photo of the beads as well as the caloric breakdown of the smoothie used can be found in the appendix.

8. Dose Rationale

The selection of the dosage for RS supplementation was based on several factors to ensure efficacy and practicality. Standard measures of glycemic response require 30-50 grams of available carbohydrates. Given that the breakfast smoothie contained this amount, adding 130 calories (32.5g) from RS was hypothesized to be sufficient for observing the desired effects without being digested (Anderson et al., 2010). Balancing efficacy with practicality, the dosage needed to be effective and realistic for regular consumption. Integrating RS beads into a smoothie format aimed to enhance participant compliance (Birt et al., 2013). Studies have shown that higher doses of RS (>25g) significantly affect appetite and energy intake. Therefore, the chosen dosage reflects these findings while remaining practical (Amini et al., 2021; Al-Mana & Robertson, 2018). The smoothie was balanced to ensure comparable total energy content, isolating the effects of RS supplementation (Shi & Maningat, 2017). The dosage also conformed to standard measures in glycemic response studies, ensuring comparability with other research (Pugh et al., 2023). In summary, the dosage of 130 calories from RS was chosen to balance scientific efficacy and practical application, making it feasible for real-world dietary practices.

9. Sample Size

Our initial target sample size was 12 participants based on similar exploratory studies in nutritional interventions, where small sample sizes have been sufficient to detect meaningful effects (Robertson et al., 2005; Higgins et al., 2004). However, due to recruitment challenges, the final sample size was reduced to 6 participants.

10. Measurements

10.1 Anthropometrics

Height (HR-100 Height Rod; Tanita Corporation of America Inc.) and body weight (HR-100, BWB-800AS; Tanita Corporation of America, Inc.) were measured after voiding the bladder. Body composition was determined using a DEXA scanner (Lunar Prodigy; General Electric). Coefficients of variation and correlation for the scanner will be 1.8% and $r = 0.99$ respectively.

10.2 Energy Expenditure

Indirect calorimetry was done to determine resting energy expenditure (REE) through the breath-by-breathe technique. Expired air will be collected into a mouthpiece and the metabolic cart will calculate REE and VO_{2max} . Measurements were taken at 2 different time points. A fasting measurement was taken for a total of 30 minutes, and a pre-lunch measurement was also taken for 20 minutes. These two measurements were repeated for each condition. The equation used for EE calculations was the Weir's $REE = (3.94 \times VO_2) + (1.1 \times VCO_2)$.

10.3 Visual Analogue Scale (VAS)

Food palatability and different sensations of appetite that are related to food wanting and motivation to eat (desire to eat and prospective food consumption) along with sensations related to hunger and fullness were measured. As such, a VAS questionnaire was completed every 30 minutes to evaluate hunger and satiety levels throughout all three conditions. A separate VAS questionnaire was also provided to assess liking of the breakfast smoothies immediately following ingestion.

10.4 Energy Intake

Food intake was measured through in-lab feeding. This was assessed by employing a previously validated lunch buffet to objectively measure *ad libitum* feeding inside a laboratory setting. Food

was offered in large amounts, and the participants were instructed that they had 20 minutes to eat until satiation was attained. All food was weighed before and after being provided to the participant. All food was weighed to the nearest 0.1g.

11. Statistical Analysis

A repeated measures analysis of variance (ANOVA) was used to compare all dependent variables found between the three experimental conditions. This type of analysis was used as there are more than two conditions and we are comparing within subjects. The within subject factors will include EI, energy expenditure, and VAS results. The ANOVA will compare values at each timepoint under each condition. The timepoints during the two-hour time trial are 60, 120, 180, 240 minutes. P-value significance will be set at 0.05.

Results

1. Participant Characteristics

Table 1: Baseline characteristics of all study participants

Category	Mean (SE)
Age (years)	21.2 (0.6)
Height (cm)	171.7 (3.9)
Weight (kg)	92.4 (13.7)
BMI	31.0 (3.8)

The CI of the mean assumes sample means follow a t-distribution with N - 1 degrees of freedom.

