Investigation into the palatability of whole wheat versus intermediate wheat grass (Kernza) flour in choice and no-choice feeding paradigms

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Abstract

The perennial grain Kernza (*Thinopyrum intermedium*) has environmental advantages over annual wheat, e.g., it grows deeper roots that improve soil stability, and water and nutrient retention. Importantly, Kernza has a distinct composition, including higher fibre and protein levels than wheat, suggesting its consumption might convey health benefits. However, composition affects palatability, which calls into question whether Kernza is as palatable as wheat. Hence, this thesis aimed to investigate the acceptance of and preference for Kernza in comparison to whole wheat flour in rats, an established feeding research model. Three variations of diets were used: mash, slurry, and pellets, to account for processing as a factor affecting palatability. Food intake experiments were followed by the analysis of the reward system's activation (using a neuronal activation marker, c-Fos) in response to a Kernza vs wheat meal. Acceptance was assessed in the no-choice meal paradigms, in which a single diet was offered to the animals. Regardless of whether meals were given either after or without prior food deprivation, and during the day or at nighttime, rats consumed similar amounts of Kernza- and wheat-based diets. Similarly, a sub-chronic 9-day Kernza vs wheat meal exposure, showed no difference in acceptance. In preference paradigms in which two diets were offered simultaneously, rats preferred Kernza over standard chow, but wheat was preferred over Kernza. Both wheat and Kernza were less preferred than hyperpalatable high-fat high-sugar pellets. Finally, the c-Fos immunoreactivity (IR) analysis in animals that have eaten a similar amount of Kernza or wheat (versus unfed controls), showed that regardless of the diet offered, activation of the ventral tegmental area, nucleus accumbens shell (AcbS), and nucleus accumbens core, is higher in response to food intake. However, consumption of wheat produced greater c-Fos IR the AcbS, which is in line with this grain being preferred over

Kernza. Overall, I conclude that Kernza is an accepted and palatable flour, however, the preference of wheat over Kernza and the reward system's activity changes indicate that Kernza is somewhat less palatable than wheat.

Acknowledgements

Firstly, I must thank my supervisor, Pawel Olszewski, for your reliability and always being happy to share your vast wealth of knowledge, along with many interesting stories and jokes. Secondly, a massive thank you to everyone in our lab that has offered me support and guidance over the last 1.5 years; Anica Klockars, Laura McColl, Tapasya Pal, Mitchell Head, Donisha Liyanagamage, Savannah Harvey, Robin Jervis, and Shaun McNeil – I have really appreciated your help, advice, and friendship, which has been invaluable throughout my research. I enjoyed learning so much from all of you and deeply admire your positivity! A special thank you to my amazing friends who always tell me to never give up, and for being so understanding - Pamela, Pammy, Emma, Greg, Ethan, Linda, and Francis. Lastly, I am so grateful to my partner Jesse - for the unconditional support you have offered me since we first met, you are the best. P.S: and thank you to Andy for being the sweetest cat-son in the world.

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List of Abbreviations

Acb: nucleus accumbens

AcbC: nucleus accumbens core

AcbS: nucleus accumbens shell

AgRP: agouti-related peptide

ARC: arcuate nucleus

BDNF: brain-derived neurotrophic factor

BS: brainstem

CART: cocaine- and amphetamine-regulated transcript

CB1: cannabinoid receptor 1

CCK: cholecystokinin

CNS: central nervous system

D1: dopamine receptor 1

D2: dopamine receptor 2

DAB: diaminobenzidine

DAT: dopamine transporter

DMN: dorsomedial nucleus

DMX: dorsal motor nucleus

EAA: essential amino acids

ECS: endocannabinoid system

FA: fatty acid

FFA: free fatty acids

FFAR1: fatty acid receptor 1

FFAR2: fatty acid receptor 2

FFAR3: fatty acid receptor 3

FFAR4: fatty acid receptor 4

GABA: gamma-aminobutyric acid

GHS-R1A: growth hormone secretagogue receptors

GI: gastrointestinal

GLP-1: glucagon-like peptide-1

H202: hydrogen peroxide

HCD: high-carbohydrate diet

HFD: high-fat diet

HFHS: high fat high sugar

HMW: high molecular weight

IR: immunoreactivity

IWG: intermediate wheatgrass

LA: lipase activity

LCFA: long-chain fatty acid

LFD: low-fat diet

LH: lateral hypothalamus

LOX: lipoxygenase

MC3R: melanocortin-3 receptor

MC4R: melanocortin-4 receptor

MCFA: medium-chain fatty acid

ME: median eminence

ML-DA: mesolimbic dopaminergic system

MOR: Mu1,2,3 receptors

NaCl: sodium chloride

NPY: neuropeptide Y

NTS: nucleus solitary tract

PFA: paraformaldehyde

PFC: prefrontal cortex

POMC: proopiomelanocortin

PP: pancreatic polypeptide

PVN: paraventricular nucleus

PYY: peptide tyrosine-tyrosine

SCFA: short-chain fatty acids

TBS: tris buffered saline

VMN: ventromedial nucleus

VP: ventral pallidum

VTA: ventral tegmental area

α-MSH: α-melanocyte-stimulating hormone

Chapter 1

Introduction

1.1 Annual Wheat: Dominating the Agricultural Landscape and Diet

Given that the global human population is forecast to reach 9.8 billion by 2050 and food production is expected to double, it is critical to ensure there is an adequate and high-quality food supply to meet the population's requirements (1,2). Annual crops serve as the foundation of our food supply, either directly or indirectly, through their use as fodder for our livestock. The bulk of these crops are cereal grains, legumes and oilseeds, and they cover approximately 69% of the world's crop lands (3). Globally, cereal grains serve as the main dietary source of carbohydrates, energy, and plant proteins, with 41% used for human consumption and up to 35% for livestock feed (4). The most widely consumed and grown cereal grains are wheat, rice and maize, with minor grains including oats, barley, rye, triticale, and millet (5). Wheat (*Triticum aestivum*) is grown more widely than any other crop (6), contributing to 28% of global crop production (7).

Grains have been a significant part of our diet since the domestication of wheat first occurred approximately 10,000 years ago; its cultivation and capability to be stored long-term enabled the feeding and survival of large populations (6). Over the last 3,000 to 4,000 years, wheat has remained a predominant component of our diet worldwide (5) due to its significance of being a main dietary source of carbohydrates, providing up to 70% of our dietary energy requirements (8). Wheat is commonly processed into various baked goods and functional ingredients such as bread, noodles, pasta, cakes, couscous, flour, and beer, but is primarily consumed via cereals and bread (6,9).

Grains were predominantly consumed in their whole form until the 19th century. Historically, the low income majority perceived refined white flour consumption as aspirational due to white bread being primarily consumed by the upper class (10). Over the last century, there has been a significant shift in global grain consumption to refined products (5,10). This shift is attributed to technological innovations such as the roller mill, which increased production and enabled large-scale processing of refined flour. The grain-refining process eliminates any contaminants that may adhere to the bran and germ, enhancing grain stability for global distribution. The increased production and availability of refined grains led to a food surplus, resulting in lowered prices and greater affordability for consumers (10).

As the global population continues to grow, there is an increasing pressure on our farms to produce unprecedented levels of food; the current scale of annual grain production has become unsustainable for the environment (3). Livestock demands have increased, driven by rising consumer demand for animal products like meat and dairy, and further strains the system (11). Large-scale production of annual grain crops has proven detrimental for the environment, harming the quality of soil, water, air, and natural ecosystems due to the modern agricultural methods required by these crops. It has become critical to address the environmental impacts of traditional grain production by exploring more sustainable alternatives to meet our grain requirements and ensure food security in the future (8). The following section will discuss a viable alternative that has potential to address these challenges.

1.2 Kernza: A Perennial Grain Alternative to Annual Wheat

In the pursuit of an alternative to annual wheat, perennial agriculture has become a growing field of interest for researchers and farmers who seek to reduce the environmental impacts

of annual wheat production. A promising candidate for a perennial grain crop is *Thinopyrum intermedium* (Figure 1.1), an intermediate wheatgrass (IWG) also known as Kernza® (which is trademarked by The Land Institute). For the sake of readability, I will refer to it as 'Kernza' throughout this document. Kernza gained popularity because it is a multi-functional crop, capable of protecting ecosystems while also providing us with a food source. This species is a distant cousin of wheat and survives for multiple years without replanting. Kernza is of particular interest due to its nutritional content, containing substantially higher fibre and protein than annual wheat grain. The environmental and health benefits of Kernza will be explained in detail in the below subsections of this document. Currently, Kernza is primarily used as livestock feed and is commercially available to farmers. However, ongoing genetic breeding and mass selection are aimed at optimising its traits for human consumption. Kernza first appeared in consumer food products in 2013. Today, several artisan brands now incorporate Kernza as a wheat substitute in a range of products, including beer, spirits, baked goods, cereal, and bread. In 2020, Perennial Pantry became the first dedicated Kernza-processed food brand.

Figure 1.1: Photograph of Kernza (*Thinopyrum intermedium*) in the research fields at The Land Institute in Salina, Kansas, USA. Photo from http://www.kernza.org.

The potential of IWG as an annual wheat alternative was recognised by The Rodale Institute, USA. They pioneered IWG development in 1983 initiating breeding programs and screening for varieties with preferable traits, such as large seeds and plant fertility. In 2001, The Land Institute, USA, obtained germplasm of these IWG varieties from The Rodale Institute and are now leading the research to further improve the plants seed size, grain quality, yield, and resistance (12). Originally from Eurasia, Kernza was introduced to North America a century ago (13) - it is now actively planted across 3,979 acres of land in the United States (Figure 1.2), including newly planted 1,698 acres in 2022 that are expected to yield in 2023. The total harvest of grain came to 454,133 pounds across 12 states in 2022, with the largest growing states being Montana, Kansas, Minnesota, and Wisconsin (14). Though Kernza is still in development, advances in breeding programs and the expansion of experimental farming of the crop could make a transition from annual to perennial grain crop farming feasible (15). Kernza's environmentally protective traits make it a more sustainable option compared to annual wheat, providing some hope for the agricultural landscape.

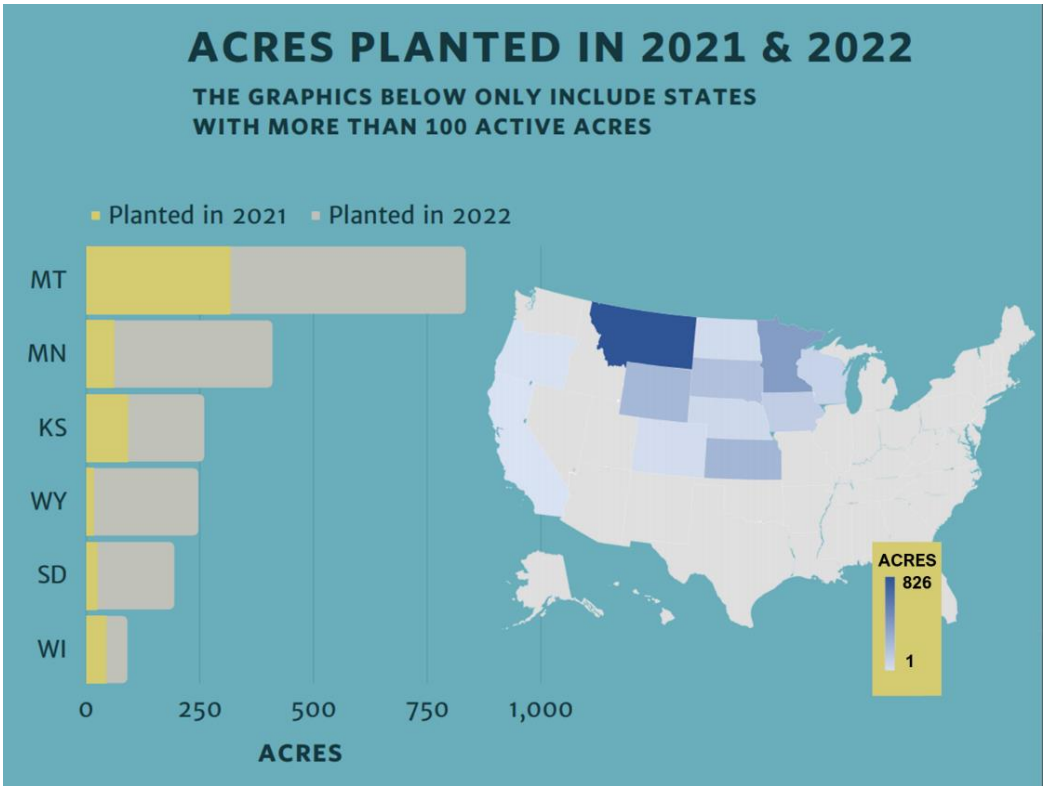

Figure 1.2: Acres of Kernza planted (left) in 2021 (green) and 2022 (grey) across 6 states in the USA (MT, Montana; MN, Minnesota; KS, Kansas; WY, Wyoming; SD, South Dakota; WI, Wisconsin). Map of USA (right) features heat-map demonstrating the acres planted in 2021 and 2022, across 12 states (14).

1.2.1 Environmental Impact of Kernza: Repairing the Damage from Annual Grain Crops

Prior to the agricultural conversion of land, diverse stands of perennial plants covered most of the world's landscape. However, these natural ecosystems, including many others, have been displaced by monocultures of annual crops (8,16). As annual crops only live for one year, their cultivation has harmed the environment through land degradation, greenhouse gas emissions, and water pollution, causing the ecosystem to fall into a state of disrepair (17). In contrast, perennial plants like Kernza provide many ecosystem services, including positive impacts on soil health, biodiversity, water quality, and climate. This is largely possible due to their inherent advantage of a deep and expansive root system, and capability of maintaining year-round ground cover (18). Maintaining ecological services are essential for environmental

health and sustainable production of food, providing benefits that impact all living organisms, not just humans.

Healthy soil is a critical component of ecosystem health, providing important services like habitat provision, water filtration, nutrient cycling, and carbon sequestration. Soil degradation is a major threat to the ecosystem and stems from the modern agricultural production of annual crops, as they require tilling each year before replanting. A combination of frequent tilling and lack of vegetative cover causes soil erosion, loss of soil organic carbon and nutrient run-off, and reduced fertility (17). This leads to a decline in agronomic productivity and production. Currently, soil erosion is occurring at a greater rate than soil formation, so is considered a non-renewable resource (19,20). Perennial grains have extended growing seasons, remaining in the ground during fallow seasons (3,20) and continue to grow for up to three years without the need for tilling (8,21). Kernza possesses dense, deep roots that grow over 3 meters long (Figure 1.3) (22), which safeguard the soil from erosion by holding it in place. This trait is a result of perennial species evolving to prioritise nutrient uptake and root longevity, critical for maximising resource conservation and root persistence. In contrast, annual species have shorter roots with a more specific root length in order to maximise nutrient acquisition during their shorter life span (23).

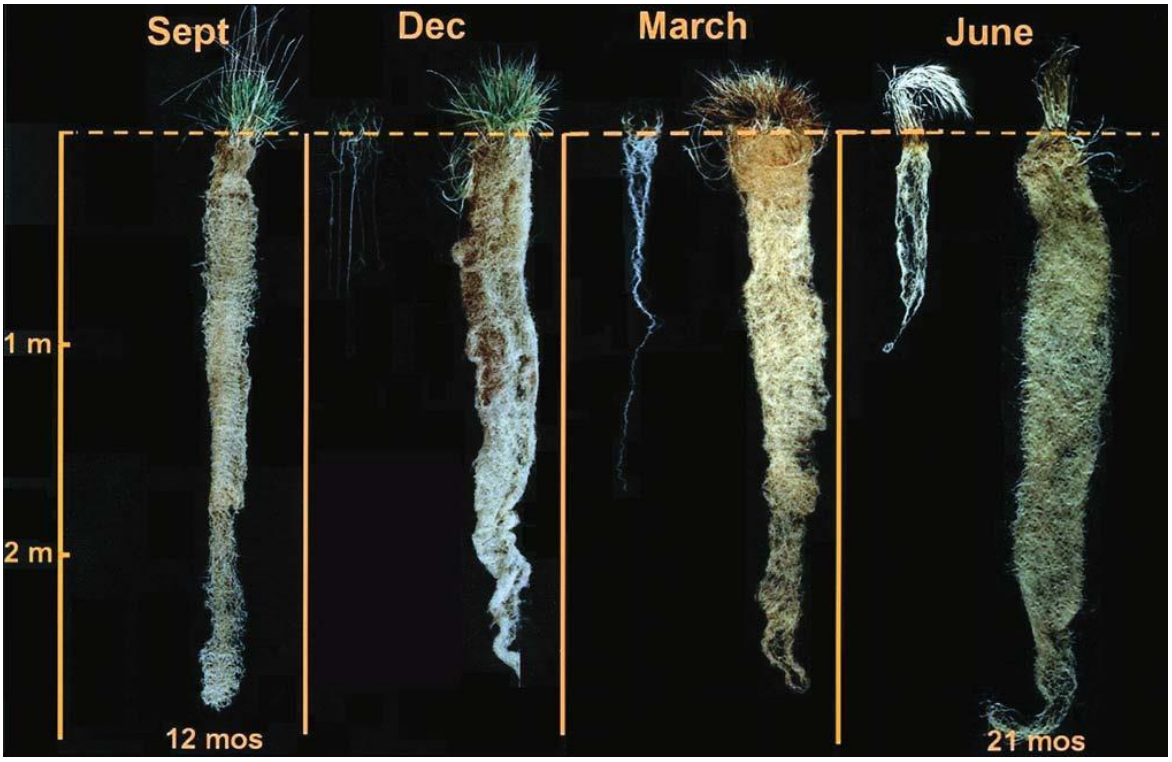

Figure 1.3: Comparison of the root systems of annual wheat (on the left in each panel) and intermediate wheat grass (on the right in each panel) at four-month intervals over a year (September, December, March, and June), beginning with 12-month-old plants. Kernza's root system is much more dense and grows deeper than annual wheat (3).

The extensive root system of perennial plants enables them to utilise and retain water and nutrients more effectively compared to annual crops (20). Kernza has shown to maintain high water-use efficiency throughout its growing season (24), which results in reduced water loss, subsurface drainage and surface runoff (15). Due to its superior nutrient retention, Kernza requires less nitrogen fertiliser compared to most other annual wheat crops. It efficiently captures any applied nitrogen which reduces nitrate leeching from the soil. This allows Kernza to be planted in fields that are prone to nitrate leeching, and prevents groundwater contamination (17,21).

Annual grain crops require careful preparation and management, spanning from seedbed preparation to harvest (3). Annual tiling and replanting of crops results in substantial input demands, including labour, pesticides, fertilisers, and energy (20). Effective management of

farming inputs is essential for sustaining agricultural production (8). A switch to perennial farming would result in a significant decrease in tillage, labour, and fuel consumption (20). This transition would lead to greater food calorie production per unit of fossil fuel used. The soil erosion caused by annual grain crops, in conjunction with emissions from modern farming machinery used to maintain these crops, contributes significantly to carbon dioxide emissions. These emissions contribute to climate change, and the temperature changes that are predicted by climate-change models will have a detrimental effect on the survivability of annual crops (3). Compared to annual grains, Kernza exhibits higher rates of carbon sequestration, net carbon uptake, and long-term carbon storage (22). Soil organic carbon significantly influences soil quality, health and functionality (25), playing a crucial role underground (26).

Kernza currently provides nutritious forage for cattle (13), but having year-round vegetation on our crop lands also provides essential habitats for wildlife (20) by reducing soil disturbance and increasing diversity of soil fauna. This includes supporting nematode communities (15) and enhanced complexity of soil microbes, namely arbuscular mycorrhizal fungi (20). These fungi are essential for increasing nutrient availability and uptake, as well as disease resistance through their symbiotic relationships with plants, all in exchange for carbon (26). Increased diversity of fauna and flora in the soil improves the soil structure and water cycling, as well as increasing plant productivity (20).

Over the last century, food prices declined due to the rises in food production (11), but have been increasing over the past decade, driven by increased demand and crop losses from weather events (6). Annual plants are more vulnerable than perennials because they must restart their growth cycle from a small seed each year, which is why perennials were

historically the dominant plants, outcompeting annuals for resources (17). Annual crops are more reliant on favourable weather conditions within a limited timeframe to grow, and are susceptible to stress due to their short growing season (3). Crop loss and increased demand are likely to continue due to climate change and loss of agricultural land to the production of non-food goods (6). In particular, the production of biofuels is increasing and is beginning to compete for the land used for food production (11,27). Most land that is suitable for growing annual crops is already in use for this purpose (27), and perennial crops provide a major benefit by their capacity to grow on erosion-prone land that annual grains cannot successfully grow on. A transition back to a perennial system has the potential to repair some of the adverse ecosystem and environmental effects caused by annual agriculture and help with long term food security for our growing population.

Since the cultivation of wheat, farmers have aimed to improve yield of annual grain crops, and as a result since the 1900s nearly 75% of plant genetic diversity has been lost worldwide replaced by genetically uniform varieties of our edible plant species, resulting in a loss of dietary diversity (Figure 1.4) (10). Perennial crops have the potential to provide more yield throughout their growth over several years, but they are not currently grown on a large scale. They are still in the early stages of domestication and have low yields compared to annual grain, as their yield decreases after the first harvest (20). For perennial crops to become adopted as a mainstream alternative, high seed yield is essential, and this is recognised by many scientists, such as The Land Institute, who working on improving Kernza's traits through genetic breeding.

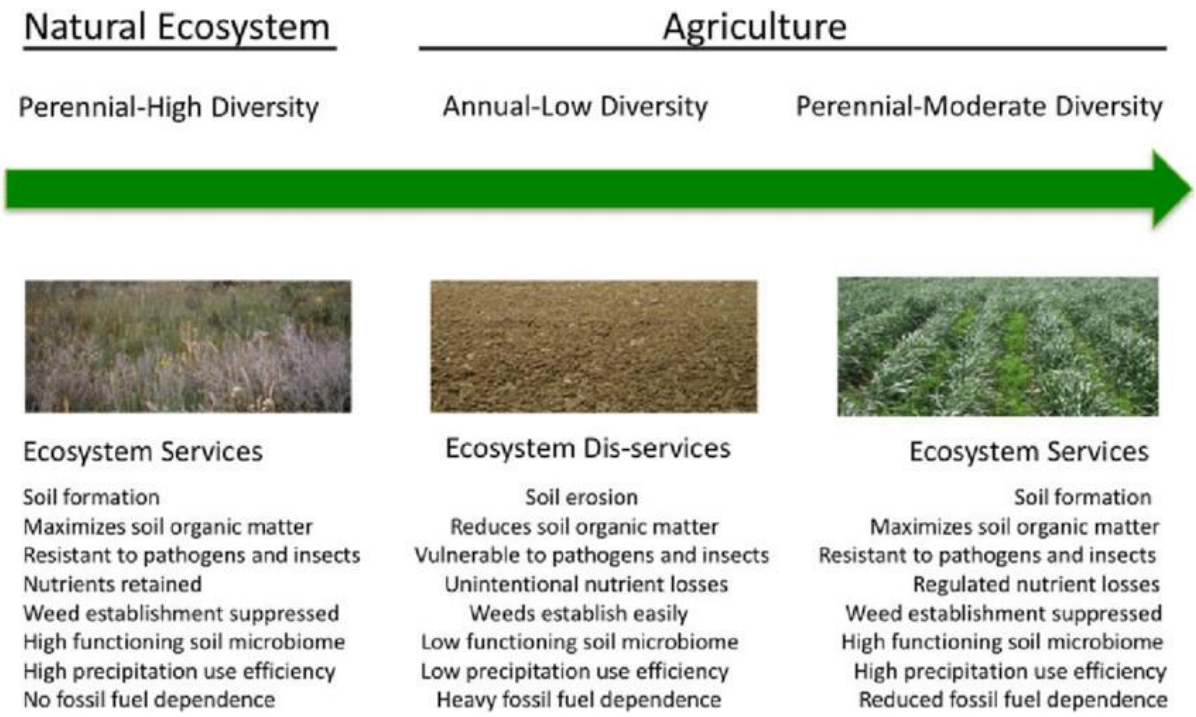

Figure 1.4: Demonstrates the eco-system services and dis-services by the transition of a natural ecosystem of high perennial diversity to a low diversity of annual crops driven by agriculture, and the aim of moderate perennial diversity (17).