N=6 with 4 females and 2 males. Weight ranged from 66.5kg to 154.9kg

2. Effects of RS on Total EI and Macronutrient Intake

A repeated measures ANOVA was conducted to assess differences in total EI and specific macronutrient intakes following the provided ad libitum meal after the breakfast smoothies. As seen in **figure 4** there were no significant differences in total energy intake (kcal) between the RS and placebo groups ($F(2, 8) = 0.137, p = 0.874, \text{partial } \eta^2 = 0.033$). Additionally, the interaction between condition and sex was not significant ($F(2, 8) = 0.904, p = 0.443$), indicating that sex did not significantly influence the energy intake results. For protein intake (kcal), the within-subjects effects showed no significant differences between conditions ($F(2, 8) = 0.211, p = 0.814$) or the interaction between condition and sex ($F(2, 8) = 0.186, p = 0.834$). Similarly, the analysis for fat intake (kcal) indicated no significant differences between conditions ($F(2, 8) = 0.197, p = 0.825$) or the interaction between condition and sex ($F(2, 8) = 0.294, p = 0.753$). The carbohydrate intake (kcal) analysis also revealed no significant differences between conditions ($F(2, 8) = 1.12, p = 0.373$) or the interaction between condition and sex ($F(2, 8) = 1.58, p = 0.264$).

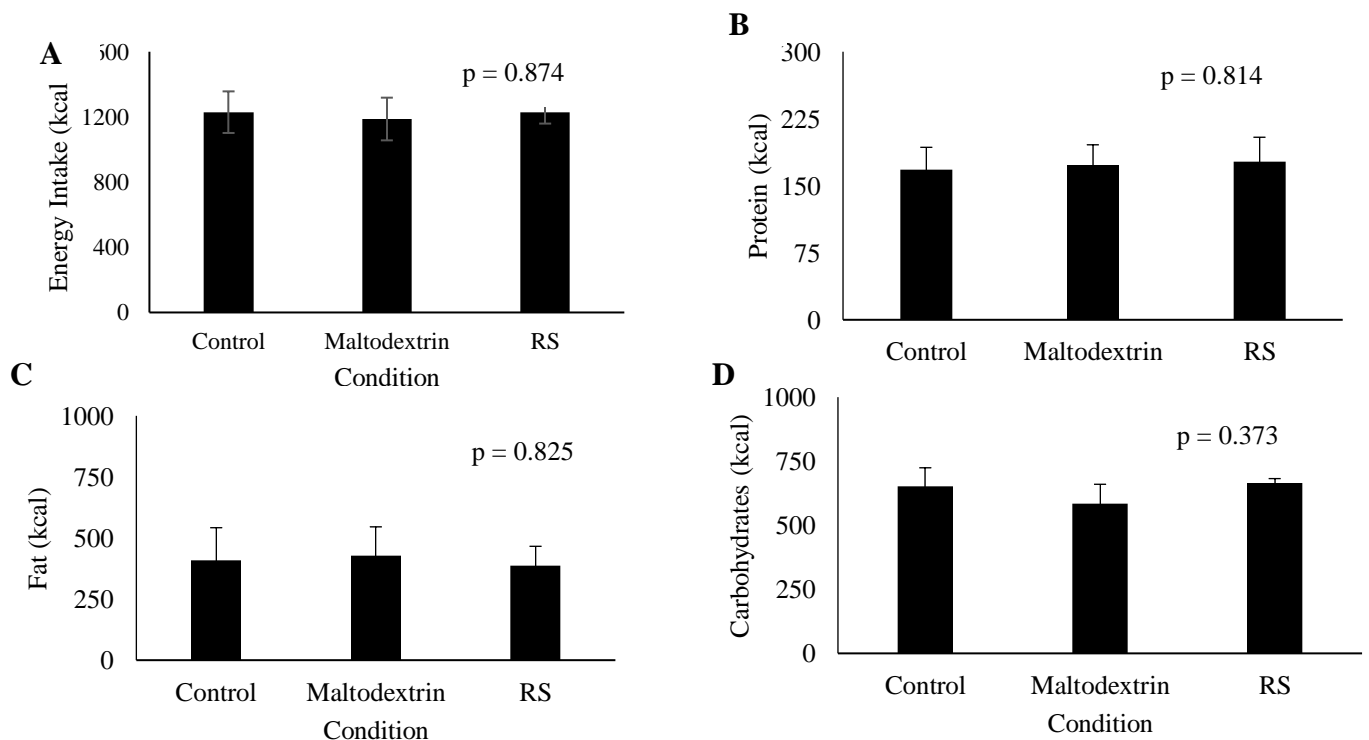


Figure 4. Energy intake (EI) and macronutrient composition (protein, fat, and carbohydrates) from an ad libitum meal following breakfast smoothies in three conditions: Control, Maltodextrin, and Resistant Starch (RS). Panel A shows total energy intake (kcal), Panel B shows protein intake (kcal), Panel C shows fat intake (kcal), and Panel D shows carbohydrate intake (kcal). There were no significant differences between conditions for total energy intake ($F(2, 8) = 0.137, p = 0.874$), protein intake ($F(2, 8) = 0.211, p = 0.814$), fat intake ($F(2, 8) = 0.197, p = 0.825$), or carbohydrate intake ($F(2, 8) = 1.12, p = 0.373$). Furthermore, no significant interactions were observed between condition and sex across all measures ($p > 0.05$). Bars represent mean \pm SE C1 = Control, C2 = Maltodextrin, C3 = RS.

3. Effects of RS on Appetite Variables & Palatability

A repeated measure ANOVA revealed significant differences in VAS scores for desire to eat over time, with large effect sizes ($F(5, 15) = 27.379, p < 0.001, \text{partial } \eta^2 = 0.901$). However, as seen in **figure 5A** no significant differences were found between conditions ($F(2, 6) = 0.742, p = 0.515, \text{partial } \eta^2 = 0.198$) or the interaction between condition and sex ($F(2, 6) = 1.714, p = 0.258, \text{partial } \eta^2 = 0.364$). For hunger VAS scores (**figure 5C**), there were significant differences over time ($F(5, 15) = 20.186, p < 0.001, \text{partial } \eta^2 = 0.871$), but no significant differences between conditions ($F(2, 6) = 0.596, p = 0.581, \text{partial } \eta^2 = 0.166$) or the interaction between condition and sex ($F(2, 6) = 1.962, p = 0.221, \text{partial } \eta^2 = 0.395$). The fullness VAS scores (**figure 5B**) also showed significant differences over time ($F(5, 15) = 11.538, p < 0.001, \text{partial } \eta^2 = 0.794$). There were no significant differences between conditions ($F(2, 6) = 0.411, p = 0.680, \text{partial } \eta^2 = 0.120$) or the interaction between condition and sex ($F(2, 6) = 0.884, p = 0.461, \text{partial } \eta^2 = 0.228$). Finally, for prospective food consumption (PFC) VAS scores (**figure 5D**), significant differences were found over time ($F(5, 10) = 17.382, p < 0.001, \text{partial } \eta^2 = 0.897$), but no significant differences between conditions

($F(2, 4) = 0.424, p = 0.681, \text{partial } \eta^2 = 0.175$) or the interaction between condition and sex ($F(2, 4) = 0.290, p = 0.763, \text{partial } \eta^2 = 0.127$).