Breeding programs help to ensure food security by increasing yield and traits that allow better crop survival under harsh environmental conditions. Historically, selection and hybridization by farmers improved the yield of wheat, and once scientific plant breeding took over, yield was increased even further (6). Thus, due to the long-term and intense seed selection for desirable traits, annual grain crops currently have a higher seed yield than perennial crops (3,8). It is essential for annual wheat to have optimal seed production and dispersal due to its shorter lifespan (28), whereas the fitness of wild perennial plants is more dependent on the survival of the vegetative structures than its seed traits. Intermediate wheatgrass propagates through rhizomes, tillers (above-ground stems) and seeds (the grain) (3). All grains have a protective hull containing an endosperm, bran, and germ (Figure 1.5). The endosperm is composed of about 50 -75% starch and 8-18% storage proteins – and the starch is the energy source for the plant embryo contained within the germ. The bran surrounds the endosperm and germ, providing protection from the environment (5). The small seed size and low yield

of perennial plants are likely a result of natural selection. Kernza has a seed size four to five times smaller than that of annual wheat (22). However, the seeds have a higher bran to endosperm ratio compared to wheat, resulting in lower starch content, but higher protein, ash, mineral and fibre content (29).

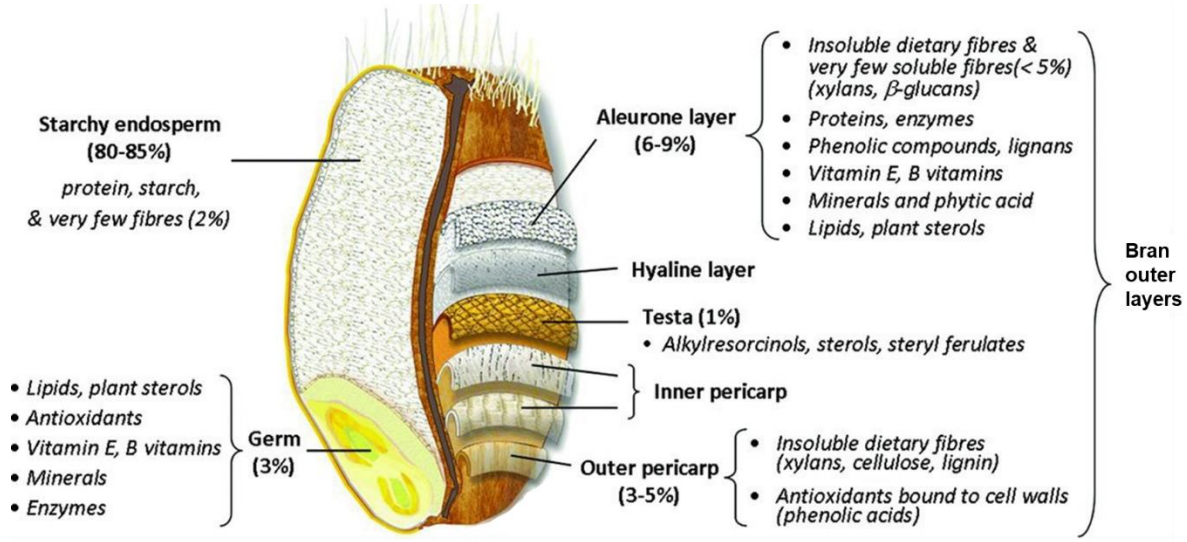

Figure 1.5: The structure of the wheat grain. The germ is the innermost component, contributing to 3% of the total grain size, and is rich in lipids, plant sterols, antioxidants, vitamins E and B, minerals, and enzymes. Surrounding the germ is the endosperm, contributing to 80-85% of the total grain size. The endosperm is mainly comprised of protein and starch, with a small amount (2%) of fibre). Surrounding the germ and endosperm is the bran, which is comprised of multiple layers, each with differing compositions. The aleurone layer contributes to 6-9% of the grain size, and contains insoluble dietary fibres, proteins, enzymes, phenolic compounds, vitamins, minerals, and lipids, with less than 5% soluble fibre content. Additional layers of the brain include the hyaline layer, testa, inner pericarp, and outer pericarp (30)

1.2.2 Kernza Consumption: Potential Impact on Health Outcomes

While Kernza's environment benefits are clear, the potential health outcomes of substituting wheat with Kernza remain uncertain due to a lack of available literature. The health outcomes of whole wheat consumption have been extensively studied, especially pertaining to the effects of dietary fibre and protein on health. When consumed in whole grain form, wheat is considered to be an important source of beneficial components such as dietary fibre, resistant starch, oligosaccharides, and antioxidants (5). Compared to wheat, Kernza contains higher

levels of dietary fibre, protein, and antioxidants, and lower levels of gluten and starch (12). Per 100g, Kernza contains 18g of dietary fibre and 19.2g of protein, compared to whole wheat which contains 10.8g of dietary fibre and 9.2g of protein (31). In New Zealand, Edmonds whole wheat flour contains 12.5g of dietary fibre and 11.6g of protein per 100g. As we know that Kernza's nutritional profile differs from that of wheat, containing a higher content of beneficial nutrients that are essential for a healthy diet, it is reasonable to consider that its consumption could also have an impact on health outcomes.

Consumption of dietary fibre has proven to have many benefits for our health (32). From the early 1800s, whole grains were recommended to prevent constipation (5). Whole grain consumption and its benefit to human health has since been extensively researched (10), and we now know that intake of whole grain food is protective against many diseases including diabetes, cardiovascular disease, cancer, and obesity (Figure 1.6) (5). Dietary fibre refers to the carbohydrates from plants that are indigestible to humans but influence our digestion and absorption of nutrients in the upper gastrointestinal (GI) tract (5) and is classified as two group: soluble and insoluble fibre. Soluble fibre consists of mainly polysaccharides found in plant cell walls, such as pectin, and these are hydrolysed and absorbed in the small intestine. Insoluble fibre are complex carbohydrates resistant to digestion, but are fermentable by bacteria in the colon, including lignin and cellulose (33). Fibre impacts the motility and transit time of the gut, help modulate the gut's immune system and influence the microflora in our colon (32). Whole grain consumption has been associated with higher diversity and growth of beneficial bacteria of the gut and colon, which are stimulated by fibre and oligosaccharides. These bacteria in turn produce short chain fatty acids (SCFA) from soluble fibre which is essential for bowel health and suppression of chronic inflammation (10,34). The bulk of

dietary fibre in grains is contained in the wheat bran, and is mostly insoluble fibre, and contains phenolic compounds that have antioxidant effects. Bioactive compounds in wheat bran have been associated with apoptosis of colon cancer cells (35).

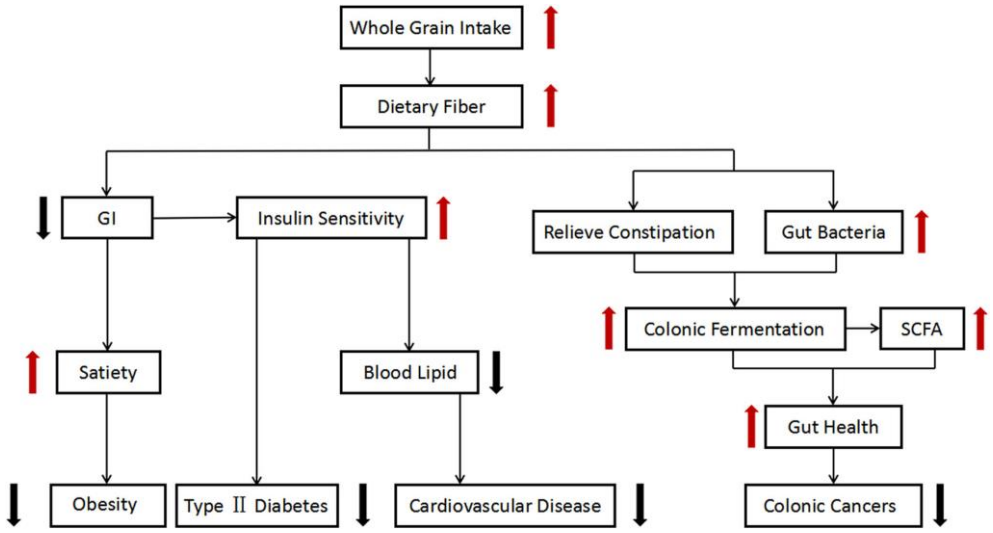

Figure 1.6: Whole grain consumption increases dietary fibre intake which is protective against major chronic diseases. Gastrointestinal (GI) signalling to the brain when the stomach is distended provides the feeling of satiety which is protective against obesity, a disease of overconsumption. Signalling also increases insulin sensitivity, decreasing risk of diabetes, and lowers the level of blood lipids, which is protective of cardiovascular disease. Dietary fibre relives constipation, provide a food source to bacteria in the gut and colon, increasing their diversity. They then provide us with short chain fatty acids (SCFA) which are beneficial to our gut health and protective against colon cancer (35).

Due to the health benefits of dietary fibre, it is recommended by the United States Department of Agriculture that whole grain foods should be consumed at least three servings a day. Despite this, Americans consume a lot fewer whole grains than the recommended daily serving, and this is similar across other countries. There is a widespread lack of consumer knowledge about the health benefits of whole grain consumption. The mass global consumption of refined grains leads to poor health outcomes due to the removal of key nutrients from the flour during processing. This results in refined grains possessing lower total dietary fibre, particularly insoluble fibre, and a higher concentration of starch due to the bran and germ being removed during the milling process (5). Refined grains also lack in micronutrients (10), and despite mandatory nutrient fortification of refined grain products in

some countries, the refinement process still results in an overall reduction of nutritional quality (36). Swaminathan et al. (37) conducted a large global study on the association between dietary intake of refined grains on health outcomes. It was found that high intake of refined grains was associated with major cardiovascular events and higher risks of mortality. Obesity also results from mass consumption of refined grains, which creates a major health concern worldwide (10).

Proteins are a fundamental part of our diet, however, dietary protein on its own does not hold nutritional value, only once it reaches the small intestine and is hydrolysed into amino acids to be utilised by the body. While our body does produce non-essential amino acids itself, some cannot be synthesised or replaced by any other dietary nutrients other than protein. Amino acids are precursors for important proteins and peptides that we need to function (38). Whole wheat bread contains high protein content compared to other breads, but it lacks some of the indispensable amino acids (39). Refined flour has even lower contents of essential amino acids than whole wheat (9). IWG contains higher levels of all essential amino acids than wheat, however similar to wheat, it is also limited by lysine (40). Overall, the protein content of whole wheat is considered inadequate for consumers (39). There are protein-fortified food products on the market, but these are not designed for older people. Our protein requirement increase as we age due to changes in metabolism and increased illnesses (41) so it is important for there to be easily accessible and familiar foods with sufficient protein content for the aging population.

Edible grains such as wheat are capable of being produced into bread and other baked goods as their flour contains a protein complex known as gluten (42). Gluten increases the hydration and swelling of starch granules when heated in a liquid, the subsequent gelatinisation is

necessary for high dough stability and obtaining a stable structure of the final baked product (43). Kernza has a much lower gluten content than wheat, in particular, lacking in high molecular weight (HMW) gluten (44), which poses some challenges when using it as the standalone flour in bread products, as the poor gluten network formation causes issues with rising in bread (12,29). However, flour lacking HMW gluten is suitable for certain applications such as flat breads, tortillas, pancakes and crackers (43). While the low gluten content may cause some challenges for consumers wishing to use Kernza flour for baking, retailers producing Kernza food products have currently overcome this by using a mix of Kernza flour with wheat flour to improve baking stability. The lower gluten content in Kernza could also be viewed as an advantage by individuals with gluten sensitivities.

1.2.3 Key Knowledge Gap: Kernza's Palatability in Comparison to Wheat

While Kernza shows a lot of promise as an alternative grain to traditional wheat through its potential health and environmental benefits, we do not know what consumer acceptance would be of this grain. The differences in nutritional composition between Kernza and whole wheat are of such that it may potentially affect acceptance and preference for Kernza, its palatability being an essential factor influencing its adoption as a staple food source. While The Land Institute states that Kernza is a palatable grain, and a small-scale sensory study using various amounts of IWG in baked goods showed acceptability among consumers (40), little is known about the flavour performance of IWG and its impact on flavour in comparison to wheat (45). There is a current lack of studies in humans and laboratory animals to determine the palatability and preference of Kernza to annual wheat. This gap in knowledge is being investigated in this thesis through a series of experiments, using a standard animal model of

food intake, for acceptance and preference testing and by investigating the reward response after a meal.

1.3 Homeostatic Mechanisms of Energy Intake

Eating is an integral part of energy homeostasis, which is our body's mechanism to maintain an energy equilibrium through balancing food intake and energy expenditure in order to maintain a stable body weight (46). This equilibrium is tightly regulated through coordinated and complex bi-directional communication between peripheral metabolic organs and the central nervous system (CNS), known as the gut-brain axis (46,47). The metabolic organs, such as the pancreas, liver, adipose tissue, muscle, and the GI tract secrete hormonal signals that act in synergy to effect eating behaviour, contributing to the short-term and long-term regulation of food intake and energy balance, as shown in Figure 1.7 (48). The short-term regulation of food intake is perceived as the sensation of satiation and leads to a decreased drive to eat as food is ingested (49), resulting in termination of eating, thus controlling the amount of energy consumed during a meal (50). Satiety is the period after satiation is reached (46), and is the fullness feeling that persists after eating, suppressing further energy intake and affecting the time between meals (50).

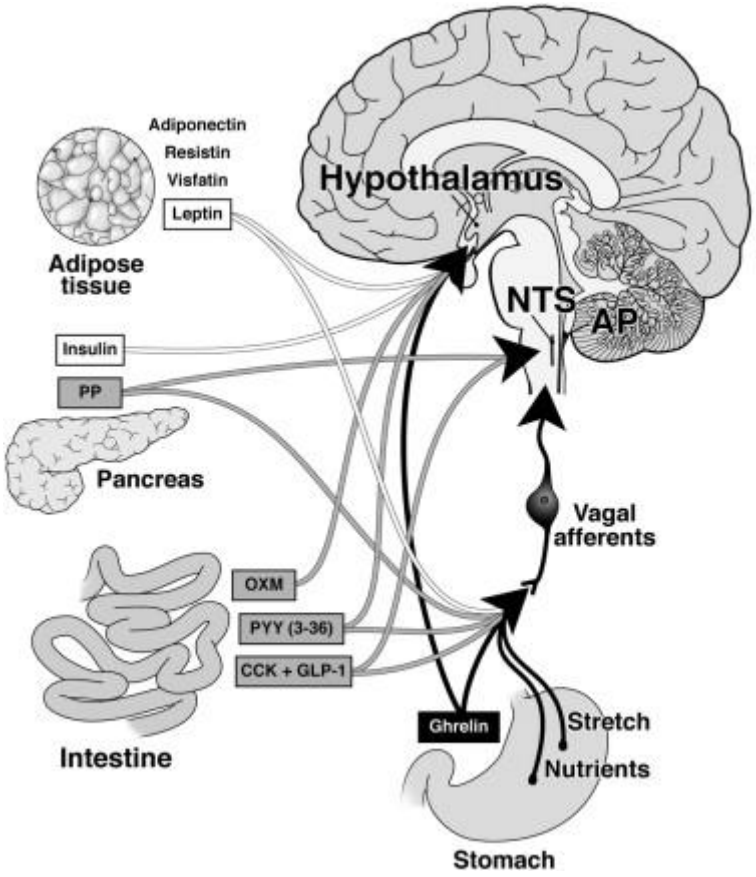

Figure 1.7: The homeostatic regulation of energy balance by the peripheral organs and their metabolites. When nutrients are sensed in the stomach, stretch receptors signal through vagal afferents to the brainstem, along with release of ghrelin which is sensed by vagal afferents and the hypothalamus as a signal of satiety. The intestines release CCK and GLP-1 postprandially to signal satiety to the vagal afferents and NTS, along with PYY and OXM. The pancreas releases PP postprandially. The adipose tissue releases leptin, along with insulin release from the pancreas to signal a negative energy balance resulting in hunger. CCK, cholecystokinin; GLP-1, glucagon-like peptide-1; NTS, nucleus solitary tract; PYY, peptide tyrosine-tyrosine; OXM, oxyntomodulin; PP, pancreatic polypeptide (51).

The mechano- and stretch-receptors of the GI tract sense the presence and energy density of food (48), as well as controlling the motility of food and transit time of digestion. Intragastric pressure occurs as a result of the ingested nutrients, known as gastric accommodation (46), a reflex in which the stomach relaxes, allowing for temporary storage of food before controlled release into the intestines. The GI tract secretes several hormones that are responsible for postprandial regulation of appetite via satiation and satiety, including cholecystokinin (CCK), pancreatic polypeptide (PP), peptide tyrosine-tyrosine (PYY), and glucagon-like peptide-1 (GLP-1) (52).

One of the most abundant neuropeptides in the brain is CCK, which is also one of the first GI hormones discovered (53). Secreted from I-cells duodenum and small intestine in response to protein or fat intake, CCK binds to vagal nerve receptors, aiding in gut motility, gastric acid secretion, gastric emptying, gallbladder contraction and secretion of pancreatic enzymes (54,55). Secretion of PP from pancreatic islet cells is mediated by the vagal nerve, to release PP into the circulation after ingestion of food, particularly of protein and fat, rising in proportion to the calorific load of a meal (52). Rapid secretion of PYY occurs postprandially from endocrine L cells in the gut to affect gut motility (52,56) and inhibits gastric and pancreatic secretion (53), and is stimulated by the presence of fat in the colon and ileum (53). Like PYY, GLP-1 is also secreted by endocrine L cells in the ileum once nutrients are sensed in the small intestine, responding in particular to fat, glucose and fermentable fibre, and degrades rapidly, slows gastric emptying and mediates CNS effects relating to satiety (48,53,57).

The vagus nerve, which provides the neuronal link between the gut and the CNS through sensory vagal afferents, provides inhibitory or excitatory effects to the stomach, and relays satiety signals from the periphery to the solitary tract nucleus (NTS) of the brainstem (BS) (46,47,52). Gastric vagal afferents innervate smooth muscle tissue in the GI and respond to distention and stretch, and mucosal afferents innervate the mucosal layer playing a role in detection of the size of food particles in the lumen through chemo-sensation when activated by intestinal nutrient infusion (58). Innervations of the vagal nerve terminals in the gut wall contain endocannabinoid receptors such as CB1 (52,59). These receptors sense increases and decreases in energy as well as gastric load (60) and modulate mobility, permeability of

epithelium, inflammation, as well as the secretion of gastric acids, neurotransmitters, and hormones (59).

The NTS is the central hub that regulates feeding behaviour through nutrient sensing (61). It relays information regarding energy balance through to homeostatic feeding circuits in the hypothalamus, which contains several interconnecting nuclei including paraventricular nucleus (PVN), arcuate nucleus (ARC), lateral hypothalamic area (LH), dorsomedial nucleus (DMN), and the ventromedial nucleus (VMN) (52,62). The hypothalamus and the BS communicate via bi-directional neuronal projections of the PVN and NTS (52). The median eminence (ME) is a circumventricular organ situated at the base of the hypothalamus, adjacent to the ARC. The ME allows nutrients and hormones to enter the brain directly that would otherwise be unable to cross the blood brain barrier (BBB), and forms a complex with the ARC (52,58). The ARC has neuronal projections to the VMN, which projects those axons to the DMN, LH, ARC, and regions of the BS (52).

The ARC contains orexigenic and anorexigenic neuronal populations that have widespread connections to other nuclei in the hypothalamus, forming a complex circuitry to decrease or increase appetite, as shown in Figure 1.8 (47). Anorexigenic neurons include proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) (52). Both of these neuronal populations project to neurons expressing melanocortin-3 and -4 receptors (MC3R, MC4R) in many hypothalamic nuclei (56) including the PVN (63) to inhibit feeding behaviour, and are activated through POMC-stimulated release of α-melanocyte-stimulating hormone (α-MSH) (62). The orexigenic neurons include Neuropeptide Y (NPY) and agouti-related peptide (AgRP) (52). Release of NPY occurs in a fasted state, and situations where there is increased energy demand, such as lactation or exercise (64). Interaction of

AgRP interaction with the melanocortin receptor, through competitive antagonist activity with α-MSH leads to inhibition of food intake (64,65). These neurons primarily receive hormonal and nutrient signals from the periphery, including that of leptin, ghrelin, insulin, and nutrients (47), which will be covered in detail in the following sections.

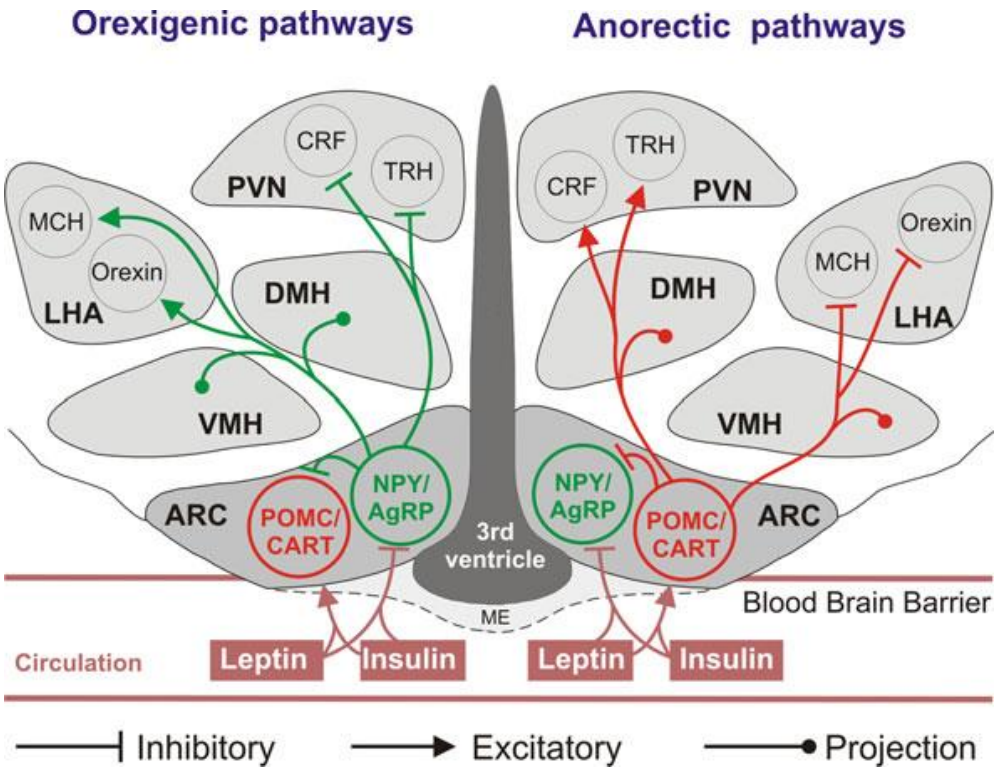

Figure 1.8: The orexigenic and anorexigenic pathways incorporating the hypothalamic nuclei involved in the neuroendocrine regulation of appetite. Leptin and insulin within circulation bypass the blood brain barrier via the ME to enter the brain, acting on neuronal populations in the ARC, where they stimulate POMC and CART (red) and inhibit NPY and AgRP (green). These signals are relayed to the PVN, VMH, DMH, and LHA. The PVN contains anorectic neurons CRF and TRH, whereas the LHA contains MCH neurons and orexin peptides. These hypothalamic nuclei have neuronal projections to higher cortical areas to modulate feeding behaviour. ME, median eminence; ARC, arcuate nucleus; POMC, proopiomelanocortin; CART, cocaine- and amphetamine-regulated transcript; AgRP, agouti-related peptide; NPY, neuropeptide Y; PVN, paraventricular nucleus; VMH, ventromedial nucleus; DMH, dorsomedial nucleus; LHA, lateral hypothalamus; CRF, corticotropin releasing factor; TRH, thyrotropin-releasing hormone; MCH, melanin-concentrating hormone (66)

In addition to the GI tract secreted hormones, nutrients are also able to relay satiety signals to the hypothalamus (52) and signal information on nutrient availability (47). Glucose increases sensations of satiety, whereas low levels of circulating glucose trigger hunger (48). Circulating glucose is stored in the body in minimal levels, tightly regulated and monitored by

the communication of the CNS with peripheral organs, driven by glucose-sensing and glucose-inhibiting neurons in the hypothalamus. These neurons are present in the ARC, VMN, as well as the NTS of the BS and the dorsal motor nucleus of the vagus nerve (DMX) (63).