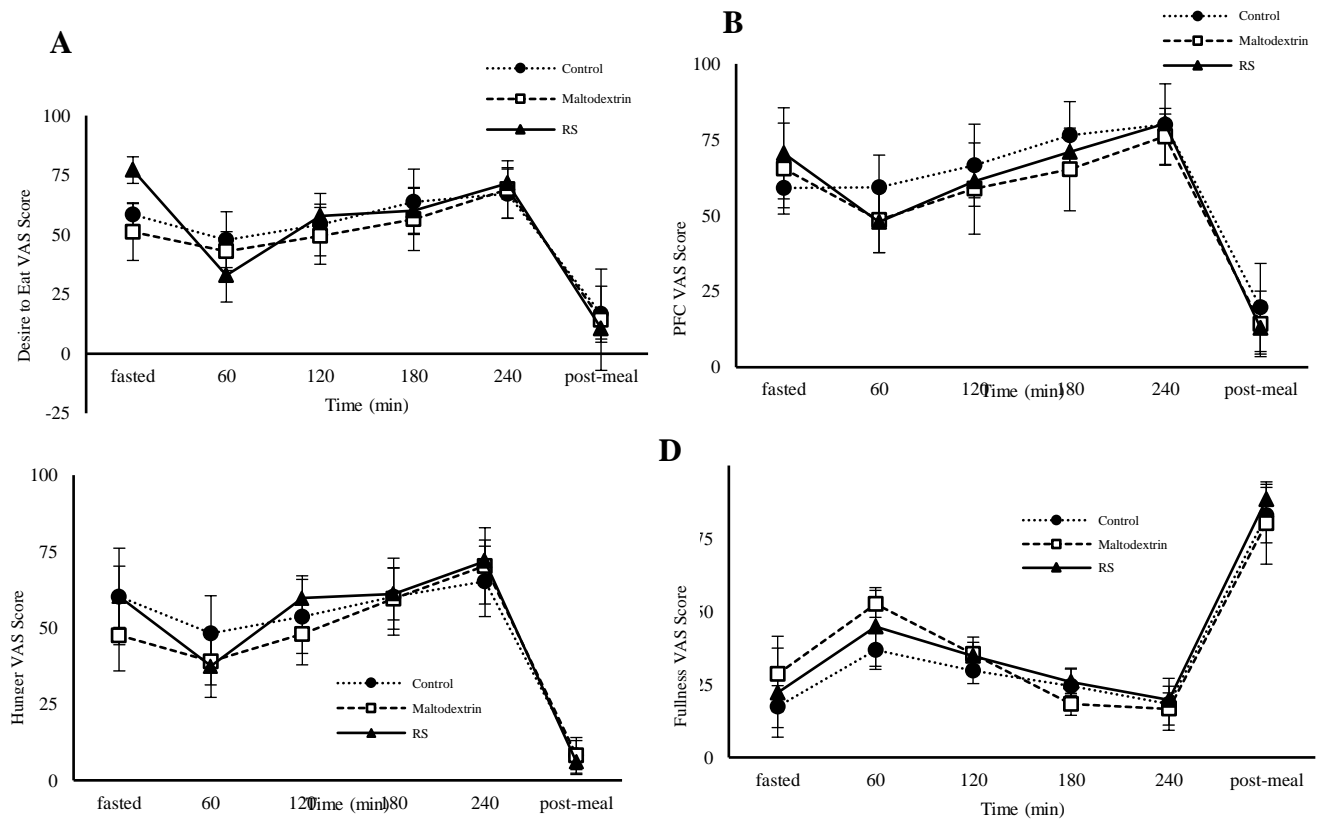


Figure 5. Visual Analog Scale (VAS) scores for appetite-related measures over time.

(A) Desire to eat, (B) Fullness, (C) Hunger, and (D) Prospective food consumption (PFC) scores were assessed at different time points during the study (fasted, 60, 120, 180, 240 minutes, and post-meal). Data are presented as mean \pm SEM for each condition: Control (dotted line), Maltodextrin (dashed line), and Resistant Starch (RS, solid line). **Desire to eat (A)** ($F(5, 15) = 27.379, p < 0.001, \text{partial } \eta^2 = 0.901$), **Hunger (C)** ($F(5, 15) = 20.186, p < 0.001, \text{partial } \eta^2 = 0.871$), **Fullness (B)** ($F(5, 15) = 11.538, p < 0.001, \text{partial } \eta^2 = 0.794$), and **PFC (D)** ($F(5, 10) = 17.382, p < 0.001, \text{partial } \eta^2 = 0.897$). However, no significant differences between conditions or

interactions between condition and sex were observed for any measure (all $p > 0.05$). Participant code (DRS06) excluded in (A).

Additionally, as seen in **figure 6**, analyses on starch palatability revealed no significant between the RS and placebo groups over time ($F(2, 6) = 1.573, p = 0.282, \text{partial } \eta^2 = 0.344$). Additionally, there were no significant interactions between condition and sex ($F(2, 6) = 0.480, p = 0.641, \text{partial } \eta^2 = 0.138$). The between-subjects effects also showed no significant differences in palatability based on sex ($F(1, 3) = 0.0338, p = 0.866, \text{partial } \eta^2 = 0.011$).

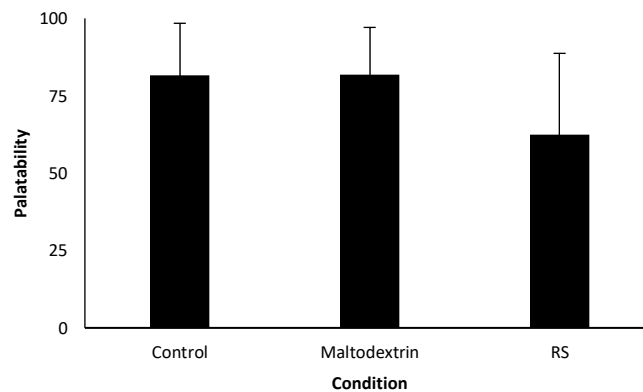


Figure 6. RS snack palatability VAS scores across all conditions. C1 = control, C2 = Maltodextrin, C3 = RS.

4. Effects of RS on EE and RER

A repeated measures ANOVA revealed no significant differences in total energy expenditure (EE) between the RS and placebo groups over time ($F(2, 6) = 2.53, p = 0.160, \text{partial } \eta^2 = 0.457$). Additionally, there were no significant interactions between condition and sex ($F(2, 6) = 2.40, p = 0.171, \text{partial } \eta^2 = 0.445$). For VO_2 , the within-subjects effects showed no significant differences between conditions ($F(2, 6) = 3.30, p = 0.108, \text{partial } \eta^2 = 0.524$) or the interaction between condition and sex ($F(2, 6) = 2.07, p = 0.208, \text{partial } \eta^2 = 0.408$). The VCO_2 analysis indicated no significant differences between conditions ($F(2, 6) = 1.15, p = 0.379, \text{partial } \eta^2 = 0.276$) or the

interaction between condition and sex ($F(2, 6) = 0.0042, p = 0.996, \text{partial } \eta^2 = 0.001$). For RER, there were no significant differences between conditions ($F(2, 6) = 0.436, p = 0.666, \text{partial } \eta^2 = 0.127$) or the interaction between condition and sex ($F(2, 6) = 0.182, p = 0.838, \text{partial } \eta^2 = 0.057$).

Table 2. Indirect Calorimetry Mean Values While Fasted and Post Snack Across all Conditions.

Measure	Control		Maltodextrin		RS	
	Fasted	Post-snack	Fasted	Post snack	Fasted	Post-snack
EE/day	1537 (106)	1570 (103)	1520 (64)	1504 (61)	1543 (122)	1671 (85)
VO2	0.225 (0.01)	0.230 (0.02)	0.223 (0.01)	0.219 (0.01)	0.230 (0.02)	0.247 (0.01)
VCO2	0.170 (0.02)	0.180 (0.01)	0.163 (0.01)	0.167 (0.01)	0.166 (0.01)	0.185 (0.005)
RER	0.751 (0.04)	0.835 (0.13)	0.714 (0.03)	0.786 (0.05)	0.717 (0.03)	0.746 (0.04)

Note. EE/day is expressed in kcal/day, VO2 and VCO2 are in L/min, RER is VCO2/VO2. (SE brackets)N=5 with 1 participant excluded because the tube was disconnected during testing.

Discussion

The objective of this study was to investigate the effects of novel resistant starch (RS) beads on appetite variables, energy intake, and food palatability in individuals living with overweight or obesity. The overarching goal was to evaluate RS as a potential dietary intervention for appetite control. Our findings did not support the hypothesis that RS supplementation would lead to significant reductions in total energy intake or improvements in appetite sensations compared to placebo groups. Despite previous studies suggesting that RS can enhance satiety and reduce energy intake, the RS group in our study showed no significant differences in desire to eat, hunger, fullness, or prospective food consumption (PFC) ratings. Secondly, contrary to our initial hypothesis, the RS supplementation did not significantly alter metabolic markers, or energy

expenditure and substrate partitioning. Finally, there were no significant differences in food palatability across the different conditions.

1. Effects of RS on Total EI and Macronutrient Intake

Our study showed that consumption of 130kcal of RS did not elicit any significant changes in total EI or specific macronutrient intakes of carbohydrates, fat, or proteins when compared to both MDS and control conditions. This coincides with several studies that have found similar results with use of RS supplementation. A study by Haub *et al.* (2010) utilized similar experimental design to our study while using RS2 supplementation on body weight and appetite control and found no significant reductions in EI compared to control groups. They hypothesized that the lack of effect could have been due to the type of RS used, the dosage, or individual participant metabolic differences. However, we report similar results using RS3, suggesting that different forms of RS may not be the cause for the non-significant effects on EI. Another review by Rashed *et al* (2021) examined RS3 supplementation with respect to both EI and metabolic markers in diabetic patients. Their review indicated that while RS3 improved glucose tolerance and increased the production of short-chain fatty acids (SCFAs), there was no significant reduction in total energy intake (Rashed et al, 2021). One possible explanation for this could be that RS3's was unable to increase satiety enough to counterbalance other factors influencing eating behavior in a free-living environment, where participants had the autonomy to choose what and how much to eat. A review by Brunstrom (2011) assessed anticipatory eating patterns and found that in *ad libitum* research settings, participants have resorted to pre-meal planning where they modified eating behaviours prior to feeding. Such anticipatory eating behaviors could have manifested as a decreased food consumption with the expectation of an abundant meal the next day (Brundstrom, 2011).

Therefore, strategic adjustments might attenuate the satiety effects of RS3, as psychological factors related to meal anticipation could overshadow the physiological signals of fullness. As a result, it is recommended for future studies to attempt to control feeding behaviour prior to study trials to ensure that pre-meal planning is not a confounding factor in EI assessments.

2. Effects of RS on Appetite Variables & Palatability

It was initially hypothesized that 130kcal of RS presented in a smoothie totaling 420kcal would elicit changes in these appetite variables based on the findings presented by Amini *et al* (2021) who reported that studies that utilized ≥ 25 g of RS showed significant reductions in rating of appetite than studies with RS doses < 25 g. However, our findings show that there were no significant differences in desire to eat, hunger, fullness, or perceptive food intake from use of our 130kcal RS3 supplement. One reason for this could be attributed to the dosage of starch provided. A study by Hollis *et al* (2014) found similar results in their assessment of healthy individuals. In the study they provided participants with a higher calorically dense breakfast (696kcal) of resistant wheat starch (RWS) muffins (16g protein, 102g carbohydrates, 24g fat, 26g fiber) and assessed subjective appetite using VAS over the course of 690 minutes, but still found no significant differences for any of the subjective appetite measures across all 4 variables. One suggested cause for these effects was attributed to differences in caloric composition of the RS breakfast provided as well as the dosage. Our study utilized 130kcal of RS3, however, based on our findings and those by Hollis *et al* (2014) and Amini *et al* (2021) it is possible that insufficient dosages of RS were provided to elicit changes in appetite variables. A study by Sandberg *et al* (2017) found that 50g of RS was necessary to significantly affect both appetite and desire to eat. Furthermore, another study by Luhovyy *et al* (2014) found that when comparing low vs high dose RS supplementation,