Essential amino acids (EAA) also play a role in energy homeostasis, through the sensing of changes in amino acid concentration by the peripheral organs and NTS (61,67). Amino acid sensors in adipose tissue allow for regulation of adipocyte differentiation and lipogenesis, and in the GI tract amino acids stimulate hormone release by intestinal endocrine cells (61). L-tryptophan and phenylalanine are significant stimulators of CCK release (32) and suppress food intake in humans, likely due to being precursors to monoamine neurotransmitters, such as serotonin, which has an inhibitory effect on appetite (48,68). Three of the EAAs, Valine, Leucine, and Isoleucine, are branched-chain amino acids (BCAAs) affect metabolism through promotion of protein synthesis (61). Leucine sensing by the NTS signals dietary protein availability, and leads to suppressed food intake through inhibition of AgRP (69). Maintaining optimal levels of EAAs is important for regulation of body weight, as EAA deficiencies lead to significant weight loss and reduced appetite, which have long-term severe health effects (61).

Adipose tissue secretes adipokines, consisting of various hormones and metabolites, including free fatty acids (FFA), leptin, and adiponectin. Adiponectin has anti-inflammatory and insulin-sensitising activity (53) as well as an effect on glucose metabolism, through action on the liver and muscle (70). Metabolism of fat in adipose tissue leads to FFA release during times of energy requirement. The PVN increases lipolysis and fatty acid oxidation through communication with the liver and adipose tissue (52). Fatty acids (FA) derived from dietary triglycerides provide us with an energy source, and also act on free fatty acid receptors (FFAR1-4) to influence energy homeostasis (71). FFAR1 are expressed in the CNS and

pancreatic β-cells, FFAR4 in the hypothalamus, adipose tissue, and taste buds, with both FFAR4 and FFAR1 expressed in intestinal L-cells (71). These receptors are activated by long-chain FA (LCFA) and medium-chain FA (MCFA), to regulate glucose metabolism, decrease NPY expression postprandially (72), increase GLP-1 secretion (73), and modulate ghrelin levels (65). Fermentation of nutrients by gut microbes releases SCFA, and are important for GI health due to their anti-inflammatory effects, with their receptors (FFAR2, FFAR3) found in intestinal epithelial cells (72). Concentrations of SCFA vary through the human GI tract, but are predominantly metabolised by microbes in the colon and cecum (74).

Appetite is also regulated through a humoral pathway, by hormone release into the circulation which modulates peptides in the gut and brain, in response to changes in the nutritional state in a slower method of communication than those mediated by the vagal efferent pathway. Ghrelin is a hormone with a range of functions, such as promoting growth hormone secretion, modulating cell proliferation and differentiation, gastric secretion, and motility, and adipogenesis. Ghrelin also regulates glucose homeostasis and inhibits insulin secretion and is well known for its role in regulating hunger as it the only known circulating orexigenic compound. Due to ghrelin's vast functions, it is produced in many different tissues in the body, however over 65% of circulating ghrelin is produced by the stomach mucosa (53,75).

Anorexigenic hormones, insulin and leptin, play a key role in ensuring that our energy stores do not get depleted, decreasing in concentration during periods of fasting (48) and promoting satiety during times of positive energy balance (46) through providing adiposity signals to the CNS (76). Insulin receptors are located in hypothalamic nuclei including the ARC, DMN, and PVN (52). Insulin is secreted by pancreas β-cells (52) as a response to elevated levels of

circulating glucose, stimulating tissue glucose uptake through binding to receptors in skeletal muscle and adipose tissue. Insulin also decreases plasma FFA by inducing lipogenesis (77). Leptin is mainly secreted by adipose tissue, and receptors for it mainly located in the ARC (52)

In a fasted state, ghrelin plasma levels are elevated (52), released by the GI tract into the circulation to pass the BBB via the vagal afferents, as well as being produced in the hypothalamus, leading to food seeking behaviour (48,78). Consequently, there is significant expression of orexigenic neurons NPY and AgRP (56), activated by ghrelin signalling via projections from the NTS to the ARC (65). Additionally, glucose circulating levels are reduced during times of negative energy balance, which alters excitability of AgRP, NPY, and POMC neurons (63). Ghrelin inhibits POMC activity (46,79), and AgRP and NPY are also able to inhibit POMC by themselves (79). Additionally, AgRP and NPY bind to melanocortin receptors, acting as inverse agonists to inhibit α-MSH (63). Ghrelin levels reduce postprandially once nutrients are present in the stomach (48).

During a meal, the hypothalamus senses gastric distention (80) along with leptin and insulin signals (65). Insulin activates POMC neurons to promote expression of α-MSH, and inhibits activity of AgRP and NPY neurons (52). Insulin secretion and circulation concentrations are proportional to recent carbohydrate and protein intake and body fat content over a 24-hour period. Insulin is transported into the CNS over a period of several hours after circulating concentrations increased (48). Leptin signalling increases POMC and CART activity and decreases NPY and AgRP neuronal activity. This increase in anorexigenic neuronal signalling results in reduced food intake, and increased energy expenditure (52,79). In addition to adipose tissue secretion of leptin, it is also secreted in the gastric epithelium, where it amplifies CCK signals in the gut increasing satiation (52). Both CCK and PYY inhibit AgRP

neurons to promote satiety, with CCK acting rapidly, but PYY acting slower in a sustained response (46), though binding to receptors in hypothalamus and BS (52) and activating POMC expression (46).

Mutations or lesions in areas critical for homeostatic energy balance have been shown to disrupt it and result in hypophagia. The LH is considered to be the feeding center, as its destruction results in weight loss and hypophagia (52), and the destruction of both the PVN and VMN result in hyperphagia and obesity in laboratory animals (52). Hyperphagic obesity occurs in both humans and mice with MC4R genetic deletions (81), and MC4R mutations contribute to around 6% of early-onset obesity cases in humans (52,82). Deficient POMC expression also results in hyperphagia due to loss of MC4R signaling (82).

Satiation and satiety are not simply dependent on ingestion of food, it is impacted by the nutritional composition and characteristics of food (83). In general, the main characteristic of food that impacts satiety is energy density, with high-fat foods tending to have the highest energy density. However, it's important to note that isocaloric foods vary in their capacity to increase and maintain perceptions of fullness, due to the differing micro- and macronutrient compositions (84). Imbalances in the diet, such as high fat intake, as well as overconsumption, lead to dysregulation of homeostatic energy balance.

Release of CCK is primarily stimulated through dietary fat and resulting amino acids from protein digestion (48), glucose also elevates CCK plasma levels but not as significantly (85). Thus, ingestion of a low-fat diet (LFD) results in sparse CCK secretion (55). Rats adapted to a high-fat diet (HFD) have decreased sensitivity to CCK (55). Elevated PYY levels have been found in many diseases characterized by weight loss in humans (56), and reduced satiety in overweight individuals has been associated with deficient release of PYY postprandially.

Studies where obese mice were fed a HFD revealed that PYY mRNA levels were similar to that of mice fed a LFD, as well the obese mice retaining sensitivity to exogenous PYY, however they had reduced PYY plasma levels, which suggests that PYY deficiency is not from decreased synthesis but from impaired release of PYY (86). Studies in mice have resulted in conflicting results on whether a HFD stimulates release of NPY in the ARC, likely due to differences in the type of fat ingested, with saturated fat shown to upregulate NPY compared to downregulation by unsaturated fat (64). However, in NPY-overexpressing mice, a sucrose diet led to a significant increase in body weight compared to control mice fed the same diet (87). When caloric intake is excessive, demand for insulin increases which leads to unresponsiveness of insulin receptors as part of a protective mechanism to prevent excessive uptake of glucose. Pancreatic β-cell function becomes impaired, releasing high quantities of insulin in an attempt to compensate for the desensitisation of insulin receptors. High fat diets contribute to insulin resistance, which is common in obesity (77).

Research has shown that energy from protein has the most significant effect on satiety, followed by carbohydrates, and that fat is the least satiating (88). Protein is the primary suppressor of ghrelin (46). Sufficient intake of dietary protein (25-30% of energy intake) aids with weight loss and long-term maintenance of weight through amino acid interactions with the specific metabolic targets required for these processes - sustaining basal energy expenditure throughout weight loss and sustaining satiety despite a negative energy balance (89). Plasma levels and secretion of PP are reduced in morbidly obese humans, and studies have shown decreased PP secretion in response to a protein-rich meal (90). Interestingly, PP is the only satiety signal that has shown to be significantly higher in anorexic individuals (68).

When peripherally administered in mice, PP reduces food intake and gastric emptying, and decreases body weight gain in genetically obese mice (91).

Certain types of fibre enhance satiation and satiety (50) by holding water in the food, lowering the energy density and rate of digestion (88). Whole grains enhance satiety for several hours after a meal and slowing down digestion and nutrient absorption (5), due to the soluble fibre absorbing water, and creating a viscous gel in the stomach resulting in stomach distention (33). Soluble dietary fibre delays absorption of glucose and fat in the GI tract and influences insulin and glucose levels postprandially (53). Consuming high levels of soluble dietary fibre has shown to decrease insulin resistance in women (92). Consumption of a high-carbohydrate, low-fibre meal decreases adiponectin concentrations, whereas adiponectin levels are increased through intake of dietary fibre (53).

Dietary fibre affects food intake through modulation of hormones in the GI tract involved in appetite regulation, namely ghrelin, PYY, GLP-1, and CCK (53). Several studies in humans have shown intake of dietary fibre decreases ghrelin levels, which could be due to increases of SCFA and reduced FFA in the colon (53). The main fermentation products of dietary fibres are butyrate, acetate, and propionate, and these are found in the present in the lumen of the gut and colon (74) and have been shown to have metabolic benefits. Butyrate provides an energy source for colonocytes (74). Increases in SCFA production reduce hepatic glucose output and improve lipid homeostasis, as well as increased PYY activity (53) and CCK and GLP-1 production (33). The consumption of resistant starch, a type of fermentable fibre, has shown to increase GLP-1 and PYY mRNA in the cecum and colon of rats (93), and GLP-1 secretions have been shown to be reduced in obese humans (57).

Acetate, a SCFA, has been shown to have anti-obesogenic effects in humans and animals. In mice, acetate has been shown to enter circulation and reduce food intake through activity in the ARC, increasing POMP expression and suppressing AgRP (94). Humans with hyperinsulinemia received intravenous and rectal infusions of acetate had significant increases in plasma PYY, along with increased plasma GLP-1 and ghrelin levels than controls (95). Diabetic, obese model rats treated with acetate had improved glucose tolerance and reduced accumulation of lipids in adipose tissue (96). When fermentable fibre is scare, microbes also ferment other dietary products such as the amino acids from proteins or fat, but these are less favourable, providing little energy for growth, resulting in reduced SCFA output and microbe activity (74). Protein fermentation mainly results in branched-chain fatty acids that originate from valine, leucine, and isoleucine (74), and these amino acids are significantly elevated in obese individuals compared to lean individuals (97).

While whole grains are considered to be satiating in comparison to refined grains, differences between the nutritional compositions of whole grains lead to differences in energy intake and as well as potential differences in satiation levels and impact on gut microbiota, however this needs to be studied further (98). Additionally, the difference in particle size between refined grain and whole grain could lead to differences in postprandial satiety. Consumption of whole grains have shown to induce a lower postprandial glucose response (99) and have positive effects on insulin sensitivity, which has been suggested results by mechanism of SCFA production and stimulation of PYY and GLP-1 secretion (100).

Energy intake is not always driven by physiological needs alone. A range of factors influence what and how much we eat, including the way food is cooked, perceived palatability, portion size, variety, and energy density. These all play a role in shaping our eating behaviour and

energy intake beyond our physiological requirements (50,57). The role of palatability in driving food intake outside of homeostatic appetite regulation, through a secondary food intake regulation system, eating for reward, will be discussed further in the following section.

1.4 Eating for Reward: The Role of Palatability in Hedonic Food Consumption

We are born with innate taste preferences, beginning with liking of sweet tastes in infancy to encourage consumption of the mother's milk, followed by the development of liking salty tastes that attract us to foods that are rich in minerals and vitamins (101). Our liking or disliking for tastes change as we age - bitter and sour tastes are innately disliked from infancy through to childhood due to an evolutionary defence mechanism to avoid toxic compounds, but as adults we may adapt a preference or tolerance for these foods (54,101,102). Genetic differences between individuals also plays a role in taste perception, with some possessing a greater sensitivity for bitter foods (103). There are certain tastes that we find more palatable than others, such as those that are sweet and fatty. The preference for these foods is present in both humans and laboratory animals (104). While the sensory preference for sweet tastes is present from birth, the preference for fatty foods often begins in childhood, particularly in Western cultures (105). This is in part due to the prevalence of the 'cafeteria diet', consisting of foods that are highly-processed and composed of high levels of fats, sugars, sodium, and other flavours specifically to enhance their palatability (102). Palatability is considered a key factor in influencing the quantity and choice of food consumed (49), and influencing our preferences for certain foods (106).

Palatability is not an inherent characteristic of food but rather a measure of the momentary and subjective pleasurable sensation that an individual experiences while eating (50). This pleasure results from the sensory properties of the food and the nutrients that contribute to

its caloric value (107), these properties include the basic tastes (sweet, salty, bitter, sour, and umami), along with the texture, mouthfeel, and flavours (Figure 1.9) (108). Flavour is a combination of olfactory, gustatory, and trigeminal sensations (54). During mastication, taste and flavour molecules in the food activate receptors in the oral and nasal cavities (109). Chemosensory neurons in the oral cavity then relay information regarding tastant detection through their projections to the ventral NTS, which goes on to relay this information to other areas in the brain (62). Taste receptors are also located in the GI tract, where specific receptors detect and bind to particular tastants, stimulating hormone release. Fat, amino acids from proteins, and carbohydrates are sensed by I-cells which release CCK in response, and receptors on these cells also sense umami and bitter tastes. The L-cells are capable of detecting sweet compounds, which stimulates release of GLP-1 (54). These hormones then enter the bloodstream (54) or act locally through vagal afferents projecting from the GI tract to the caudal area of the NTS (58).

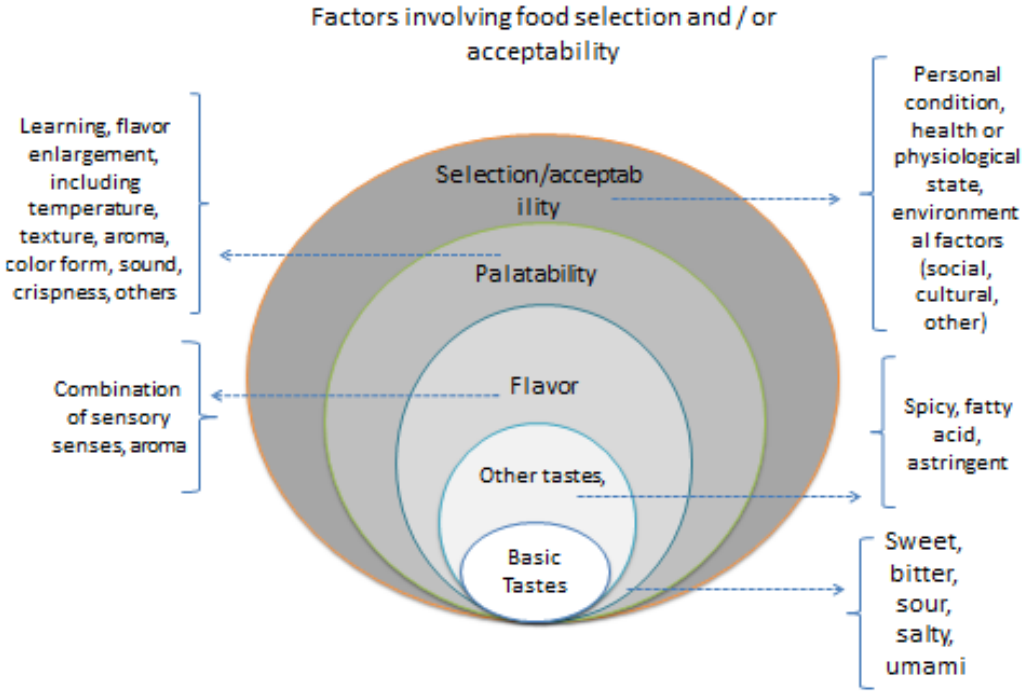

Figure 1.9: Multi-layered factors contributing to food selection and acceptability. At the core are the basic tastes of food: sweet, bitter, sour, salty, and umami, followed by other tastes such as spicy, fatty, acidic, and astringent. These tastes contribute to the overall flavour, which is a combination of sensory senses including the aroma of the food. Collectively, these orosensory properties contribute to the overall palatability of the food, which is the most significant contributor to food acceptance and preference. Palatability is additionally influenced by the temperature, colour, sound, and texture of food. Differences between individuals health or physiological state, and the social or cultural factors affecting the environment in which they consume the food also contribute to the overall food selection (110).

The duration of oral exposure to food is important for perceived taste intensity, which is determined by the texture of food and the rate that it can be eaten. A solid food containing the same amount of sucrose as a liquid solution is perceived as less sweet than the liquid and would require double the amount of sucrose to match in taste intensity. Additionally, the texture and taste intensity of food are predictors of the nutritional content and impact meal duration and total intake (111). Liquid substances provide a lower satiation response compared to solid foods, due to shorter orosensory exposure, resulting in a later meal termination (112). Foods with harder textures take longer to eat and are more energy-dense with lower water content than food with softer textures, leading to earlier meal termination.

Foods of high sweetness and saltiness intensity have the same effect, as foods with low taste intensity likely signal low nutrient density and aren't perceived to be as satiating (111). Palatability is closely connected to the energy density of foods, with fat providing the most concentrated source of dietary energy (109).

The orosensory properties of fat, such as the texture and fat-soluble volatile flavour molecules, contribute to a food's palatability and acceptability (109). Fats in foods provide a mouthfeel sensory experience that is hard to reproduce in low fat foods (108). Fat creates creamy and smooth sensations in dairy products, moistness in cakes and crispiness and crunchiness in fried and baked foods (109). Consumption of fats are not usually studied in isolation from other ingredients, due to lack of palatability or flavour when consumed solely by itself, as well as the fact that dietary fats are usually consumed in combination with other ingredients (113). Sweetness is capable of masking the effect of fat in foods (105) as well as masking bad tastes (101). Preference of sweet solids and liquids tend to follow an inverted U-curve with added sweetness increasing preference initially, and decreasing when food becomes too sweet, but this curve is decrease in children who have an innately higher preference for sweet taste than adults (109). Studies of hedonic responses in humans to sweet and fatty tastes using cream, milk, and sugar samples, showed that the response was dependent on the relative proportions of sucrose and fat in the test food samples. The hedonic preference ratings increased alongside increasing the dairy fat content in the samples, but for sugar, the preference declined with increasing sucrose content. When sucrose was added to the high-fat samples, it significantly enhanced the preference ratings (113). The fat to sugar preference ratios in food have been linked to body weight extremes, with morbidly obese women preferring higher fat to sugar ratio, and those with anorexia

preferring the inverse, higher sugar to fat (109). Sex differences also affect food preferences. Men differ to women in their preference of high fat foods, preferring foods high in fat and protein such as meat dishes, whereas women prefer foods high in carbohydrates and fat that are more sweet such as baked items (114).

Palatability of food is dynamic; it typically declines as the food is ingested, but increase with food deprivation (88). Sensory-specific satiety is the changing response to the sensory properties of food throughout its consumption, decreasing compared to uneaten food. The pleasantness of food changes immediately after consumption, as demonstrated by sensory-specific satiety studies in humans, where food is consumed *ad libitum* in a state of hunger with taste rating occurring periodically throughout the meal. This suggests that the sensory stimulation accompanying ingestion is the primary contributor to sensory-specific satiety. Pleasantness also includes visual sense, when sweets of one colour are eaten, the pleasantness of the taste of that colour declines, but not of the other uneaten colours despite all sweets only differing in colour, not taste. As well as texture, with pasta decreasing in pleasantness as a particular shape was eaten. However, taste-specific satiety provides the strongest effect (115).

The abundance and affordability of highly palatable and energy-dense food in modern society has resulted in an obesogenic environment that facilitates excessive caloric intake (49,116,117). The cafeteria diet contributes to a large proportion of our dietary energy in the Western diet (109). Cafeteria diets produce hyperphagia in humans, which has also been observed in rodents (118). Foods high in fat are more preferred amongst the obese population (109). Rats prefer and overeat high-fat foods, and gain weight from these diets despite when energy consumption is not elevated (119). Consumption of HFDs promote

increased caloric intake over than those high in carbohydrates, as well as increased weight gain. This has been demonstrated in rats that were fed a HFD *ad libitum*, either orally or via intragastric feeding, and exhibited greater intake than those fed a high-carbohydrate diet (HCD) (104). While palatability factors into the hyperphagic effects of HFD, these foods promote overeating through other mechanisms independent of that, such as postingestive effects and caloric density (120). When rats are given access to sucrose or glucose solutions along with standard chow, they consume 60% of their total calories as sugar, increasing their energy consumption by 20% (119).

Highly palatable food is also capable of altering eating behaviours such as eating patterns and meal size. Human studies have shown that larger meal size and duration were associated with higher levels of palatability, with the highest palatable meals being 44% larger than the meals rated lowest for palatability (121,122). Rats fed a cafeteria diet snacked more often and ate less meals than rats which were fed standard chow, as well as an overall increased caloric intake, and became obese (123). This behaviour is also present in studies of obese men and women, with increased snacking and energy intake than controls, associated with high fat and sugar foods such as cakes, cookies, chocolate and desserts (124).

Overall palatability and acceptance of food is also influenced by an individual's sensory capabilities, metabolic state, and the environmental context in which the food is consumed (88). Some individuals are more vulnerable to overconsumption than others due to genetic, metabolic, physiologic or psychological differences (116). They may find it difficult to control their energy intake, despite awareness of negative health outcomes or possessing a desire to limit their consumption (125). This has massive implications for our health, as hedonic food intake is a key contributing factor towards binge-eating and obesity diseases (126). While food

intake is primarily controlled through the homeostatic system, consumption of highly palatable food activates reward pathways in the brain that also plays a role in feeding behaviour and drive food intake beyond caloric need.

The reward system in the brain is activated by rewarding stimuli, and produces feelings of satisfaction, euphoria, and pleasure. These stimuli are either natural, such as food, sexual behavior or social interactions, or artificial, such as drugs and alcohol (127). The function of the reward system is to direct behaviour by reinforcing actions that lead to positive outcomes, and avoiding actions that lead to negative outcomes (127). Through learning the value of rewards, humans are able to use that information in decision-making without the need for conscious processing of contextual cues (128). Reward consists of three major components: *liking*, *wanting*, and *learning* (Figure 1.10). These normally occur together but function through different brain systems (129). The *liking* refers to the hedonic impact of the reward and is separate from the experience of conscious pleasure. Similarly, *wanting* is the feeling of desire, separate from a conscious, cognitive desire (130). L*earning* involves forming predictive associations of the stimuli with anticipation of reward (131).

Reward-related cues or conditioned stimuli are attributed a value by the incentive salience *wanting* (131) which is behind the motivation and decision-making driving the consumption of the reward, a function of the mesolimbic brain systems (129,131). *Wanting* usually occurs with the other two components of reward (*liking* and *learning*) but is also able to be dissociated from them (129). Repeated artificial reward activation leads to long-term consequences and disruptions in the reward system, such as a loss of interest in natural rewards, or in the terms of drug and alcohol abuse, addiction development (127). Incentive-sensitisation is a result of long-lasting changes in the mesolimbic systems and blunted

pleasure response. This leads to *wanting* to occur despite a decrease in *liking*, and intense cue-triggered cravings (129,130). This sensitisation leads to relapse for drug addicts despite long periods of detoxification (130), and in the case of eating disorders leads individuals to compulsively seek and crave food despite not receiving pleasure from it (129).

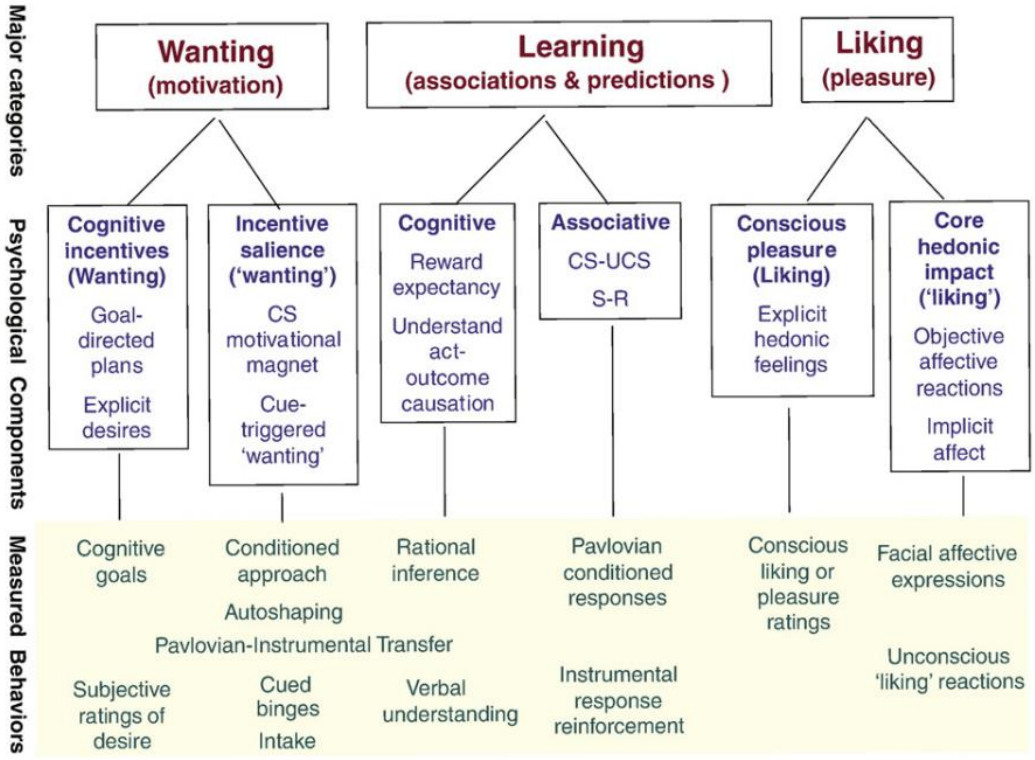

Figure 1.10: The three main components of reward: wanting, learning, and liking, with the psychological components of each and how the motivated behaviours are measured in scientific experiments. Incentive salience is attributed to rewards and their predictive cues and triggers *wanting*. The hedonic impact of the reward contributes to the *liking*. Learning occurs through associations of conditioned stimuli (CS) and unconditioned stimuli (UCS) (Pavlovian conditioned responses), and predictions of reward (132).

There is commonality between the neural networks and structures triggered by palatable food consumption and of those implicated in drug abuse, including the exaggerated responsiveness of the reward system to drug cues in addicted individuals aligning with that of obese individuals to food cues (133,134). Highly palatable food activates reward circuitry through release of various neurochemicals that induce hypothalamic hunger signals while inhibiting satiety (135), and this reward increases the chance of the food being consumed

again (136). Consumption of sweet or fatty food elicits a strong pleasure response that triggers addiction-like responses in brain reward circuitry, driving compulsive eating. Repeated consumption leads to changes in this circuitry akin to those induced by drug consumption (125,137,138). This system is known as the mesolimbic dopaminergic (ML-DA) system and is responsible for motivated behaviors and reward and includes two key areas: the ventral tegmental area (VTA), and the nucleus accumbens (Acb), which modulate reward through their connectivity to other brain areas including the pre-frontal cortex (PFC) and amygdala (131).

The sensory properties of the food combined with the hedonic impact contribute to the *liking* (130). Proximal stimuli, such as the food's taste, colour, smell or texture, become associated with sensory afferent inputs and endogenous neuroendocrine and physiological changes in response to food (88). Liking can be detected in behaviour and neural signals generated in subcortical areas of the brain (129). When food is consumed for the first time, dopamine transmission increases in the brain, eventually being released in response to cues associated with food reward once food exposure has occurred more often, as a predictor of reward (112).

The reward circuits and homeostatic feeding circuits are integrated and cross-talk with each other (Figure 1.11) (128,139). *Wanting* and *liking* can be modulated by internal physiological states – hunger increases the desirability of food, and satiation dampens the pleasure response (130). Some of the hormones involved in endocrine signalling for nutrient availability, such as ghrelin, PYY, and insulin, also act in part on corticolimbic structures involved in the reward system triggered by food intake (127,128). Ghrelin has been associated with the activation of reward circuity (46) as well as GLP-1 (127). Food cues such as sight or

smell of a food, and external environmental cues including food packaging and advertisements, tend to be salient and meal initiation by increasing the feeling of hunger and CNS-GI tract signalling in preparation for the expected meal, stimulating secretion of ghrelin and dopamine transmission, leading to impairment in self-regulation of food intake (112).

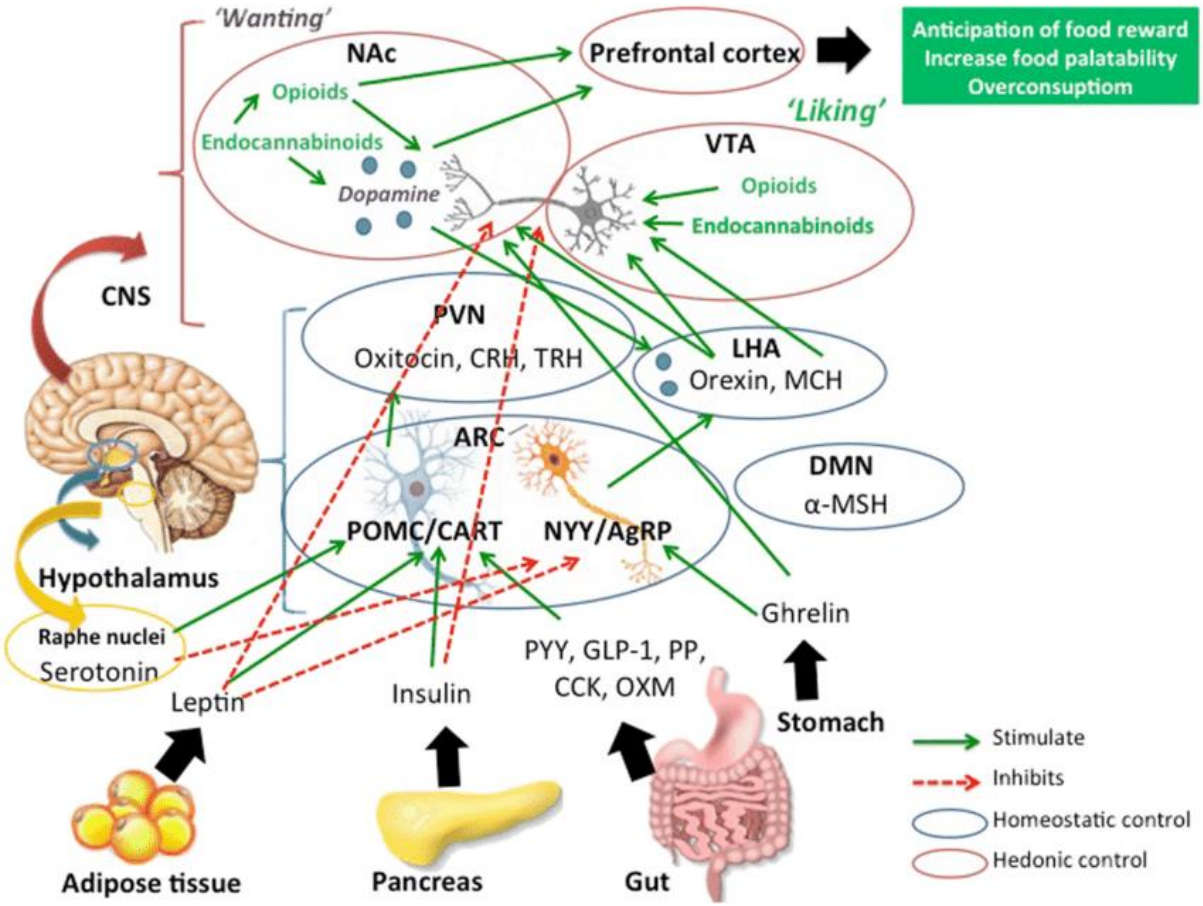

Figure 1.11: The crosstalk between homeostatic and hedonic food intake regulation. The LHA plays a role in both homeostatic and the mesolimbic dopaminergic reward system and modulates hedonic food intake through its connectivity to the VTA and NAc. The VTA and NAc are important for the *wanting* and *liking* of food, and through the NAc connectivity to the prefrontal cortex lead to food reward anticipation and increased perceived palatability of food which result in overconsumption. Dopamine, opioids, and endocannabinoids signalling molecules are involved in this process, as well as signalling molecules from peripheral organs involved in homeostatic energy balance, such as leptin, insulin, and ghrelin. LHA, lateral hypothalamus; VTA, ventral tegmental area; NAc, nucleus accumbens (140).

Intake of protein and carbohydrates are tightly regulated through negative feedback systems, whereas fat intake induces positive feedback through greater orexigenic signaling in the brain and circulation, increasing the desire to consume more fat (136). Consumption of HFHS diets

increase NPY mRNA and decreases POMC mRNA in the ARC (141). Rats fed a LFD for 8 weeks had significant expression of c-Fos in the NTS when fed lipids through an intragastric gavage, compared to rats maintained on a HFD (142). The reward system influences our perception of food; studies in humans have shown that individuals who enjoy sweet flavours remember them as sweeter than they actually are, potentially contributing to cravings for more sweet foods (143). Ultimately, the connectivity of the VTA to the Acb play a key role in hedonic food intake and are commonly the focus of studies that investigate effects on palatable food on the reward system.

1.4.1 Anatomy and Neurochemistry of the Ventral Tegmental Area and Nucleus Accumbens

The VTA is situated in the midbrain and is composed of a heterogenous population of neurons, including dopaminergic, gamma-aminobutyric acid-ergic (GABAergic), and glutamate neurons, but is primarily characterised by its dopaminergic neurons that project throughout the brain contributing to the reward system through behavioural reinforcement. The neurons of the VTA make direct contact with many other brain areas, namely including the PFC, LH, ventral pallidum (VP) and Acb (144). Dopamine neurons in the VTA express receptors for insulin, leptin and brain-derived neurotrophic factor (BDNF) (62), and neuronal projections from the LH to the VTA release orexins (62).

The Acb lies parallel to the brain's midline and is a part of the ventral striatum. Structurally, it is round though dorsally flattened. It is a major downstream target of dopaminergic projections from the VTA and substantia nigra, and contains glutamatergic projections from the amygdala, hippocampus, thalamus and PFC (145). The Acb is structurally and functionally divided into two components: the outer shell (AcbS) and inner core (AcbC) (146). The AcbS

and AcbC differ in their histology, anatomical connections, and efferent and afferent projections. The AcbC has neurons projecting through to the motor system, starting at the VP, which has subsequent projections to the substantia nigra and subthalamic nucleus (147,148). The AcbS is more 'limbic' than the AcbC, with efferent projections to the VTA, LH, and amygdala (147,148). The AcbS is considered a transitional zone between the extended amygdala and the striatum, due to immunohistochemical and connectional similarities. Both the extended amygdala and AcbS have rich populations of neurotensin, cholecystokinin and opioid peptides, afferent connections from the basolateral complex of the amygdala efferent, and connections to the LH (145). Additionally, the AcbS received direct projections from the NTS of the BS (107). The distribution of neuroactive substances and receptors differ between the AcbS and AcbC, with the former containing more serotonin receptors, serotonin and dopamine, and the latter containing more GABA receptors (145). There are dense levels of insulin receptors in the AcbS that overlap with μ-opioid receptors (107). The μ-opioid receptors in the Acb and VTA potentiate dopamine signaling through direct action on Acb receptors (149). Opioid hedonic hotspots contribute to 10% of the Acb, 30% of which are a part of the AcbS (129).

Another system involved in regulating both homeostatic and hedonic food intake, along with energy expenditure and storage, is the endocannabinoid system (ECS). The ECS consists of endocannabinoid peptides, receptors and enzymes, and functions through modulating both neuropeptide activity in the hypothalamus and dopamine release in the Acb, and through bi-directional communication of sensory fibres in the brainstem-duodenum connection (60). In the brain, CB1 receptors regulate both homeostatic and hedonic food intake. Insulin plays a role in maintaining dopamine homeostasis in the Acb; in this region there are insulin receptors

that promote dopamine release but also enhance dopamine transporter (DAT) activity, contributing to the effects of satiety and decreasing palatable food intake (62). The malfunctioning of orexins and insulin are implicated in the development of obesity. There is a growing body of literature on how hedonic food consumption alters brain functioning through the reward system, particularly the effects in the VTA and Acb. Food cues and consumption of highly palatable food, such as those high in fat and sugar, activate similar areas in the human and rodent brain through the ML-DA pathway, thus rodents are a common model to study the reward response. This is frequently done though c-Fos immunohistochemistry, which is a marker of neuronal activity.

1.4.2 The Role of the Ventral Tegmental Area and Nucleus Accumbens in Feeding Reward

The VTA plays a role in learning, memory, and reward processing (144), and is involved in the *wanting* of reward, driving behaviour and motivation to seek reward. Mice fed an acute HFD had differentially activated dopamine neurons in multiple subregions in the VTA, demonstrating that different areas of the VTA may have distinct roles in hedonic eating (150). Rats with VTA lesions had decreased consumption of a preferred and palatable sucrose solution when compared to sham-operated rats, but there was no change in consumption of non-preferred bitter tastes (151).

The Acb predominantly functions as a gratification centre, involved in the processing of motivation and reward (152). The AcbC is involved with the cognitive aspects of learning, such as goal-directed behaviour, exploration, and motivated behaviour, whereas the AcbS plays more of a role in the hedonic aspects of learning, such as reward evaluation and acquisition (147,148). Fat and sugar intake increases c-Fos activation across the Acb in rats, including the

core and shell (153). Bassareo & Di Chiara (154) examined the different roles of the AcbS and AcbC in motivated behaviour in rats. Using micro-dialysis, they measured the effect of food stimuli on dopamine levels in these areas. They found that dopamine levels increased solely in the AcbC when the rats were presented with a box filled with a palatable snack, compared to rats who received an empty box. Eating the snack increased dopamine levels solely in the AcbS. When dopamine-deficient mice are given sucrose and saccharin solutions, they still find it to be more palatable than water and prefer the solutions as much as wild-type mice. However, they did not return to the solutions as often and consumed less in total than wild types. This demonstrated that dopamine is not necessary for reward or preference but is more important for goal-directed behaviours (155). Dopamine transmission from the VTA to the Acb assigns an incentive salience to the reward and cues related to delivery of the reward, that trigger the wanting, and subsequently shapes behaviour to obtain the reward and promotes learning (131,156). Bernal et al. (157) demonstrated that antagonists of dopamine receptors (D1 and D2) in the AcbS significantly decreased fructose-conditioned flavour preferences in rats, as well as increasing the extinction of these preferences, but did not fully block consumption.

The LH acts as a central region involved in reward seeking and feeding behaviour through its connectivity to the VTA and Acb. Mice that are acutely exposed to a HFD have increased c-Fos activation in their VTA, Acb, and LH (150). Nieh et al. (158) investigated the LH-VTA pathway in mice. They identified two types of neurons, those projecting from the LH into the VTA (Type 1) and those projecting from the VTA to the LH (Type 2). Through dual-adenovirus labelling and electrophysiological recordings, they were able to monitor activity of these neurons, while they assessed the behaviour of mice trained to respond to a reward-predictive

cue of sucrose. Additionally, through use of optogenetics to disable Type 1 or Type 2 neuronal activity, they were able to increase and decrease sucrose consumption. Through this, they found that Type 1 neurons were responsible for encoding the conditioned response of reward seeking, and when inhibited, the food seeking behaviour was reduced but sucrose consumption was not fully eliminated, and they found that Type 2 neurons encoded the predictive cues and errors for reward.

Opioid peptides and their receptors play a crucial role in the reward system, where they reinforce behaviours associated with reward and contribute to neuroadaptations implicated in drug abuse. They are also involved in the homoeostatic intake system through interactions with other neuropeptides such as orexins (159). Opioids enhance the palatability of foods that are rich in fat, sugar, or salt (107). The main family of opioids involved in the reward system are μ-opioids which modulate food palatability by acting at the Acb and VTA though the ML-DA system (141,159). Localised 'hot spots' have been found in the Acb and VP, and interact together to amplify *'liking'* and '*wanting'* responses to sweet tastes, through simulation by μ-opioids (160). A 'cold spot' has also been identified in the AcbS, where simulation by opioids suppresses the hedonic response to sweet taste (161). The endogenous opioids system is involved in the rewarding effects of taste in rats, with consumption of palatable food increasing c-Fos activation in the LH, VTA, AcbC, and AcbS. When an injection of Naltrexone, an opioid antagonist, is given to rats alongside palatable food, they show decreased c-Fos response in the VTA, AcbC, and AcbS (162). Naltrexone has also shown to reduce the intake of high-fat and sweet foods in humans (163).

Fasting also increases activation of brain reward systems and creates a bias to the response to high calorie food in humans (164), and administration of intravenous ghrelin has shown to

increase this response (165). Intravenous administration of ghrelin activates the VTA, Acb, and LH network (166). Several studies in rats have shown that ghrelin increases consumption of plain chow, acting on the VTA and Acb through the ML-DA pathway (167,168). When ghrelin is microinjected into the brains of operant-trained rats, it increased their behavioural motivation to seek sucrose reward, but only when injected directly into the VTA, not the Acb (168). Local administration of ghrelin into the VTA causes Acb dopamine release, particularly in the AcbS, in mice, due to the presence of growth hormone secretagogue receptors (GHS-R1A) in these areas, and has also shown to increase alcohol consumption in mice (127).

Orexins regulate wakefulness, arousal, and appetite. They also regulate dopamine neurons and their response to palatable food and drugs (62), but only modulate food intake through acting in the VTA, not in the Acb. Orexin neurons in the mouse LH respond to an acute HFD through innervation of the VTA. When orexin signalling is blocked through an antagonist, both food intake and c-Fos activation in the VTA were reduced. However, the orexin antagonists did not block HFD induced c-Fos expression in either the AcbC or AcbS, indicating that the Acb is an initial target of activation by a HFD, and occurs independently of orexin signalling (150). Cone et al. (78) found that when rats were fed sugar pellets at irregular intervals, the retrieval of the pellets resulted in a dopamine concentration spike in the AcbC. They showed that ghrelin acted centrally in the LH to regulate brief spikes of dopamine in the AcbC but not in the VTA, and that orexin neurons in the LH and VTA mediated ghrelin's effects on food intake and dopamine signalling. The activation of CB1 receptors affects the salience and motivational value of food. The ECS can be overactive in those with obesity, leading to increased intake of palatable foods, as well as reducing energy expenditure (60).

Administration of CB1 antagonists in rats decreased their intake of a highly palatable canned cream whip (169).

The hedonic response to palatable food is a complex combination of orosensory and post-ingestive effects. Studies where rodents have been fed through gavage have shown to not activate the ML-DA, and that oral stimulation from the diet is necessary for mesolimbic activation (150,170). Sclafani (117) found that energy intake of sweet and fatty flavours was modulated by post-oral nutrient factors – by enhancing the reward value of the associated flavour stimuli along with conditioning flavour preferences. Additionally, the amount of food consumed also matters, with rats showing to have increased dopamine based on the amount eaten (171). Studies using sham feeding in rats have shown that the orosensory effects of palatable food alone can contribute to its reward value, when isolated from the post-ingestive effects. This is done by draining the food solution out of the stomach before post-ingestive effects take place (172,173). Hajnal et al. (172) demonstrated that sham feeding of sucrose in rats increased levels of dopamine and its metabolites in the Acb, and that the increase functioned in a concentration-dependant manner, with higher concentrations of sucrose increasing dopamine release in the Acb through increased intensity of orosensory stimulation. Similarly, Liang et al. (173) found that sham feeding of corn oil to rats also resulted in a significant increase of dopamine release in the Acb, similarly to that of sucrose ingestion. Rats given a highly palatable powdered diet had significant activation of their ML-DA system compared to a non-palatable powdered diet. Upon ingestion, there was increased levels of extracellular dopamine and its main metabolites in the AcbS for those that consumed the palatable powder (174). However, food hydration impacts the amount consumed, with rats consuming less saccharides in powder form than when in a solution (117).

Hedonic food consumption has long term impacts on our health, and overeating reduces the responses to palatable food. Diet-induced obesity can lead to an impaired functioning of the homeostatic and hedonic systems, through dysfunction of hormonal signals from adipose tissue and CNS regulation. Individuals with insulin resistance, and leptin or BDNF deficiency, have higher levels of obesity. Insulin infusions into the VTA has shown to decrease food intake in rats (62). Intranasal insulin has also been demonstrated to reduce the attractiveness of palatable food through modulation of the mesolimbic pathways in humans. In a study by Tiedemann et al. (175), human participants, who had fasted overnight, were shown pictures of palatable foods and asked to rate them. Compared to the placebo group, those that received intranasal insulin rated the attractiveness of the foods as lower, and through fMRI showed decreased neural activity in the Acb and VTA, through the inhibition by insulin of the forward projections from the VTA to the Acb. Additionally, insulin-resistant individuals, whom had lower baseline activity in their Acb in response to food cues than those with normal insulin activity, did not show any significant changes in brain activity upon receiving intranasal insulin.