there were significant differences in subjective appetite variables in the high dose group (22.5g RS) compared to both controls (11g and 0g RS). As a result, it is possible that future studies should assess differences in RS dosing to see if they could elicit more significant effects on appetite ratings. However, it must also be noted that aside from increasing doses, perhaps future studies should also attempt at discovering a more calorically efficient supplement as there also comes a point where significant increases in supplement doses correlated with reductions in EI could also be attributed to the caloric load of the supplement rather than its overarching biological effects.

With regards to palatability and overall appraisal of the RS breakfast smoothie, it was hypothesized that consumption of the RS snack would be palatable and liked by participants. Accordingly, we found no significant differences in liking of the RS snack in comparison to control conditions, suggesting that the snack was neither liked nor disliked by participants. This prompts future studies to continue to attempt at finding a form of RS supplementation that is liked by consumers.

3. Effects of RS on EE and RER

Our final objective for this study was to assess the effects of RS on total EE and RER. It was hypothesized that 130kcal of our RS supplement would elicit a lower post-prandial EE and RER based on the initial findings of Heijnen *et al* (1995) who suggested that 27g of RS per day were sufficient to lower dietary-induced thermogenesis by an average of 90 KJ/5h. However, our study found no significant differences between the RS and control group, which seems to support the current consensus in the literature to date. A review by Higgins (2014) confirmed that there remains inconclusive data and suggested that it is possible that RS could change total EE via its fermentation process, but the studies conducted were too short to capture the true effects (Higgins *et al.*, 2014). It is well established that the fermentation of RS in healthy adults takes from 6-8

hours, which could vary further in individuals living overweight or with obesity. They also mentioned that post-meal EE peaks approximately 90 mins after RS ingestion compared to 30 mins in digestible starches, suggesting that RS could elicit changes in EE, but studies have not provided sufficient time frames to find significant effects (Higgins et al., 2014). Therefore, these findings seem to suggest that further studies should continue to assess whether different measurement times or starch modalities, such as type or dosage, could elicit significant reductions in REE (Higgins, 2014).

Aside from the analysis of EE, one noteworthy finding from this analysis was the slight decrease in RER found between the post-snack maltodextrin and RS groups, respectively. Although statistically insignificant, it is interesting to note that there was a decrease in RER post-snack following RS supplementation compared to in the maltodextrin group. This could be attributed to a slower release of glucose with the RS supplement, however these findings remain purely speculative and would require statistical significance to be further supported.

Limitations, Strengths, and Recommendations

An important limitation of this study was the small sample size ($n=6$). With only six participants, it is much more challenging to detect real effects of RS supplementation on the outcomes measured. This reduced power increases the risk of Type II errors, where true effects are missed because the study lacks the sensitivity to identify them. Additionally, small sample sizes can lead to exaggerated or underestimated effect sizes. For example, the large effect size observed for VO₂ (Partial $\eta^2 = 0.524$) might be an overestimation or underestimation of the true effect, simply due to the variability inherent in such a small sample. This makes it difficult to generalize the findings to a broader population complicating the interpretation of results and limiting the ability to draw

firm conclusions about the efficacy of the RS supplementation. Our *a priori* aim was to have closer to 12 participants to elicit our anticipated results, however, due to participant attrition, scheduling variables, and COVID protocols, we were unable to gather a sufficient study population. Additionally, one of our participants had to be removed from the study due to weight fluctuations beyond the inclusion criteria set for our study, and another participant's data was excluded from several analyses due to insufficient data obtained from the VAS questionnaire and malfunctioning of the metabolic cart.