Corderia et al. (176) examined the function of BDNF on feeding behaviour in mice, finding that BDNF in the VTA is important for regulating hedonic food intake through positive modulation of the ML-DA system. Mice that had central deletion of BDNF had increased intake of a HFD, and impaired dopamine release in the AcbS and dorsal striatum, and when treated with a dopamine receptor (D1) agonist, it normalised their intake of the diet. Geiger et al. (177) induced obesity in rats through a cafeteria-style diet high in carbohydrates and found that they had lower basal dopamine levels than normal weight rats. Additionally, the obese rats released dopamine only when they consumed food that was highly palatable, and

not when consuming plain laboratory chow. This is in contrast to normal weight rats that exhibit dopamine release after consumption of plain chow (174). This indicates that the obese rats have a strong preference for the highly palatable food, likely due to obesity induced changes in the functioning of the ML-DA system.

Rats that are predisposed to obesity also exhibit lower dopamine levels and signalling than obesity-resistant rats, likely due to differences in gene expression of genes that regulate dopamine synthesis, and it is thought that compensatory hyperphagia occurs in order to elevate dopamine levels (178). Davis et al. (179) examined the effect of a HFD and obesity on behaviour and dopamine turnover in rats. Rats underwent operant conditioning to psychostimulant reward cues, and to press a lever for sucrose. Rats that were fed a HFD had decreased dopamine turnover in their ML-DA system, and in the Acb, they also exhibited behavioural changes through reduced preference of the psychostimulant reward cue and decreased sucrose seeking.

Neuroimaging studies have shown that obese individuals respond differently to high-calorie food cues than lean individuals. They exhibit differences in brain activity in areas associated with emotion, motivation and reward, and it is thought that an overactive reward system may lead to an exaggerated reactivity and sensitivity to food cues (134,180). Stoeckel et al. (134) conducted a study of the motivational effects of food cues in obese women. They presented pictures of high- and low-calorie foods to obese women and a control group and used fMRI to investigate brain activation in regions involved in the reward system. They found that the high-calorie pictures significantly increased activation in these brain areas in the obese women than those of the control group, including the Acb, ventral striatum, VP, PFC, amygdala, and hippocampus. Using a similar method, Carnell et al. (180) found greater

functional connectivity in the midbrain-VTA region in obese women than lean women, in response to both photographic and audio high-calorie food cues. In addition to reward hypoactivity, withdrawal from long-term exposure to a cafeteria diet has shown to alter gene expression in brain areas that are associated with stress. Rats maintained on this diet for 15 weeks were switched back to standard chow, and this increased mRNA levels of corticotropin-releasing hormone in the hypothalamus (181).

Dietary factors are capable of inducing epigenetic alterations, which increased risk of obesity in future generations - human studies have shown that total energy intake and diet composition during pregnancy and throughout lactation increase epigenetic modulation of obesity development (182). Chronic consumption of HFD leads to reward hypofunction. Ong & Muhlhausler (183) studied the hereditary effects of a HFD in rats and found that female offspring may retain a higher propensity for diet-induced obesity than males. Dams were fed junk food for the period beginning one month pre-pregnancy until the end of lactation. Their female offspring had higher body fat mass than controls, and when exposed to junk food, exhibited higher expression of dopamine receptors and DAT in their VTA. However, this upregulation of dopamine signalling did not persist when they were maintained on a LFD. Several studies of chronic HFD intake in mice have linked differential DNA methylation to altered expression in reward system areas of the brain, including dopamine genes, D1 and D2, in the Acb and VTA (184), as well as dysregulation in the opioid system exhibiting decreased expression of Mu receptors (MOR) in the VTA, Acb, and PFC (185).

While some individuals may have a propensity to become obese due to hereditary factors or genetic syndromes, the large scale of obesity across Western culture is largely due to the changes in our environment that have occurred over the recent years (82). Due to the mass

availability of junk food that is designed to be highly palatable and the increased processing and refining of foods with decreased nutritional benefits, food choices are often made based primarily on the pleasure derived from consumption. As wheat is such a staple food for the global population, it could only be advantageous to provide consumers with an alternative that has greater nutritional benefits. However, due to the importance of palatability in food choice, it is important to understand how Kernza may differ to whole wheat in that regard based on what we know about its nutritional composition.

1.5 Potential Impact of Kernza's Nutritional Composition on Palatability

While foods high in protein and fibre offer significant health benefits, they often have an unpleasant flavour which impacts consumer's willingness to incorporate them into their diets. The organoleptic properties of whole grain foods (the taste, texture, appearance, and smell) have been reported to be a barrier to their intake, resulting in it being less popular among consumers (186). The bitter taste and coarse texture of wheat bran likely contributes to the bitterness sometimes associated with whole grain bread, and is thought to negatively impact consumer acceptability and consumption (187). Flavour is difficult to control for as it can be affected by food ingredients and compounds, processing methods and storage conditions (188). The flavour of cooked foods is significantly influenced by the Maillard reaction; heat causes amino acids and sugars to undergo a complex series of chemical reactions, creating new compounds collectively known as an aroma profile. These profiles create a spectrum of discernible flavours, some of which may be desirable while others not so much (189).

Eight bitter compounds have been identified in the crust of whole wheat bread, developing from dough fermentation and Maillard reactions. Whole wheat bread contains a much higher concentration of Maillard compounds compared to bread made from IWG, this difference

likely due to the lower starch content of IWG (40). Paravisini et al. (45) conducted a comparative analysis of the aroma profiles between IWG and whole wheat bread crusts (Figure 1.12). Their findings revealed that both breads shared primary aroma profiles, but that there were significant differences in intensity, with wheat bread containing higher levels of aroma compounds (187). The dietary fibre content in grains is thought to influence the Maillard reaction by suppressing the formation of aroma compound intermediates (45). Phenolic compounds have also been linked to the bitterness of whole grains due to altering reactions of the Maillard pathway (187). The difference in chemical composition of Kernza could lead to discernible differences in its flavour for consumers in comparison to whole wheat products, however it is unknown to what extent this would impact its consumption. Human studies have shown that repeated exposure to healthier food, such as pulses which are high in fibre, can increase in palatability over time, thus potentially increasing their intake (190). Additionally, after 6-week exposure to whole grain food products, individuals reported a higher rating of liking, texture and flavour and a willingness to include whole grains in their diet, compared to refined grain foods (191).

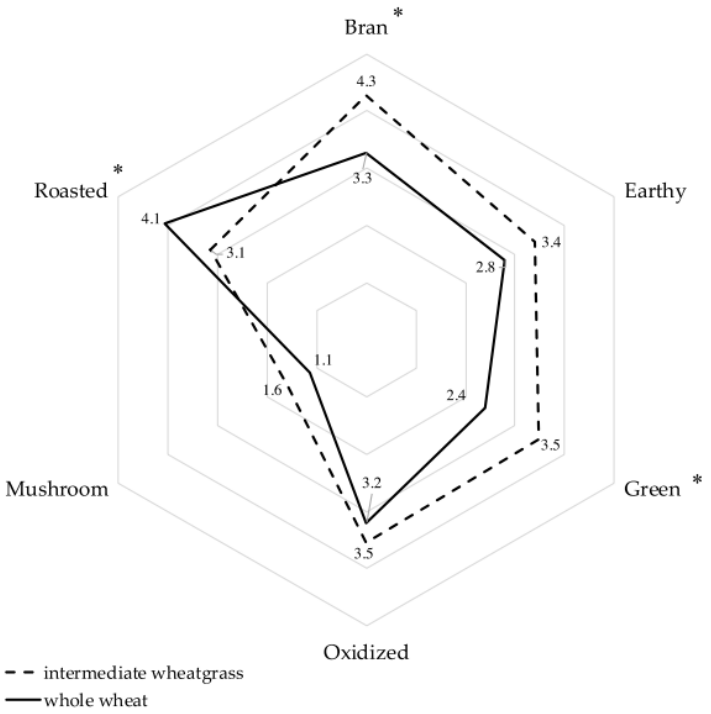

Figure 1.12: Sensory descriptive analysis of aroma profiles of whole wheat and intermediate wheatgrass bread crusts. * shows significance of the sample on aroma intensity (45).

Proteins indirectly influence the flavour of food through binding to flavour compounds, which are released when we chew (188). When protein content is too high, it negatively impacts the flavour. Protein-fortified food often tastes unpleasant and causes a sensation of dry mouth, which impacts customer acceptance of these products (41). Studies have shown that rats that were adapted to receive low-no-protein diets, exhibited decreased food intake of medium-high protein diets, which was attributed to poor palatability of high protein diet (192). Human studies have shown that cooked rice grains containing high protein content were found to be less palatable than those with lower protein (193). Additionally, cooked pasta containing high protein content was also found to be less palatable than both high fibre pasta and normal pasta (194). When proteins bind with polyphenols in food under high temperatures, they sometimes produce favourable or unfavourable flavours, their interaction is also responsible for the oxidative browning reactions that occur during cooking (195), for example the browning of bread crusts. Heat causes proteins to aggregate, leaving a lower accessible

surface area for binding but this may not necessarily impact flavour significantly as potential conformation changes of the proteins allow for increased flavour binding in this state, but this has varied across studies (188). As wheat is such a fundamental part of our diet but lacks in protein, research is being conducted on the prospects of using edible insects that are high in protein to fortify wheat products. However, products like this could negatively impact perceived palatability of the food based on consumers preferences, particularly in Western countries where insects are not commonly eaten (39). It would be beneficial to introduce a grain with higher protein content to the market for widespread consumption, such as Kernza.

Dietary fats are considered very palatable among most mammals despite not having a strong inherent taste, and this is due to the appealing sensory experience they evoke when consumed (196). The fat content in IWG exceeds that of wheat (12), which leads to potent flavours when the flour is baked due to the Maillard reaction. This enhancement in flavour is a desirable quality for many food products. However, the increased fat content in grains like IWG presents challenges during storage. Enzymes naturally present in the grains break down fats, leading to negative changes in flavour (45). Lipase is a ubiquitous enzyme in nature responsible for hydrolysing fats into free fatty acids (FFA), and this leads to the development of rancid odours in grains. Additionally, lipoxygenase (LOX), an enzyme with anti-inflammatory properties, catalyses the formation of unstable hydroperoxides through a series of reactions that may lead to the development of flavour-active volatile compounds. Studies on a collection of IWG varieties have shown them to contain low LOX activity, however, a few varieties exhibited high lipase activity (LA). It is hypothesised the combination of LA and the high fat content in IWG grains could contribute to potential rancidity (12,45). These

challenges could be overcome through continued genetic breeding aimed to lower LA, and careful determination of shelf life of Kernza-based products (12).

Satiation is affected by food texture, with increased chewing leading to lower *ad libitum* food intake (197). Foods with high fibre content and large particle size increase the effort and time required during chewing, ultimately increasing the energy expenditure required to break it down into smaller particles (84). In addition to personal taste preferences, the coarse texture of whole grain foods leads consumers to opt for less nutritious, refined grain alternatives (186). In a small study conducted by Becker et al. (40), consumer responses to baked foods that incorporated various percentages of IWG were assessed. Bread produced with IWG was perceived to have a coarser texture and was less preferable to whole wheat bread. However, other baked goods, such as cookies containing IWG, were perceived to have a more favourable texture and were well received by consumers. Gluten content has an impact on the texture and taste of bread, resulting in low consumer acceptance of gluten-free bread, except for those who are life-long consumers (198). Gluten-free bread lacks certain Maillard compounds and shares similarities with Kernza of low HMW gluten, which not only impacts the texture of bread but also the flavour formation (45). While grains with higher bran content provide a lot of nutritional value, it is important to consider the sensory characteristics they impart to food and the resulting impact it may have on acceptance from consumers, who already prefer refined grain foods over whole wheat options.

1.6 Aim

Kernza, as a perennial grain crop, which provides benefits to the environment over annual wheat. Its extensive root system stabilises the soil, retains water, and reduces nitrate leeching and carbon dioxide emission. Additionally, Kernza differs in its nutritional composition, and it

is a better source of fibre and protein than annual wheat. Due to this, Kernza may provide some health benefits over wheat, particularly for metabolic health. However, these differences in composition may impact the palatability of Kernza (thereby affecting the acceptance of and preference for diets rich in this flour), the issue which has not been investigated thus far.

Therefore, the **overarching goal** of this set of studies was to determine whether Kernza is palatable in relation to whole wheat flour. The experiments were performed in adult male laboratory rats, a standard animal model utilized in food intake research on eating for palatability. The overarching goal was accomplished by addressing the following specific aims:

Specific aim 1: Determining the amount of Kernza consumed by animals given this tastant in a single, episodic meal, in which no other food was simultaneously available (no-choice scenario). This was done in non-deprived/sated rats (consumption driven primarily by palatability) as well as in hungry animals (consumption driven by a combination of energy needs and palatability). The no-choice paradigm indicates acceptance of a given diet. Additional groups of rats were given instead of Kernza a whole wheat flour-based meal or a meal consisting of highly palatable high-fat high-sugar chow (HFHS) or standard ("bland" chow pellets).

Specific aim 2: Determining whether the acceptance for the Kernza vs wheat flour diet remains stable if either of the diets is offered over a period of 9 days (sub-chronically). This parameter allows us to infer whether acceptance of Kernza (vs wheat) is modified by prolonged consumption of the tastant.

Specific aim 3: Determining the amount of Kernza consumed by animals given this tastant in a single, episodic meal, in which the animals had a choice between Kernza and whole wheat flour (or between Kernza and HFHS chow or Kernza and standard “bland” chow). The choice paradigm allows us to examine preference for Kernza relative to other diets that differ in palatability.

Specific aim 4: Determining whether consumption of a Kernza meal (compared to a whole wheat flour meal) affects neuronal activation in key brain regions associated with reward and feeding behaviour – the VTA, AcbS, and AcbC. Typically, a greater palatability of a meal translates to a higher level of brain activity in the reward system. Neuronal activation was established by immunohistochemically staining brain tissue with the antibody directed against a protein product of a neuronal transcription factor, c-Fos.

Chapter 2

Materials and Methods

2.1 Animals

All animals used in this study were adult (12-month-old) male Sprague Dawley rats. They were single-housed in standard plastic cages with wire lids in a temperature-controlled (22°C) facility with a 12:12 hour light dark schedule (lights on at 08:00). Standard laboratory chow (Sharpes Stock Feed, New Zealand; 3.6 kcal/g) and tap water were available ad libitum unless otherwise stated. Animals were maintained in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. Ethics approval was granted by The University of Waikato Animal Ethics Committee for all studies and experiment procedures described.

2.2 Feeding studies

2.2.1 Flour-based diets

Two types of flour were used throughout this study: Kernza (FGI, St. Paul, MN), and whole wheat flour (Edmonds, New Zealand). Three different flour-based diets were used in this study: mash (raw flour and water), slurry (cooked flour and water), and pellets (cooked flour, water, and beef lard). The caloric densities of each diet are visible in Table 2.1.

Animals were previously exposed in their home cages to all flour-based diets to avoid neophobia. Animals were weighed at the onset of each feeding study so food intake could be accurately calculated based on body weight. All experimental meals took place between 9:00 to 11:00 unless otherwise stated. Due to the viscosity of both the mash and slurry meals, they

were unable to be served in the normal food hopper in the animal's cages. Thus, they were served in small plastic feeders that hooked into the wire lid of the animal's cages, situated directly in front of their food hopper. All animals used in this study were accustomed to eating out of the feeders. Animals used for experiments with two simultaneously presented food choices we also pre-exposed prior to the experiments to receiving two meals on either side of their food hopper. Water was available ad libitum for their entirety of all studies.

Pellets were prepared by mixing either whole wheat (3.30 kcal/g) or Kernza (3.57 kcal/g) flour with tap water and beef lard (8.84 kcal/g) and baked at a high temperature until solid. The final composition of the pellets was 87.55% flour and 12.45% fat. The caloric density of the whole wheat and Kernza pellets was 4.05 kcal/g and 4.23 kcal/g, respectively. The pellets were stored in an airtight container for up to one month. The pellets were given to the animals in their normal food hopper.

The mash was made fresh prior to each experimental meal and was prepared by thoroughly mixing raw flour (either whole wheat or Kernza) and tap water together. The flour to water ratio was adjusted to ensure that the Kernza and whole wheat diets were isocaloric, resulting in a final caloric density of 1.2 kcal/g.

The slurry was made fresh prior to each experimental meal. First, tap water and raw flour (either whole wheat or Kernza) were thoroughly mixed. The mixture was then cooked in the microwave for approximately 3 minutes and stirred every 30 seconds, until the temperature reached 60-70 degrees Celsius, indicating that gelatinization had occurred. It was then cooled to room temperature before serving. The water content for the slurry doubled that of the mash mixture, to allow for gelatinization and water evaporation. This is reflected in its lower

final caloric density of 0.73 kcal/g for whole wheat slurry, and 0.71kcal/g for Kernza slurry. Like the mash, the flour to water ratio was adjusted to ensure both flour variations were isocaloric.

Table 2.1: Total caloric density of the whole wheat and Kernza flour-based diets used in this experiment: pellets, mash, and slurry.

	Pellets	Mash	Slurry
Kernza	4.23 kcal/g	1.2 kcal/g	0.72 kcal/g
Wholemeal	4.05 kcal/g	1.2 kcal/g	0.72 kcal/g

2.2.2 Episodic intake of individually presented mash

To avoid neophobia the animals were previously exposed to receiving either whole wheat or Kernza mash for 1 hour on 2 days prior to the experiment using the plastic feeders. For assessment of food intake in response to energy deprivation, the animals were deprived of standard chow overnight (food removed at 16:00 for 18 hours). The next day the animals were individually presented with either whole wheat or Kernza mash (n=11-12/group) for 1 hour. Food intake was measured after 1 hour using a digital scale and expressed in grams.

To assess non-energy deprived episodic intake of the flour mash, standard chow was removed 1 hour prior to and during the experimental session. Animals were individually presented (n=12/group) with either whole wheat or Kernza mash. Food intake was measured after 1 hour using a digital scale and expressed in grams.

2.2.3 Episodic intake of individually presented slurry

To assess the palatability of cooked flour animals that had been previously exposed to receiving either whole wheat or Kernza slurry for 1 hour on 1 day prior to the experiment using plastic feeders. On the experimental day, standard chow was removed from the food hoppers and the animals (n=11/group) were individually presented with either whole wheat

or Kernza slurry for 1 hour. Food intake was measured after 1 hour using a digital scale and expressed in grams.

2.2.4 Episodic intake of individually presented pellets

After acceptance of the raw and cooked flour diets in wet form (mash and slurry) was confirmed, the next experiment was to assess whether the animals would find a cooked solid flour diet palatable. Prior to the feeding studies using flour pellets, the animals had been accustomed to being transferred to a clean, bedding-free cage for 1 hour on 3 days. This was necessary as to weigh any spillage of pellets after the experimental meal. Animals had also been previously exposed to receiving whole wheat or Kernza pellets in their food hopper.

First, food intake in response to energy deprivation was assessed. Standard chow was removed from the animal's cages overnight (at 18:00 for 16 hours). The next day, the animals were transferred to a bedding-free cage for 1 hour and individually presented with either whole wheat or Kernza pellets (n=14-13/group). Spillage from the pellets was recorded after the feeding session, and food intake was measured after 1 hour using a digital scale and expressed in grams.

The next experiment was to assess non-energy deprived consumption of flour pellets during the animal's normal mealtime (at night), as all other experiments were conducted during the animals rest period (day). Thus, after pre-exposure to the flour pellets at dark onset for a 3-hour meal for 1 day, standard chow was removed from the cage, the animals were individually presented on the experimental day with either whole wheat or Kernza pellets (n=14-13/group) at onset of dark in a bedding-free cage for 3 hours. Spillage from the flour pellets was recorded after the feeding session, and food intake was measured using a digital scale and expressed in grams.

To compare the non-energy deprived intake of flour pellets at dark onset to the usual experimental time (during the day), once again the animals that had been accustomed to consuming flour pellets in bedding-free cages were individually presented with either whole wheat or Kernza pellets (n=13-14/group) for 1 hour, with standard chow removed during the experimental session. Spillage from the flour pellets was recorded after the feeding session, and food intake was measured using a digital scale and expressed in grams.

2.2.5 Sub-chronic daily intake of mash meal

A sub-chronic study of non-deprived individually presented diets was conducted, to assess whether the animals would maintain interest in the flour-based foods over a longer period. The mash diet was used for this experiment due to ease, and all animals used for this experiment had been previously accustomed to eating the mash from the plastic feeders. The animals (n=11/group) were individually presented with either whole wheat or Kernza mash for 2 hours per day, over 9 days. Outside of the 2-hour experimental meal, they had ad libitum access to standard chow. After each session, food intake was measured using a digital scale and expressed in grams.

2.2.6 Episodic intake of simultaneously presented mash

The preference of whole wheat compared to Kernza flour was assessed by simultaneously presenting the animals with both foods. Animals were previously exposed to receiving both whole wheat and Kernza mash in plastic feeders for 1 hour on 2 days. The feeders containing the mash had randomised placement between either the left or right side at the front of their cage. On the experimental day, the animals were simultaneously presented with whole wheat and Kernza mash (n=23) in separate plastic feeders for 1 hour, with standard chow removed

from the food hopper. After the experimental session, both feeders were removed and weighed, and intake was expressed in grams.

2.2.7 Episodic intake of simultaneously presented slurry

Following the same experimental protocol described above, animals were pre exposed to simultaneously presented whole wheat and Kernza slurry for 1 hour on 3 days. On the experimental day, standard chow was removed from the food hopper and the animals received both whole wheat and Kernza slurry (n=16) for 1 hour, randomised between the left and right side of their cage. Afterwards, both feeders were removed and weighed, and intake was expressed in grams.

2.2.8 Episodic intake of simultaneously presented pellets

The final preference study involved simultaneously presentation of whole wheat and Kernza pellets. Animals had been previously exposed to simultaneously receiving both flour pellets in bedding-free cages for 1 hour on 1 day. On the experimental day the animals (n=25) were transferred to a bedding-free cage and given both whole wheat and Kernza pellets subdivided in their food hopper for 1 hour, randomised between the left and right sides of their cage. Standard chow was removed for the duration of the experimental session. The food intake was then measured after the session using digital scales and expressed in grams.

To assess the palatability of flour pellets compared to the animal's standard laboratory chow (Sharpes, New Zealand; 3.6 kcal/g), the animals were previously exposed to receiving one of the flour pellets (either whole wheat or Kernza) alongside their standard chow pellets, for 1 hour on 2 days, in bedding-free cages. On the experimental day, the animals were transferred to a bedding-free cage with no access to standard chow, 1 hour prior to the experimental

session. The animals (n=13/group) were then simultaneously presented with standard chow and flour pellets (whole wheat or Kernza) for 1 hour. After the experimental session, the remaining standard chow and flour pellets were weighed along with any spillage using digital scales and expressed in grams.