Another limitation of this study was the large range of BMI values accepted in the study. We had set the criteria for individuals living with overweight (BMI >25) or obesity (BMI > 30), however, this resulted in large BMI fluctuations ranging from as low as 25.85 to 49.68 kg/m². It's been found that individuals of greater size will elicit different changes in total EE, appetite ratings, and glycemic response (Dakin et al., 2023) creating potential for concern especially considering our limited sample size. (weight-based supplementation). Additionally, another limitation of this study was the short duration of our intervention. Our study spanned a total of 6 hours which was anticipated providing ample time for digestion of RS and to elicit effects on our objectives. However, several other studies have started to implement broader time frames for data collection which could serve to answer further questions with regards to the effects of these RS on appetite and energy markers. Specifically, a paper by Guo *et al* (2021) conducted a broad-spectrum review on multiple RS supplementation papers and indicated that a possible reason for a large proportion of insignificant findings could be attributed to the difference in the intervention durations. Specifically, a study by Dodeska *et al* (2016) utilized a quasi-experimental design of RS3 supplementation over the course of 12 months and found significant effects on participant weight reduction, whilst other papers that assessed the same weight loss markers (over the course of 12

weeks at the most) did not find any significant effects (Guo *et al.*, 2021). This suggests that further studies may benefit from implementing different intervention durations to fully understand the potential effects of RS supplementation on overall EI, appetite markers, and EE.

Conclusion

In sum, our study explored the effects of RS supplementation on appetite sensations, EI, and REE in individuals with overweight or obesity, compared maltodextrin (MD) and control conditions. Our findings did not reveal significant differences in total EI, desire to eat, hunger, fullness, PFC, or EE such as REE, VO₂, VCO₂, RER. While these results suggest that RS, in the dosage and form used, did not significantly impact the measured parameters, this does not preclude the possibility of meaningful effects under different conditions, as larger population sizes and varied intervention designs may provide clearer insights into the role of RS in appetite regulation and metabolic health.

General Discussion & Future Perspectives

The global prevalence of obesity and related metabolic disorders has reached alarming levels, prompting extensive research into various dietary interventions aimed at managing weight and improving metabolic health. Among the strategies explored, the use of RS has gained attention due to their potential role in modulating appetite, reducing EI, and enhancing metabolic markers such as PYY and GLP-1. However, contrary to expectations based on previous literature, the results of our study did not reveal significant differences between the RS, MD, and control conditions with respects to appetite sensations, EI, or metabolic outcomes. Key measures such as total EI, desire to eat, hunger, fullness, and PFC showed no significant changes attributable to RS

supplementation. Similarly, metabolic markers including REE, VO₂, VCO₂, and RER were not significantly altered by RS. These findings suggest that, within the parameters of this study, RS supplementation may not exert a strong effect on appetite regulation or metabolic processes in individuals living with overweight or obesity.

1. Anticipatory Feeding Behavior and Its Impact on Study Outcomes

One potential explanation for the lack of significant findings could be the role of anticipatory feeding behaviors (Brunstrom, 2011). It is possible that participants, knowing they were involved in a study focused on dietary interventions, may have adjusted their eating patterns before the controlled feeding sessions. This anticipatory behavior could mask the true effects of RS supplementation, as participants may have altered their intake in anticipation of the study, thus reducing the observable impact during the actual sessions (Brunstrom, 2011). Future studies should consider methods to control for such behaviors, perhaps by standardizing pre-study meals or by implementing strategies that minimize participants' ability to alter their eating habits prior to the intervention.

2. Weight-Based Dosing of RS Supplements

Another factor that may have influenced the outcomes is the dosage of RS used in the study. While the dosage was selected based on prior research, it may not have been sufficient for all participants, particularly those with higher body weights (Higgins, 2014). Our study had a BMI range of 25.85 to 49.68 kg/m², which prompts for a larger possibility of error associated with these variances. Larger individuals may require higher doses of RS to achieve the desired physiological effects of enhanced satiety and reduced EI. The concept of weight-based dosing is well-established in

pharmacology and could be applied to nutritional interventions like RS supplementation (Barras, 2017). As a result, future research should explore the use of weight-adjusted RS dosing to determine whether this approach can yield more significant and consistent results across a diverse population.

3. Acute vs. Long-Term Supplementation

In addition to the dosage of RS provided, the duration of RS supplementation in this study was relatively short, focusing on acute effects over the course of a single session compared to two control conditions. While this approach allowed for the examination of immediate responses to RS, it may have been insufficient to capture the potential long-term benefits of sustained RS intake. Dietary interventions often require extended periods to manifest their full effects, particularly in terms of appetite regulation, energy balance, and metabolic health. For instance, Guo *et al* (2021) indicated in their review that an important future step in the assessment of RS supplementation on TEE, appetite profiles, EI, and metabolic biomarkers (GLP-1 and PYY) is in the duration of trials as they could account for additional delayed effects of RSs. As a result, future studies should consider longer-term supplementation protocols to assess whether prolonged RS intake can lead to more pronounced and lasting changes in appetite control and energy expenditure.

4. Limitations of the VAS Questionnaire

The use of the VAS questionnaire, while common in appetite research, presents certain limitations. VAS relies on subjective self-reports, which can be influenced by various factors, including the study environment, participant expectations, and even the time of day. While VAS is a valuable tool for assessing immediate sensations, it may not fully capture the complexity of appetite

regulation, especially in a controlled lab setting where participants might not behave as they would in a more naturalistic environment. Additionally, VAS does not account for the psychological and emotional factors that can influence eating behavior, which could lead to an underestimation or overestimation of true appetite levels (Douglas and Leidy, 2019). Future research should explore the integration of more objective measures (metabolic markers, gastric emptying studies) to complement VAS assessments and provide a more comprehensive understanding of appetite regulation.

5. Challenges in Monitoring Food Intake

Another significant limitation of the study was the challenge of accurately monitoring food intake, both in the laboratory and in free-living conditions. In the lab, while it is possible to control and measure food intake precisely, this environment is inherently artificial and may not reflect real-world eating behaviors. Participants may alter their intake simply because they are aware they are being observed (McCambridge et al., 2014). Conversely, however, in free-living conditions, it becomes difficult to monitor food intake accurately, as self-reported food diaries are prone to inaccuracies, underreporting, and recall bias (Breit et al., 2023). The inability to monitor participants' food intake outside the lab means that the study could not account for potential compensatory eating behaviors that might occur between sessions. As a result, future research can explore the idea of an in-ward study with multi-day observations using advanced dietary assessment tools, such as wearable devices or mobile applications that track food intake in real-time, to enhance the accuracy and reliability of dietary data.

6. Overall Assessment and Future Directions

Despite the limitations, this study contributes to our understanding of the potential role of RS in dietary interventions. While our RS supplement used did not produce significant effects under the conditions of this study, it is essential to recognize that dietary interventions are complex and multifaceted. Our results suggest that RS may not be sufficient on its own to produce significant changes in appetite or energy intake in individuals with overweight or obesity. However, the study also highlights several areas where RS supplementation could be refined and optimized, such as adjusting the dosage based on body weight, extending the duration of supplementation, and combining RS with other dietary strategies to enhance its effectiveness. The findings also delineate the importance of considering the broader context in which dietary interventions are implemented. Factors such as anticipatory feeding behaviors, the limitations of self-reported measures, and the challenges of monitoring food intake in free-living conditions can all play a crucial role in shaping the outcomes of dietary studies. By addressing these challenges in future research, we can gain a more accurate understanding of how RS and other dietary components influence appetite regulation, energy balance, and overall metabolic health. Additionally, another noteworthy insight to the study was the caloric assessment of the RS supplement. Based on previous literature, it was assumed that the RS beads contained 4kcal per gram due to the structural similarities to other carbohydrates (Shi & Maningat., 2017), however, other evidence has suggested that this assumption can be challenged. An earlier study by Nugent (2005) had suggested that due to RS's ability to withstand digestive processes. Nugent suggested that RS should hold a 2kcal/g value, however, newer studies did not seem to exercise this notion further. However, given the results obtained from this intervention, it is noteworthy to explore this concept further in future studies.

Moving forward, several key areas should be prioritized in future research. First, studies should explore the effects of weight-based dosing to determine whether larger individuals require higher RS doses to achieve the desired outcomes. Second, extending the duration of supplementation to assess the long-term effects of RS on appetite and metabolism is crucial. Third, the integration of objective measures alongside self-reported tools like VAS will provide a more comprehensive assessment of appetite regulation. And finally, the true caloric value of RS and their effects on energy expenditure.

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APPENDIX

14/12/2023

Université d'Ottawa

Bureau d'éthique et d'intégrité de la recherche

University of Ottawa

Office of Research Ethics and Integrity

CERTIFICAT D'APPROBATION ÉTHIQUE | CERTIFICATE OF ETHICS APPROVAL

Numéro du dossier / Ethics File Number	H-06-20-5885
Titre du projet / Project Title	Novel resistant starch beads in appetite control and regulation of body energy
Type de projet / Project Type	Recherche de professeur / Professor's research project
Statut du projet / Project Status	Renouvelé / Renewed
Date d'approbation (jj/mm/aaaa) / Approval Date (dd/mm/yyyy)	15/10/2021
Date d'expiration (jj/mm/aaaa) / Expiry Date (dd/mm/yyyy)	14/10/2024

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Bolthouse Farms Mighty Mango



Per 290 kcal

- Carb: 67.1g
- Fat: 0.56g
- Fiber: 2.23g
- Sugars: 58g
- Protein: 2.23g