Another preference experiment was conducted to compare the preference of flour pellets to a calorically dense and highly palatable HFHS chow (Research Diets #D12451; 4.73kcal/g). Prior to the experiment, the animals had been exposed for 1 hour on 2 days to simultaneously receiving HFHS chow and either whole wheat or Kernza pellets, in bedding-free cages. On the experimental day, the animals were transferred to a bedding-free cage, with no access to standard chow for 1 hour. The animals (n=13/group) were then presented simultaneously with HFHS chow and flour pellets (either whole wheat or Kernza) for 1 hour. Food intake of the HFHS chow and flour pellets was measured after the experimental session along with any spillage, using a digital scale, and expressed in grams.

2.3 Neuronal activity

2.3.1 Perfusion

Eighteen rats were subdivided into three groups (n=6/group). At 09:00 their standard chow was removed, 1 hour prior to the experimental meal. Two groups then received approximately 20g each of either whole wheat mash or Kernza mash under non-energy deprived conditions. The third group was used as a baseline (control) and received no meal. The meals were fully consumed in 20 to 40 minutes, after which the animals were anesthetised 60 to 90 minutes later (to allow for peak c-Fos expression) via an intraperitoneal injection of 35% urethane. This was followed by an intracardial perfusion with 50mL saline and then 500mL 4%

paraformaldehyde (PFA) solution. Next, the brains were dissected and stored in PFA for 48 hours for postfixing at 4°C, then transferred to tris-buffered saline (TBS) until sectioning.

2.3.2 Immunohistochemistry

The brains were sectioned through the coronal plane (60 µm, free floating sections) using a vibratome (Leica, Germany) and stored in TBS at 4°C. The sections were stained for c-Fos. The sections were transferred to agitators at room temperature, and an initial four washes in TBS, followed by a 10-minute treatment of 3% H_20_2 and 10% methanol in TBS. After another four washes in TBS, the sections were incubated overnight in the primary rabbit c-Fos antibody (1:4000; Synaptic Systems, Australia) in supermix (0.25% bovine skin gelatin and 0.5% Triton X-100 in TBS) at 4°C.

The following day, the sections were retrieved, washed four times in TBS, and incubated at room temperature in goat-anti-rabbit secondary antibody (1:400; Vector Laboratories, Burlingame, CA, USA) in supermix for 90 minutes. After another four washes in TBS, the sections were incubated for another 90 minutes in avidin-biotin complex (ABC) solution (1:800; Elite Kit, Vector Laboratories, Burlingame, CA, USA) in supermix. The sections were washed again in TBS, before developing them in a TBS solution of 0.05% diaminobenzidine (DAB), 0.3% nickel sulphate, and 0.001% H_20_2 for 10 to 20 minutes.

The stained sections underwent a final wash in TBS and mounted onto gelatinised glass microscope slides, before being air dried at room temperature for 48 hours. The slides then underwent the process of ethanol dehydration (70, 90 and 100 percent concentrations), in ten-minute intervals before a final bath in xylene (Merck KGaA, Darmstadt, Germany) for 20 minutes. Coverslips were then applied with mounting media, Entellen (Merck, KGaG, Darmstadt, Germany).

2.3.3 Analysis of neuronal activity

The brain sections were photographed with a camera attached to a light microscope (Nikon Eclipse 400) and analysed through ImageJ software. Allen Brain Atlas was used as a reference for mapping areas of the brain. The following areas were analysed: VTA, AcbC, and AcbS. Density of the c-Fos immunoreactive (IR) nuclei (per mm^2) were counted and averaged per animal and then per group. All data analyses were performed in GraphPad Prism using one-way ANNOVA and two keys post hoc test. Student's t-tests were used to analyse 2 group comparisons. Data were expressed as mean±SEM. Differences were considered statistically significant for $p < 0.05$.

Chapter 3

Results

3.1 Feeding Studies

3.1.1 Energy-deprivation feeding studies

Energy deprived animals given episodic access for 1 hour to one diet of whole wheat or Kernza mash showed no difference in intake (Figure 3.1A, B, C). Similarly, the animals showed no difference in energy-deprivation intake when episodically given one diet of whole wheat or Kernza pellets (Figure 3.2A, B, C).

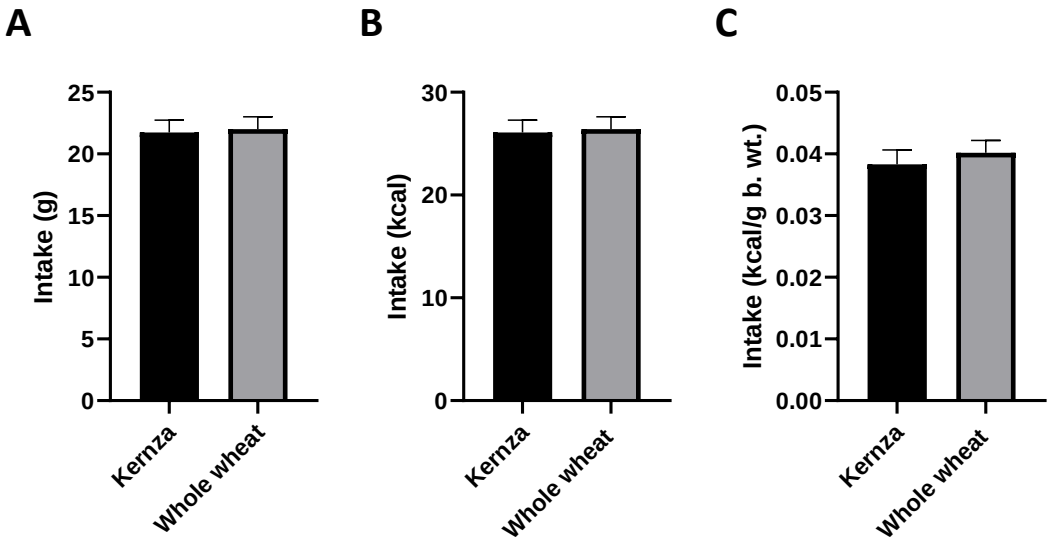

Figure 3.1: Episodic intake of individually presented Kernza and whole wheat mash in energy-deprived animals (n=11-12/group). Standard chow was removed 18 hours before the 1-h meal. Intake expressed in terms of grams (**A**), kcal (**B**), and kcal/g of body weight (**C**).

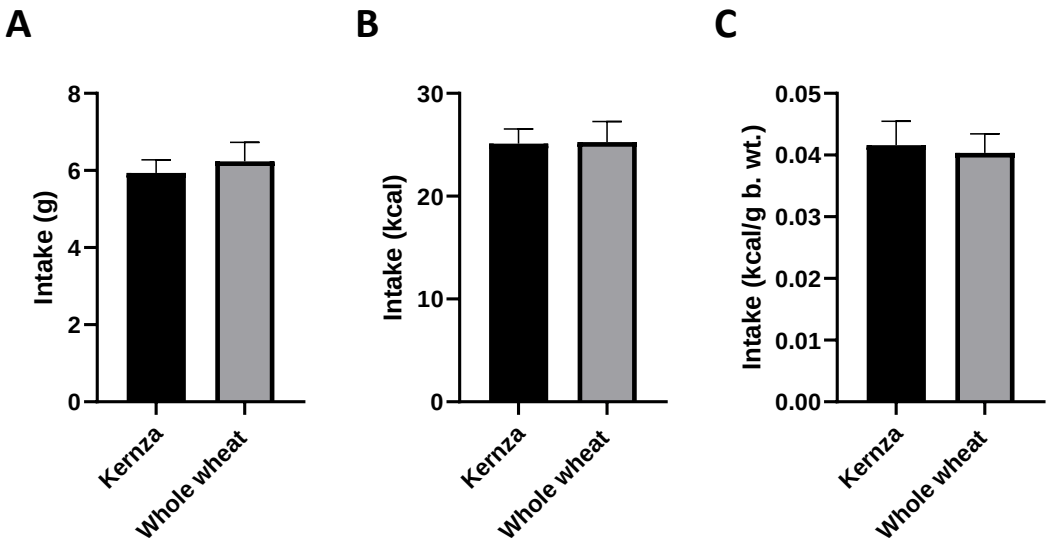

Figure 3.2: Episodic intake of individually presented Kernza and whole wheat pellets in energy-deprived animals (n=14-13/group). Standard chow was removed 16 hours before the 1-h meal. Intake expressed in terms of grams (**A**), kcal (**B**), and kcal/g of body weight (**C**).

3.1.2 Sub-chronic feeding study

Animals were presented with one diet of whole wheat or Kernza mash for 2-hour feeding sessions over 9 days. There was no difference in intake between the two diets (Figure 3.3A, B, C). The average amount of mash consumed each day trended slightly upwards over time but was relatively consistent.

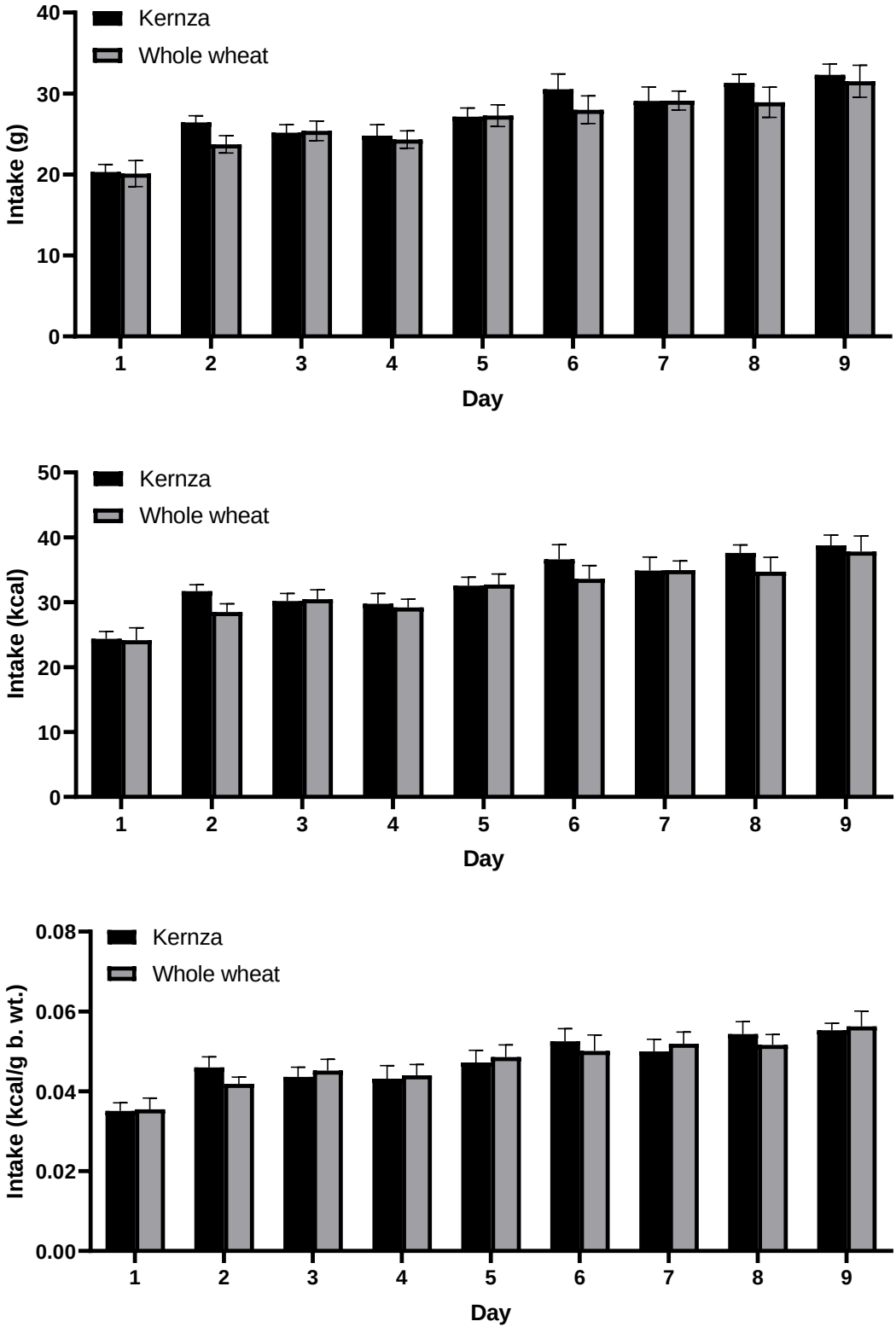

Figure 3.3: Sub-chronic intake of individually presented Kernza and whole wheat mash in non-deprived animals. Meals were provided for 2-h daily for 9 days (n=11/group). Intake expressed in terms of grams (**top**), kcal (**middle**), and kcal/g of body weight (**bottom**).

3.1.3 Episodic intake of individually presented diets in non-deprived animals

When rats were presented with one diet of whole wheat or Kernza mash for 1 hour, there was no difference in intake between the two diets (Figure 3.4A, B, C), and this was alike for the slurry (Figure 3.5A, B, C), and the pellet (Figure 3.6A, B, C) diets, including when the pellet diets were offered for 3 hours at dark onset (Figure 3.7A, B, C).

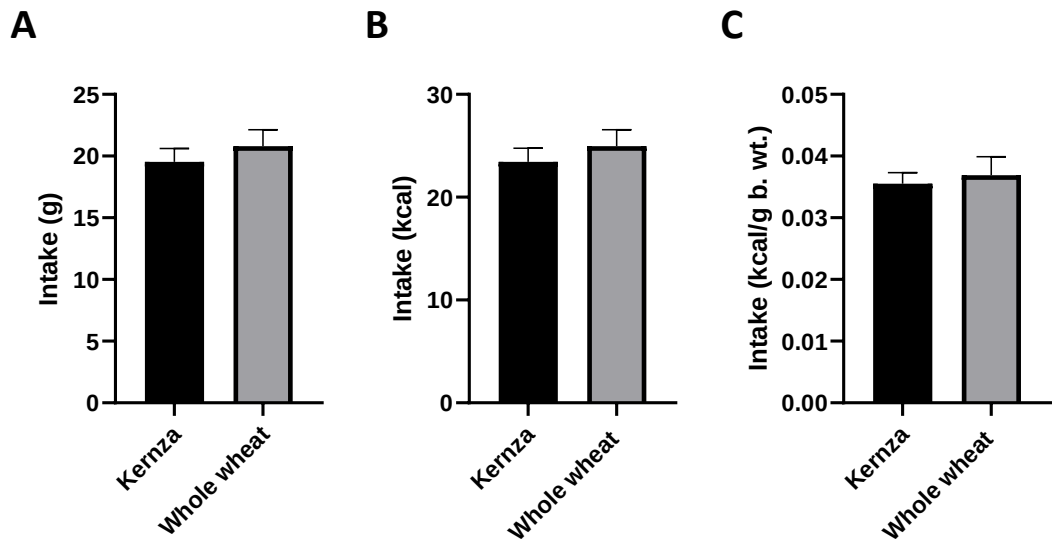

Figure 3.4: Episodic intake of individually presented Kernza and whole wheat mash in non-deprived animals (n=12/group). Standard chow was removed 1 hour before the 1-h meal. Intake expressed in terms of grams (**A**), kcal (**B**), and kcal/g of body weight (**C**).

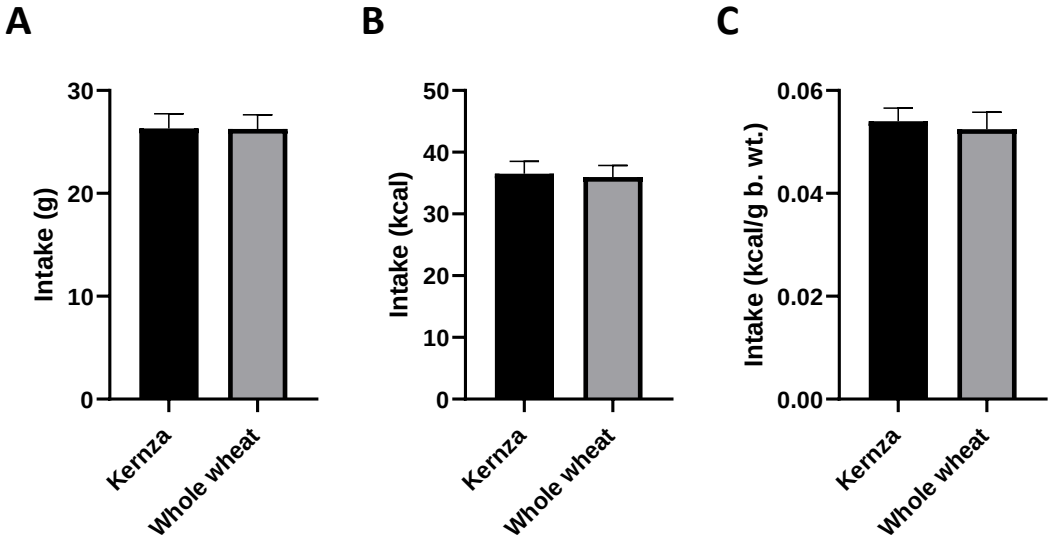

Figure 3.5: Episodic intake of individually presented Kernza and whole wheat slurry in non-deprived animals (n=11/group). Meal was provided for 1-h. Intake expressed in terms of grams (**A**), kcal (**B**), and kcal/g of body weight (**C**).

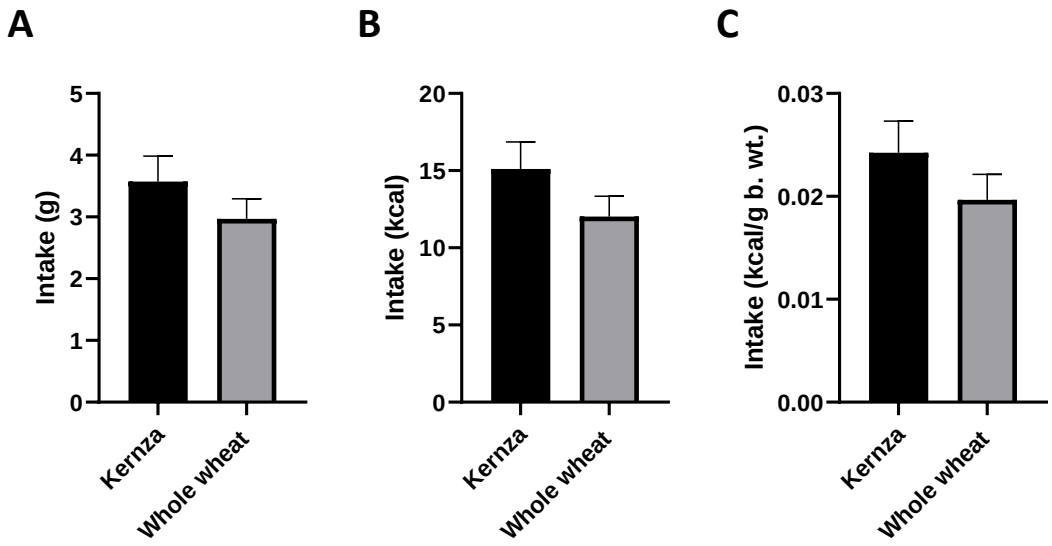

Figure 3.6: Episodic intake of individually presented Kernza and whole wheat pellets in non-deprived animals (n=13-14/group). Meal was provided for 1-h. Intake expressed in terms of grams (**A**), kcal (**B**), and kcal/g of body weight (**C**).

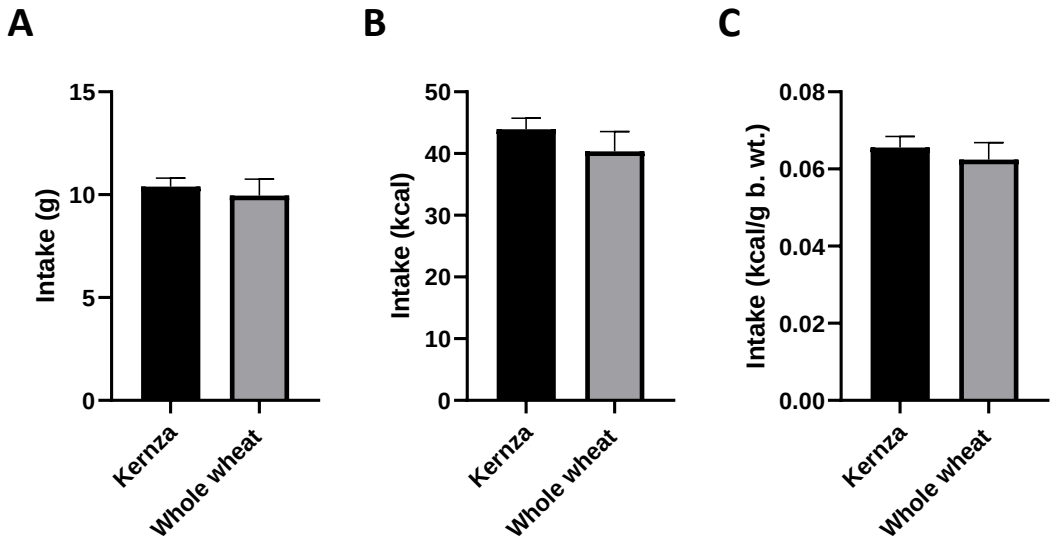

Figure 3.7: Episodic intake at dark onset of individually presented Kernza and whole wheat pellets in non-deprived animals (n=14-13/group). Meal was provided for 3-h. Intake expressed in terms of grams (**A**), kcal (**B**), and kcal/g of body weight (**C**).

3.1.4 Episodic intake of simultaneously presented diets in non-deprived animals

When rats were episodically presented with two diets of whole wheat and Kernza mash for 1 hour, they consumed significantly more whole wheat mash (intake in grams, p=0.001, Figure 3.8A; intake in kcal, p=<0.0001, Figure 3.8B; intake in kcal/g b. wt, p=<0.001, Figure 3.8C) than Kernza mash. When rats were episodically presented with two diets of whole wheat and

Kernza slurry for 1 hour, they consumed significantly more whole wheat slurry (intake in grams, p=0.006, Figure 3.9A; intake in kcal, p=0.004, Figure 3.9B; intake in kcal/g b. wt, p=0.010, Figure 3.9C) than Kernza slurry. When rats were episodically presented with two diets of whole wheat and Kernza pellets for 1 hour, there was no difference in intake between the two diets (Figure 3.10A, B, C).

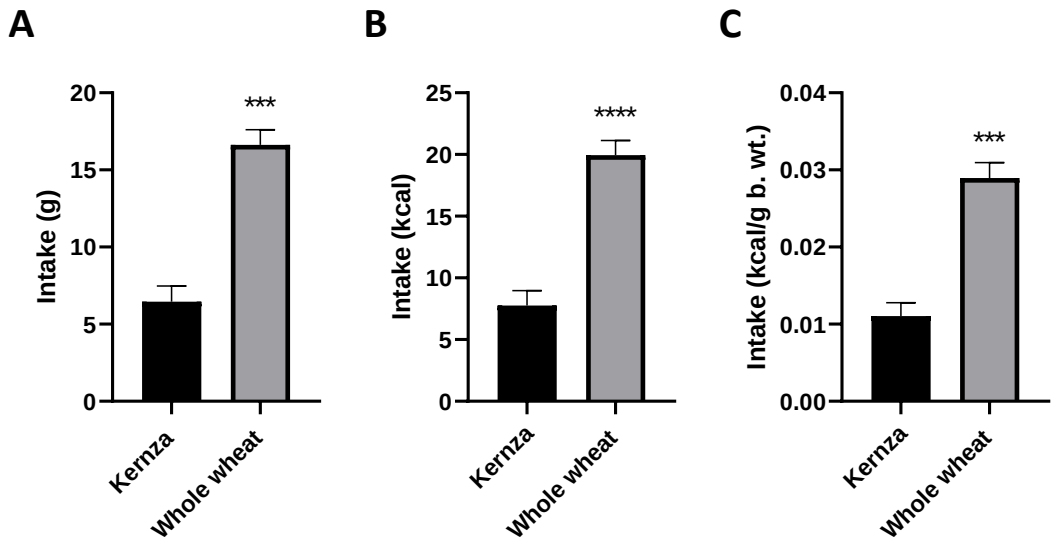

Figure 3.8: Episodic intake of simultaneously presented Kernza and whole wheat mash in non-deprived animals (n=23). Meal was provided for 1-h. Intake expressed in terms of grams (**A**), kcal (**B**), and kcal/g of body weight (**C**). *** p=<0.001; **** p=<0.0001.

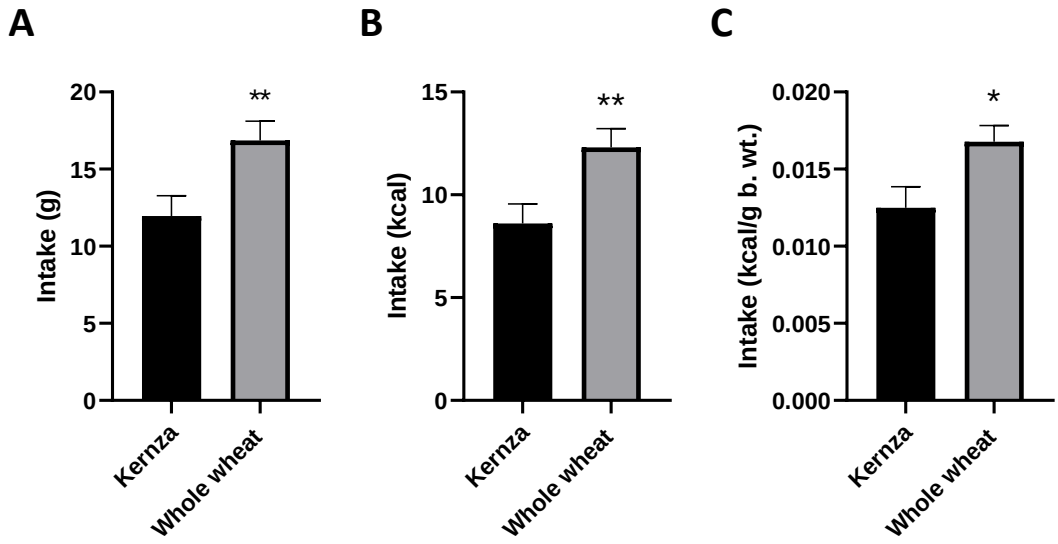

Figure 3.9: Episodic intake of simultaneously presented Kernza and whole wheat slurry in non-deprived animals (n=16). Meal was provided for 1-h. Intake expressed in terms of grams (**A**), kcal (**B**), and kcal/g of body weight (**C**). * p=0.010; ** p=0.004 (**B**) p=0.006 (**A**).

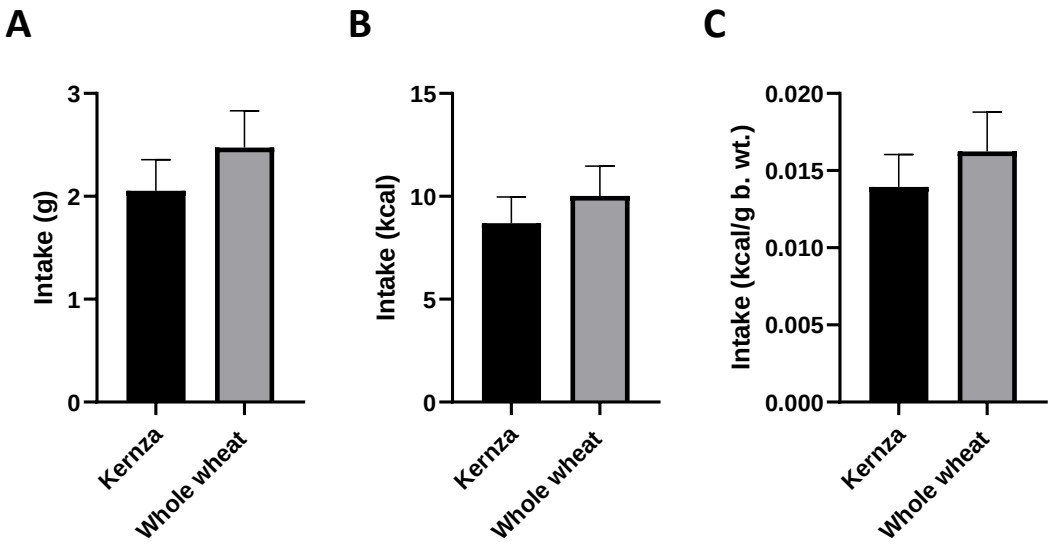

Figure 3.10: Episodic intake of simultaneously presented Kernza and whole wheat pellets in non-deprived animals (n=25). Meal was provided for 1-h. Intake expressed in terms of grams (**A**), kcal (**B**), and kcal/g of body weight (**C**).

In the preference test for the bland standard chow, compared to whole wheat or Kernza pellets, there was a significant intake of both whole wheat (intake in grams, p=<0.001, Figure 3.11A; intake in kcal, p=<0.0004, Figure 3.11B; intake in kcal/g b. wt, p=<0.0001, Figure 3.11C) and Kernza pellets (intake in grams, p=<0.0001, Figure 3.12A; intake in kcal, p=<0.0001, Figure 3.12B; intake in kcal/g b. wt, p=<0.0001, Figure 3.12C) compared to standard chow.

In the preference test for the highly palatable HFHS chow compared to whole wheat or Kernza pellets, there was significantly higher consumption of HFHS chow than both whole wheat (intake in grams, p=<0.001, Figure 3.13A; intake in kcal, p=<0.0001, Figure 3.13B; intake in kcal/g b. wt, p=<0.0001, Figure 13.3C) and Kernza (intake in grams, p=<0.001, Figure 3.14A; intake in kcal, p=<0.0001, Figure 3.14B; intake in kcal/g b. wt, p=0.0001, Figure 3.14C) pellets.

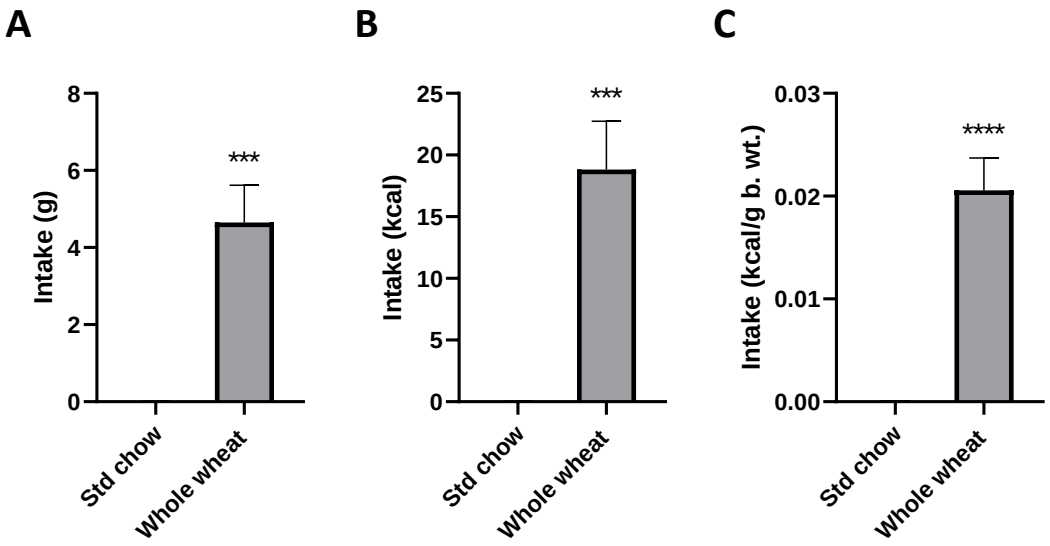

Figure 3.11: Episodic intake of simultaneously presented standard chow and whole wheat pellets in non-deprived animals (n=13). Standard chow was removed 1 hour before the 1-h meal. Intake expressed in terms of grams (**A**), kcal (**B**), and kcal/g of body weight (**C**). *** p=<0.001 (**A**) p=<0.0004 (**B**); **** p=<0.0001.

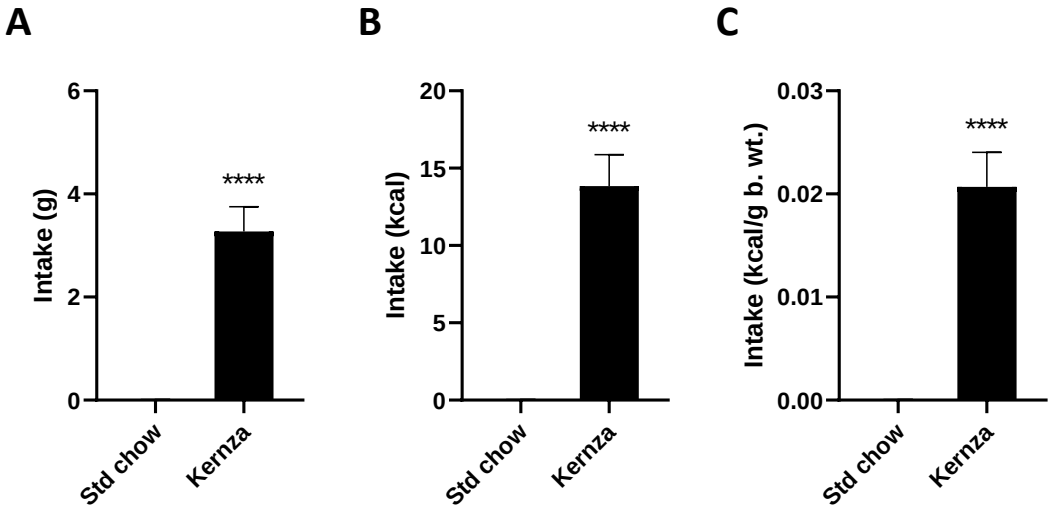

Figure 3.12: Episodic intake of simultaneously presented standard chow and Kernza pellets in non-deprived animals (n=13). Standard chow was removed 1 hour before the 1-h meal. Intake expressed in terms of grams (**A**,), kcal (**B**), and kcal/g of body weight (**C**). **** p=<0.0001.

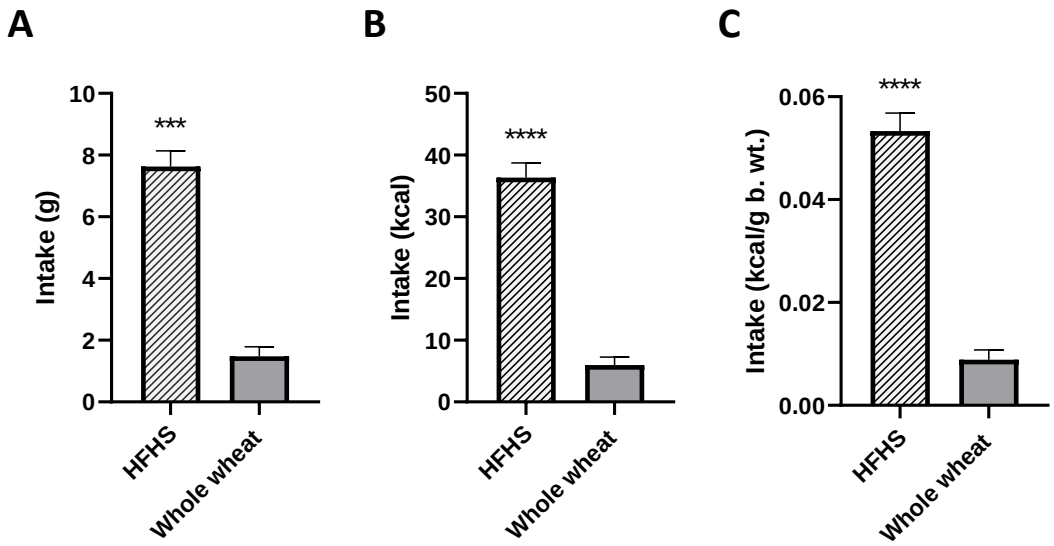

Figure 3.13: Episodic intake of simultaneously presented HFHS chow and whole wheat pellets in non-deprived animals (n=13). Standard chow was removed 1 hour before the 1-h meal. Intake expressed in terms of grams (**A**), kcal (**B**), and kcal/g of body weight (**C**). *** p=<0.001; **** p=<0.0001.

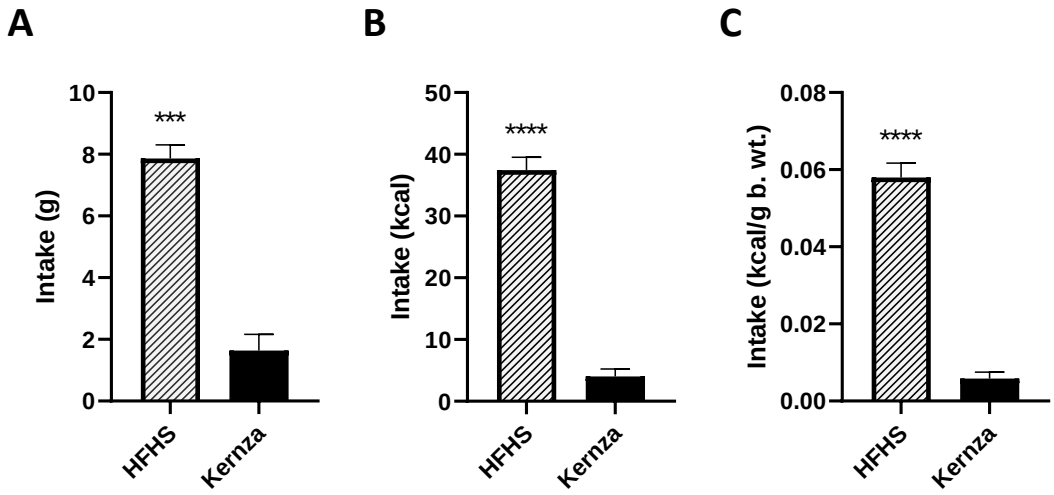

Figure 3.14: Episodic intake of simultaneously presented HFHS chow and Kernza pellets in non-deprived animals (n=13). Standard chow was removed 1 hour before the 1-h meal. Intake expressed in terms of grams (**A**,), kcal (**B**), and kcal/g of body weight (**C**). *** p=<0.001; **** p=<0.0001.

3.2 c-Fos Immunoreactivity

Assessment of c-Fos IR in areas of the brain related to reward and feeding behaviour included the three areas: AcbC, AcbS, and VTA. In the VTA, there was a significant difference in c-Fos IR between the baseline and Kernza group (p=0.006, Figure 3.15A) and greater difference between the baseline and whole wheat group (p=<0.001, Figure 3.15A). However, there was no significant difference in c-Fos IR between the Kernza and whole wheat group. In the AcbC,

there was a significant difference in c-Fos IR between the baseline and Kernza group (p=0.05, Figure 3.15B), and a greater difference between the baseline and whole wheat group (p=0.007, Figure 3.15B), but no difference in c-Fos IR between the Kernza and whole wheat group. In the AcbS, there was a significant difference found in c-Fos IR between all groups. The baseline group had significantly less c-Fos IR than the Kernza group (p=0.007, Figure 3.15C), and even lesser compared to the whole wheat group (p=<0.001, Figure 3.15C). The whole wheat and Kernza group also showed a difference in c-Fos IR (p=0.02, Figure 3.14C).

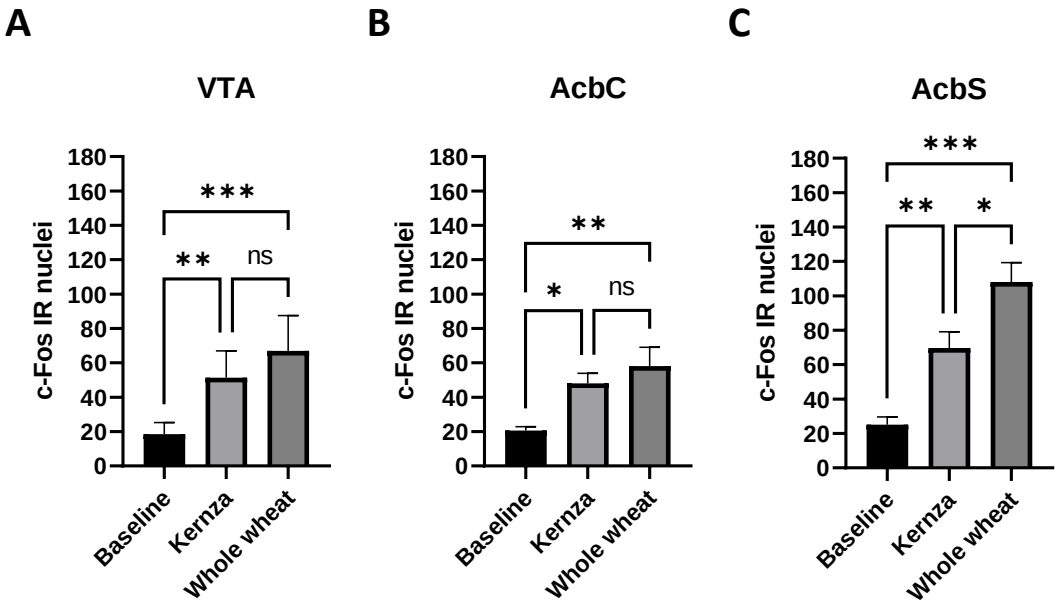

Figure 3.15: Effect of 20g intake of whole wheat or Kernza mash compared on c-Fos immunoreactivity (IR) compared to (unfed) baseline in non-deprived animals (n=6/group). Density of Fos-positive nuclei (per 1mm^2) averaged per individual animal, and per group. VTA, ventral tegmental area; AcbC, nucleus accumbens core; AcbS, nucleus accumbens shell. * p=0.02 (**C**) p=0.05 (**B**); ** p=0.006 (**A**) p=0.007 (**B**, **C**); *** p=<0.001.

Chapter 4

Discussion

Since the onset of agriculture, crop cultivation has been primarily focused on maximizing yield within a given area of land. This is not surprising given the prevalence of food scarcity and malnutrition throughout human history, with access to nutritious diets considered to be a luxury. Modern agricultural techniques and utilisation of food science has revolutionized our ability to mass-produce high-energy, palatable, and affordable foods, the abundance of which finally allows us to shift our attention beyond crop yield and towards the impact that specific crops have on our environment and ecosystem.

Wheat is one of the most abundant crops and is a dietary staple in the Western world. However, despite its many nutritional benefits as a source of calories and macro- and micronutrients to billions of people worldwide, this has come at a cost to the environment. As wheat is an annual crop, its cultivation is associated with, among others, degradation of soil health through erosion and inefficient water retention, and an increased carbon footprint. Thus, substitution of wheat with an alternative crop that is more environmentally sustainable is of great importance.

Kernza has been proposed as a promising replacement crop for wheat. However, while there are clear environmental benefits linked to Kernza cultivation, our understanding of the impact that consumption of this grain has on consumers' metabolic and appetite parameters remains extremely limited. The current thesis provides a significant leap in our knowledge of the effect of Kernza versus whole wheat on appetite. Namely, it defines Kernza as a highly acceptable and palatable grain, which is readily ingested by hungry and sated animals. While being less

preferred than whole wheat in choice paradigms, it is nonetheless eaten even in the absence of caloric needs, underscoring its gustatory attractiveness. This is reflected by Kernza's ability, when provided as a meal, to increase (albeit not as robustly as wheat consumption does) the activity of the reward system.

In the first set of experiments, I focused on examining acceptance for Kernza and found that this grain was highly accepted (similar to wheat). Acceptance refers to the willingness and ability of the laboratory animals to eat a new type of food that is introduced into their diet and continuation to eat it over time, as well as their physiological adaptation to it. Acceptance of a new food is influenced by palatability, nutritional content, and the physical characteristics of the food. Taste is as important for nutrient sensing and food intake, with taste cues impacting food acceptance and avoidance behaviour (199–201). Novel tastants are less palatable than familiar ones, and palatability increases as the tastant becomes more familiar (201,202), with the responsiveness of taste-hedonic neurons changing between novel and familiar tastes (203). Neophobia occurs when animals are exposed to novel foods, as a safety mechanism to protect them from illness or death. Laboratory animals are pre-exposed to novel foods prior to experiments to allow for their adjustment, initially consuming small amounts that increase over time once the novel tastant is accepted as safe (201,204,205). Aversive conditioning of a novel tastant is the strongest if illness occurs within the first 2 hours of ingestion, with the effect weakening over time (204). Greiner & Petrovich (206) compared habituation to novel food in food-deprived female and male rats, finding that once male rats became habituated to the novel taste they developed a preference for the novel food, and that female rats had suppressed habituation to novel tastes which was sustained throughout the study.

In my studies, non-energy deprived rats demonstrated the same level of acceptance for both whole wheat and Kernza. This was true for all flour diets (mash, slurry, pellets) however the pellets had the lowest average intake compared to the mash and slurry, which may be due to the addition of lard to the pellets. When laboratory animals have no choice of a diet, they will consume the diet, however the amount of consumption amount depends on the palatability of the diet – if they are given a bland one, such as standard chow, they consume enough to satisfy their energy requirements, but not beyond that (207). On the other hand, when animals find a diet to be palatable, the food provides an incentive value (208) and they often eat beyond their energy requirements, overconsume these foods when they are freely available, and readily exhibit operant behaviours to obtain these foods, such as lever pressing and maze running (117,168). Sclafani & Springer (207) maintained rats on a cafeteria diet until they became obese, then switched the diet to a less favourable one (with added quinine, a bitter compound) and found that the rats consumed fewer calories despite their body weight and exhibited diminished operant behaviour for the food. As Kernza- and wheat-based diets were readily consumed by animals even when they were not deprived of energy - this underscores the palatability of Kernza.

The taste and palatability of carbohydrates (sugar or polysaccharides) contribute to overeating in rats when these foods are offered in addition to standard diets (209). However, rats have shown to compensate for this overconsumption through self-restricted intake of standard chow, ultimately balancing their energy intake and maintaining a normal body weight (210–212). As shown by Avena et al. (210), rats with *ad libitum* access to standard chow will binge-eat HFHS chow when it is provided in a 2-hour time frame, and over several weeks come to consume the majority of their daily energy intake during that time period. The

same is true for rats offered sucrose solution (210), or vegetable shortening (212), and could be due to a reduction in the reinforcement efficacy of standard chow due to exposure to palatable food (211).

It is of importance to note, that in all but one of my studies, rats were exposed to the flour diets at a time of day when spontaneous intake does not usually occur – feeding experiments are often conducted in non-fasted rats during the light period to avoid hyperphagia of the dark period, thus the properties of the food matters more during the light period (174,213), implying that consumption is due to the palatable nature of the food (150). One experiment in this study was conducted during the onset of the dark period, and the rats consumed over twice the amount of flour pellets as what was consumed during the light period. Again, there was no difference in acceptance between whole wheat or Kernza.

Scheduled, intermittent access to palatable food may lead to increased reward sensitivity. Moore et al. (214) alternated the diet of rats by feeding standard chow for 5 days and sucrose for 2 days and found increased DAT mRNA in the VTA of these rats during the sucrose phase. They also found that the rats had a blunted response to *d*-Amphetamine, displaying lower *d*-Amphetamine heightened locomotor activity, and a lower efflux of dopamine during the sucrose phase. The impact of a restricted feeding schedule on dopamine turnover was examined in rats by Hajnal & Norgren (215). Rats were either pre-fed sucrose or water for 2 hours before receiving standard chow. After one week, microdialysis was performed in response to standard chow, with rats that received sucrose having higher dopamine turnover in the Acb than those that received water. Dopamine release was not habituated over time as seen when steady access to palatable food is provided, and further studies have also demonstrated the same persistent release of dopamine, specifically in the AbcS, and this

effect has been attributed to the specific paradigm of intermittent feeding and palatable food (216).

In addition to palatability, previous experience with a diet can condition eating behaviour and impact acceptability of foods. Reed & Friedman (217) tested the acceptability of corn oil in rats that were previously fed isocaloric HFD or HCD, with some of the HFDs altered with added carbohydrates, high fibre, or low fibre content. Rats that were fed the pure HFD and the HFDs containing low or high fibre demonstrated the strongest preference for corn oil, over those on a high-fat-added carbohydrate diet and the HCD fed group. They also preferred the oil when it was simultaneously presented with sucrose. The rats on the high-fat-added carbohydrate diet consumed the same amount of oil as the rats in the pure HCD. These findings demonstrated that previous exposure to a HFD led to the greatest acceptance of novel high-fat foods.

Food-deprivation induces alterations to the physiological and motivational state, by increasing food acceptance, intake, and attractiveness of certain tastes, and the acceptance and rejection of food can also depend on its hedonic features (49). Thus, it was important to distinguish if there would be any differences in the acceptance of Kernza and whole wheat in energy-driven or pleasure-driven consumption, to indicate that palatability of these flour diets was not due to the impact of hunger or energy needs alone.

Acute food deprivation leads to enhanced food intake, as demonstrated in a study by Warwick & Synowski (218), where rats were deprived of food for 24 hours before receiving a high-fat solution, of which the consumption was one third higher in calories than that of non-deprived rats. Additionally, food-deprivation has also been shown to enhance acceptance and preferences for food, by increasing attractiveness of certain nutrient flavours and increasing

motivation to obtain food to return to a positive energy balance. Rats that are food-deprived have stronger preferences for fatty tastes, followed by carbohydrates, over sweet tastes (218,219), as fats and carbohydrates provide a better source of energy (217). Warwick & Synowski (218) have also shown that in no-choice tests preceded by 24-hour food deprivation, there was enhanced acceptance of high-fat solutions over high-carbohydrates solutions, with rats consuming twice as much of the high-fat solutions. They also investigated the effect of food deprivation on high-fat and high-carbohydrate preference in a choice test. Rats consumed significantly more high-fat than high-carbohydrate solution when simultaneously offered, and this preference was not visible in rats that were non-deprived. When Sclafani et al. (220) offered rats either a high-fat or low-fat cake under non-deprived conditions, the cakes were found to be equally palatable. However, when the rats were food-deprived they significantly preferred the high-fat cake, likely due to the caloric density and deprivation-enhanced fat preference. Sclafani & Ackroff (219) found that deprived rats prefer polycose over sucrose, in contrast to the preference of non-deprived rats for sucrose. Additionally, they demonstrated that the sucrose preference could be reversed back to polycose or oil through food-deprivation.

As well as increasing the attractiveness and preference for certain foods, food deprivation also increases the palatability of all foods (139,221) by altering the anticipatory and consummatory response to palatable food through sensitisation of the reward system (139,170,181). Changes in gene expression occur in the ML-DA region of the brain in response to acute food deprivation (154). Studies have shown that rats deprived of food for 24 hours have decreased levels of POMC in the ARC (222), and 48-hour deprivation decreased secretion of POMC peptides in the ARC, and increased NPY and AgRP mRNA in cerebral spinal fluid

(223). Brady et al. (224) also found increased NPY mRNA and decreased POMC mRNA in the ARC in rats after 4 days without food, and that this effect was greater in rats that were food restricted. Hypothalamic μ-opioid receptors play a role in driving the preference and consumption of high-fat diets. Barnes et al. (221) found that 48-hour food deprivation significantly increased mRNA expression of μ-opioid receptors in the ARC, VMN, and LH of rats, though they did not find this effect in 12- and 24-hour fasts. After 48 hours the deprived rats were given simultaneously access to a HFD and LFD, consuming significantly more of the HFD. Additionally, when another group of 48-hour deprived rats were administered an μ-opioid receptor antagonist before gaining access to the two diets, they consumed significantly less of the HFD. Colantuoni et al. (225) alternated intermittent food deprivation with meals of palatable food, depriving rats of food for 12 hours during the early-dark period and providing access to standard chow in addition to a 25% glucose solution for the following 12 hours. In the following weeks, glucose intake increased two-fold and was mostly consumed within the first hour of access. After several months they found significant upregulation of D1 and μ-opioid receptors, with increased D1 receptor binding in the AcbS and AcbC, and μ-opioid receptor binding in the AcbS.

In an energy-deprived state, rats accepted whole wheat and Kernza diets equally. They consumed a higher amount in grams of mash than pellets, as the mash is lower in energy density than the pellets, however the total intake in kcal and kcal/g b.wt was the same for both experiments. As each of these diets were consumed whether the rats were hungry or sated, and there was no difference in acceptance between Kernza and whole wheat, it demonstrates that energy deprivation was not a factor in acceptance, with these diets

providing enough incentive for the rats to increase their energy intake when sated, indicating that they found the diets to be palatable.

The novelty effect of food can lead to its preference compared to when the food is part of normal diet (226). When rats are first exposed to palatable food, they consume up to twice as much as that of rats with a history of consuming the food. However, chronic intake of palatable food coupled with the absence of variety has shown to eventually decrease motivation to consume palatable food over time (211,227,228). This has been demonstrated by South et al. (229), who exposed rats that were previously maintained on a cafeteria diet to a palatable food (biscuits and high-fat chow) during test sessions and found that the consumption of the palatable foods decreased over time, whereas rats maintained on standard chow ate more of the palatable foods than the cafeteria-maintained rats. Chronic intake of a HFD has been shown to decrease the incentive reward value of palatable foods, decreasing preference for sweet flavours in mice (230), and is associated with decreased CB1 and D1 receptors in the Acb in rats, leading to a prolonged latency and reduced motivation to consume sweet foods during operant tasks (227).

It was important to establish whether Kernza and whole wheat intake would be consistent over time. The acceptance of the Kernza and whole wheat diets observed in the episodic meals was maintained when the mash diet was offered sub-chronically, under non-deprived conditions. Thus, the consumption of the flour diets in the previous no-choice paradigm was not just from the novelty of the diet.

In addition to novelty, the sensory properties of the food can also impact long term intake. This was seen by Lalanza & Snoeren (118), when rats maintained on a HFHS chow developed hypophagia after several weeks, which was thought to be due to the lacking variety in sensory

properties of the chow. They reversed this effect thorough addition of novel aspects to the HFHS chow, including differing the presentation of the food and providing the rats with a free choice between HFHS chow and standard chow. Naim et al. (231) found that when rats had their diet adulterated by aversive taste stimuli they initially ate 12-16% less than controls which were fed an unadulterated version ad libitum. In the first 8 days the rats showed an inhibition in feeding efficiency, however by day 9, underwent a compensatory process in which their feed efficiency increased above the control group, and by day 15 exhibited the same feed efficiency as the controls. As the intake of whole wheat and Kernza maintained stability when offered sub-chronically, and the motivation to consume the diets was not decreased over time, it demonstrates that the rats found the flour to be palatable and to have no aversive orosensory properties.

The rats in my study demonstrated the same level of acceptance for both Kernza and whole wheat diets for the episodic and sub-chronic intake experiments, whereas in preference testing where the animals were provided with the choice of Kernza and whole wheat diets, there was a clear preference for the whole wheat flour over Kernza. This was evident across all three flour diets (mash, slurry, and pellets). However, whilst there is a clear preference for whole wheat to Kernza reflected in the average total consumption of the diets when provided together, the animals still consumed Kernza in the presence of whole wheat which reflects that while Kernza is less preferred, it is not entirely unpalatable.

Cereal preference in wild brown rats (*Rattus norvegicus*) were studied in pseudo-natural environments by Barnett & Spencer (232), who found that the rats had a strong preference for wholemeal (93% extraction) compared to whole wheat (English, white variety) and wheat germ, and whole wheat was substantially preferred to white flour. It is thought that the white

flour was least preferred due to its inferior nutritional composition. However, even though there was a significant difference between intake of the preferred and non-preferred food, the rats still consumed an appreciable amount of the less preferred food. Rats and mice both preferentially eat the germ of grains, which may be due to a combination of nutrient sensing and textural properties (233). Carlson & Hoelzel (234) found that when rats were given whole corn kernels, they precisely ate the germ, and left behind the starchy part (when sufficient food was available). Some rats still consumed the starchy part, but all rats left behind the yellow part and skin of the corn kernel, which has a harder texture than the germ and starch, however they then ate these after the components were soaked in water. Fromentin et al. (235) offered two novel diets to rats, one of which was devoid of amino acids and the other unmodified, and found that the rats recognised this within a few hours and developed an aversion to the amino acid deficient diet. Interestingly, as rats are capable of sensing toxicants as well as nutrients in food, studies have shown that they show a significant preference for organically fertilised wheat compared to wheat that is inorganically fertilised, indicating that they sense variations in wheat grain quality impacted by the crops health status (236). Thus, the ability to sense the nutritional properties of food plays an important role in what animals choose to consume.

The consistency of the preference for whole wheat over Kernza regardless of the formulation of the diet. This indicates that whole wheat was more palatable to rats than Kernza, no matter how the Kernza flour is presented. However, while the preference was clear, it was not statistically significant in the pellet preference experiment as it was for the mash and slurry. Additionally, the preference difference present between Kernza and whole wheat slurry was less significant than the preference difference found between Kernza and whole wheat mash.

Ultimately, the preference for whole wheat over Kernza decreased slightly with each stage of processing (from raw to cooked, to cooked with lard). This may be due to changes to the flavour of Kernza flour that occur through cooking and gelatinisation. It also makes it reasonable to consider that the bitterness of Kernza may have been slightly masked by fat, and the possibility that the preference for whole wheat may disappear if Kernza was offered in a more complex diet. Rats show a preference for solutions that have a mixed taste, for example polycose and saccharin (non-sweet and sweet taste) over other solutions (237). In addition to the potential of fat to mask any unpleasantness of Kernza's flavour, the nutritional composition of Kernza differs to whole wheat as such that it may have influenced preference. Kernza contains less starch than whole wheat, but is higher in fibre, protein, and fat. The higher bran to endosperm ratio of the Kernza grain also impacts the texture of the flour compared to whole wheat flour.

While starch is tasteless to humans, rats are capable of detecting starch in fluids when in concentrations as little as 0.5%, and preferentially drink fluids containing starch than fluids without starch (238). Like sugar solutions, polysaccharide solutions such as polycose induces overeating in rats, and it is thought that they are attracted to the 'starchy' taste. However, this does not apply to dry powders, likely due to the rapid digestion of carbohydrates in gels and solutions as opposed to when in powered form (239). Ramierz (238) conducted a preference test where they offered raw and cooked corn or potato starch to rats. They found that the rats were not efficient at discriminating between the two and had no preference. However, when Ramirez (240) further investigated preferences between various starches in raw and cooked form, they found that rats had a strong preference for cooked potato and rice starch compared to its raw form but showed little or no preference between cooked and

raw corn or wheat starch. Additionally, when these rats were trained to avoid raw starch, they also avoided cooked starch – until they were offered a choice between both, then they avoided the raw starch only. While it's not possible to know whether there were any differences in taste between the starch of Kernza and whole wheat, cooking the flour may have changed other aspects of the flavour profile, however in these experiments it is more likely that the fat combined with flour when cooked into pellets played more of a role in the lesser preference between the two flours compared to the significant preference when in mash and slurry form. Despite the varying significance of preference for whole wheat across flour diets, it was still preferred, and although the rats find Kernza palatable when offered as a sole diet, it is less palatable than whole wheat. However, it is important to note that palatability is a complex phenomenon and is influenced by many factors, not only the sensory properties of the food and nutritional content, but also the individual preferences of the rats. During this study, some rats did indeed show individual preferences for either whole wheat or Kernza. Further research would be needed to fully understand the differences in palatability between Kernza and other flours.

When Kernza and whole wheat was offered alongside standard chow, which is also grain based, the rats showed a strong and equal preference for the flour diets, consuming only the flour diets and not touching the chow, indicating they find the flour diets to be much more appealing than their standard chow. Rats have been recognised to find the standard laboratory chow diet to be bland, so Rolls et al. (241) attempted to increase the novelty of the usually offered diet, by changing the taste or smell of either water or standard chow and found that this significantly enhanced intake in rats. Rats also show a preference for almost

all foods with added sucrose over foods they find bland or boring (242), so the results found in the standard chow preference test are not surprising.

In my studies, the rats significantly preferred the HFHS chow when offered alongside Kernza or whole wheat, which was to be expected as this chow is highly palatable to rats. Other preference studies where rats were offered the choice between high-fat and high-carbohydrate solutions, they showed a significant preference for the high-fat diet, consuming two thirds more fat than carbohydrates (218). Interestingly, the rats still consumed the Kernza and whole wheat diets in the presence of the HFHS chow, indicating that they found the flour diets somewhat palatable even while in the presence of a highly palatable food. Although the intake of the flour diets was low, they did not ignore it completely, as seen previously in the standard chow preference test. The consumption of the flour diets in the presence of HFHS chow could be due to factors such as nutrient sensing, variety, or the orosensory properties of the diets. Choice is important for persistent hyperphagia, and this effect is greater as the variety of food presented increases (228,241). Additionally, the palatability of a given food decreases during consumption relative to uneaten foods (243). As rats readily consumed Kernza and whole wheat diets when satiated and not only when presented with a choice between the two, it is further evident that they find both flours palatable, due to the consumption of these diets when in the presence of a highly palatable HFHS chow.

Some studies have shown rats to reverse an initial preference for unknown reasons after continued consumption of test-foods, indicating that food preferences are also dependent on the metabolic state of the subject at a given time (213,244) and that there are changing responses to the sensory properties of food once it has been consumed (117,118,201). Rats generally do not consume solutions with high concentrations of sodium chloride (NaCl), but

these solutions are accepted by sodium-deficient rats (245). Mice show a significant preference for fried potatoes (cooked in corn oil or lard) compared to boiled potatoes, however as the absorption of circulating dietary fats takes time, this preference was thought to be due to the orosensory properties of the food (246). Rats choose to consume sugar over wheat, but the preference reverses if they are fed sugar before being tested with wheat. The decrease in sugar intake is thought to be due to the orosensory properties of the diets and sensory-specific satiety, as both foods are similar in their nutritive properties, with both being carbohydrates (247). Rats also overconsume starch-derived polysaccharide solutions, and have shown to consume more of this than of sucrose solutions in short-term choice tests, except when sucrose is in high concentrations (248).

The preference tests in which HFHS chow or standard chow was provided with a flour diet, were conducted with the flour in pellet form. It is possible that the rats enjoyed the texture of the flour pellets compared to that of the HFHS chow, as the flour pellets were crunchy whereas the HFHS chow is smooth and creamy. Rats have an innate tendency to gnaw on objects, though this behaviour is non-specific to hunger. One particular study successfully reinforced this behaviour through hypothalamic stimulation, providing gnawable objects as rewards to operant learning. When stimulation occurred during a consumption of a powdered chow meal, the rats stopped eating and gnawed on a board (249). Obese hyperphagic rats decrease food intake significantly when they are provided their diet in powdered form, however this is not seen in dynamic hyperphagic rats (250).

While palatability derived from the flavour of food is critical for determining food preference (251), as well as the nutritional value of the food and any previous experience with it, preference is also influenced by the positioning of the food in the rats cage (place preference)

(232). To overcome place preference issues in diet preference testing, the positioning of experimental diets are systematically interchanged with each meal, as rats have shown to differ between left and right dominance (244). All the diets offered in these choice experiments were alternated between the left and right sides of the cages in each test to mitigate any place preference that may be present, in addition to the animals being pre-exposed to this situation.

The palatability of Kernza and whole wheat flour was further confirmed in the c-Fos IR experiment. The rats that consumed a meal of Kernza or whole wheat flour had significant activation of the VTA, AcbS, and AcbC, compared to the rats that did not consume a meal, which had low baseline activity in these reward areas. This activity in the reward system is to be expected as Kernza and whole wheat have been established as accepted and palatable diets in the previous experiments and indicates that they find both foods rewarding.

This is in line with the current knowledge of palatable food consumption activating the mesolimbic pathway, particularly the VTA and Acb (106,139,252), and this is evident even with acute exposure to palatable food. This system is activated even in the absence of caloric intake, with rats whom are simply expecting chocolate showing significant increases in c-Fos in the VTA compared to those expecting their standard chow (253). Additionally, sham-drinking of sucrose for 1 hour has been found to significantly elevate c-Fos expression in Acb (254), specifically in the AcbS, but not in the AcbC (255,256) VTA, or LH (255). Two hour exposure to a high-fat meal is enough to increase c-Fos IR in the hypothalamus, including the PVN, DMN and LH, but was activation was most intense in the VTA and Acb (150). Consumption of HFHS chow increases expression of GABAergic neurons in the VTA.

Additionally, when these neurons are photo-stimulated in mice for 12 hours prior to a meal, they drive excess consumption of HFHS chow (257).

However, while an acute meal of both flours provided significant activation in the reward system compared to baseline, there were differences in the activation between brain areas and diets. Rats that consumed the whole wheat had significantly higher activation in the AcbS than those that consumed Kernza. These results also align with the results of the preference studies discussed above, where whole wheat was strongly preferred to Kernza. Both groups that ate the flour meal had a similar level of activation in the VTA to that in the AcbC, whereas the AcbS showed the most activation across both groups.

Different diets have shown to differentially activate the reward system. Dela Cruz et al. (153) examined differences in c-Fos IR in reward regions of the rat brain after 1 hour exposure to novel solutions, including corn oil, fructose, glucose, and saccharin, and these were compared to water consumption in controls. Corn oil was found to significantly increase c-Fos IR in the VTA compared to water intake, whereas corn oil, glucose, and fructose significantly increased activation in the AcbC, though not in the AcbS. Saccharin resulted in the same or less activation than water in these areas. As stated previously, the AcbS is associated with hedonic aspects of learning and reward acquisition, playing a preferential role in reward function due to the dopaminergic projections from the VTA. Park & Carr (162) provided rats with a palatable meal of shortening and sucrose, combined with standard chow, and found this increased c-Fos IR in the VTA and LH. Additionally, when this was consumed in a meal-paired environment, it further increased c-Fos IR in the VTA, as well as an increase in activity in the AcbS.

The effects of drugs of abuse on the reward system has been thoroughly studied, and as mentioned previously, consumption of palatable food similarly activates the same areas of the brain (133). Studies in rats have shown that self-administration of morphine and cocaine significantly increased extracellular dopamine in the AcbS, compared to the AcbC (258) and that nicotine, whether administered systematically or locally within the VTA, increased extracellular dopamine and c-Fos IR in the Acb (259). Consumption of caffeine, which is known for its reinforcing properties within the ML-DA, increases dopamine output by antagonising adenosine receptors in the Acb and has been demonstrated to increase motivation to obtain palatable food (260). Large but not low doses of caffeine increase c-Fos IR in the Acb of rats (261), and Retzbach et al. (260) found that chronic administration of moderate doses of caffeine (5mg/kg) increased operant behaviour in rats to obtain sucrose, and increased c-Fos in the Acb. Colantuoni et al. (262) investigated the role of intermittent food-deprivation in developing a dependency on palatable food; in this case a 10% sucrose solution. They found that injection of Naltrexone after the palatable meal led to withdrawal symptoms as demonstrated by the rats behaviour, which was accompanied by a decrease in extracellular dopamine by 82% of baseline in the Acb, which has also been demonstrated in studies on the effects of drugs of abuse (127).

Peciña & Berridge (263) used c-Fos plumes to map subregions of the AcbS that mediate 'liking' in the rat brain, and through microinjections of morphine found that the hedonic reaction to oral infusions of sucrose could be localised to the medial caudal subregion and adjacent ventromedial structures, which has also been demonstrated with a HFD (264). These subregions of the AcbS project to the VP, which together encode hedonic value of food (254,263) and drive drug seeking behaviour (265,266). The medial subregion of the AcbS was

further investigated by Van Der Plasse et al. (267) using deep brain stimulation in rats to assess the effect of bilateral stimulation on the AcbC, and lateral and medial subregions of the AcbS on food intake and the behavioural response to palatable food. Only stimulation of the medial AcbS significantly increased chow intake, up to 250% of that of baseline. Operant responding for sucrose was not significant for any of the three areas, however it was highest in the lateral AcbS. However, when a progressive ratio scheduling of reinforcement was used, the rats receiving the highest intensity of stimulation to the lateral AcbS showed significantly decreased motivation for sucrose. When sucrose was presented with water, there was no change in preference for sucrose regardless of stimulation intensity or group. These results clearly distinguish the role of the AcbS (particularly the medial subregion) in modulating food intake, and the AcbC in motivation to consume. Thus, the concurrent activation of the AcbC and AcbS in rats who consumed the flour diets (each group consumed 20g each) indicates that the food was rewarding to consume. The activation of the AcbC demonstrates that Kernza and whole wheat possess similar levels of attractiveness and motivational properties, but differ in hedonic response, demonstrated by significantly increased AcbS activation in the whole wheat group.

While these current results answered our questions and provided insight pertaining to Kernza's palatability, it is clearly only the beginning of scientific discovery for this crop. There are many aspects of Kernza that remain unexplored, including any effects it may have on metabolic and digestive outcomes. Due to the uniqueness of Kernza's nutritional composition, such as high fibre, protein, and low gluten, it would be interesting to conduct a long-term study to deduce the impact of Kernza on satiety and body weight. As this study only measured Kernza intake sub-chronically, it would be of benefit to use a chronic feeding

paradigm to study Kernza in the future. Male rats were used in this study due to being the standard model for feeding studies, as well as having increased food intake and lack the hormonal fluctuations of female rats. However, there may be differences in the response of female rats, or other animals models such as mice, to Kernza. Additionally, these studies were conducted on rats 12 months of age, whereas additional studies using juvenile or aged rats, may be helpful to understand how age-related changes impact preference and Kernza intake. These aspects have not been addressed in this current study and warrant further investigation. Therefore, this work is considered to be a preliminary step in exploring the effects of Kernza intake.

Overall, the data obtained in the current set of studies allow me to conclude that Kernza is palatable; it is as acceptable as whole wheat in no-choice meal paradigms, although it is less preferred than whole wheat (but more preferred than standard chow) in food-choice scenarios. Kernza consumption enhances activity of key brain areas that govern palatability-driven feeding; however, this increase is not as pronounced as that of wheat intake.

Chapter 5

Conclusion

- Kernza is accepted in all episodic no-choice paradigms, including by energy-deprived or non-energy deprived rats, and during dark or light hours. This acceptance is equal to whole wheat flour.
- Rats accept Kernza flour when offered as a mash (raw with water), slurry (cooked with water), or pellets (baked with lard).
- Kernza acceptance is maintained when offered sub-chronically for 9 days, and equal to whole wheat flour.
- Kernza is highly preferred to standard chow in choice paradigms, but less preferred to HFHS chow and whole wheat flour.
- A 20g meal of Kernza increases c-Fos IR in reward areas of the brain in rats; the AcbC, AcbS, and VTA. However, this activation is less than that of whole wheat consumption, which was significantly higher in the AcbS.

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