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Food Matrix and Gherlin Hormone

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Abstract

Obesity has been brought up to be a major leading cause of diseases and mortality in developed countries, with diet and exerciserecommendations failing to control rising obesity epidemic levels. Energy homeostasis is a complex multi-centred system composed of a network of neurons, regulated by circulating satiety hormones through binding to special receptors. Ghrelin, the only orexigenic satiety gut hormone secreted primarily from the stomach, has been proven as a regulator of appetite, food intake, and energy homeostasis.

The aim of the present study was to investigate the postprandial effects of the food matrix on circulating levels of the active-form of ghrelin "acylated-ghrelin" in healthy volunteers. Two randomized crossover studies with iso-energetic experimental meals with the only difference being the food matrix were performed. A total of 24 participants were divided into two parts; 16 participants in trial A of the study consumed almond extracts and almond emulsion; comparing two oil bodies where the first covered by a naturally found protein "oleosin" while the other covered by a synthetic protein "casein". In the following pilot trial B, eight participants consumed raw almond and almond oil in iso-energetic meals comparing a naturally intact food matrix versus almond oil that lacks a food matrix. Blood samples were withdrawn at baseline, 1 hour and three hours following the meal consumption for measuring the circulating plasma acylated ghrelin levels as well as blood lipid profile.

There was no significant difference in post-prandial acylated ghrelin levels within both trial A and B, although there was a greater tendency for the intact natural structure of the raw almond seed to lower the acylated ghrelin levels more than that seen in the almond oil meals. A significant difference in the post-prandial triglyceride levels was noticed in both trials following consumption of almond products, but no correlation with the change in the acylated ghrelin level was evident.

In conclusion, our studies suggested that food matrix have no significant impact on postprandial levels of satiety hormone "acylated ghrelin", despite the bioavailability and bio-accessibility of lipids being higher in subjects consuming almond products with no matrix.

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Chapter 1- Introduction

Introduction

Living in a modern environment has boosted the obesity epidemic to adopt an annual increasing trend through the availability of extremely palatable calorie-dense food and the lack of physical activities in our daily routines. According to the World Health Organization 2013, 1.4 billion adults have been categorized as overweight and approximately 500 million as obese (World Health Organization, 2013). Obesity ranks fifth in the global death causing diseases and the consideration of being one of the greatest health challenges of the 21st century.

Through the ages our bodies evolved mechanisms that maintained our body weight mainly in the case of famine, unfortunately not in the case of excessive availability of palatable fatty food. The first laws of thermodynamics suggest that, "Body weight cannot change if, over a specified time, energy intake and energy expenditure are equal" (Hill, Wyatt, & Peters, 2012). Consequently in the case of any irregularity in this balanced energy system such as energy intake exceeding energy expenditure or vice versa. Leading to disorders of feeding behaviour in order to compensate and retain the system to its natural balanced state.

What we are concerned with in this study is the positive imbalanced energy that arises when energy intake exceeds energy expenditures, the excess energy will be stored as fats. This unbalanced energy system has been more common recently due to the high availability of high-fat fast foods in which the new generation of children have been raised on, which can lead to obesity and its related diseases such as cardiovascular disease, type 2 diabetes and metabolic syndrome if not being ignored.

From a different perspective, obesity is considered as a preventable disease that can be reversed by weight loss. Although it might sound easy, but for many people this requires conditions that might be harsh to attain, such as tough physical activities or lowering calorie intake by managing hunger, in other words starving themselves. Therefore more attention needs to be drawn to better understanding the mechanism ofweight regulation, which will assist us in developing alternative therapeutic approaches that are easy, effective and reliable in tackling the growing obesity pandemic and identifying potential targets for treating this pandemic.

Our body is continuously under a frequent state of hunger that is satisfied by occasionally eating, consuming food at that occasion will make us feel satisfied through several hunger suppression mechanisms that our body performs as a result of the presence of nutrients in the gastrointestinal tract. This satisfying effect disappears with time allowing the hunger desire to take over. Two major factors have gained more attention in unravelling appetite-regulating mechanisms that might resolve obesity problems and maintain a uniform body weight: engagement in physical activity and controlling our food intake.

In this study we will focus on food intake regulations by examing the effects of food microstructure on gut satiety hormones, and how this will affect our feeding behaviour and satiety. According to Perry, Zhang, Sun, Saleh, and Wang (2012) the best place to start looking for obesity treatment is in the body's largest endocrine organ, the gastrointestinal tract, due to its important role in regulating energy homeostasis through its secretory gut hormones and their influence on the hypothalamic regulating feeding behaviour system.

1.1 Gut-Brain Alliance

Meal termination due to satisfaction of appetite during the consuming of a meal is called satiation, while the feeling of fullness after consumption of the meal is known as satiety, fading of this satiety with time will lead to the initiation of a new meal (de Graaf, Blom, Smeets, Stafleu, & Hendriks, 2004). Coordinating these two complementary complicated processes is significant in matching energy intake and expenditure, and hence maintaining body homeostasis.

The complex appetite regulatory system exerts its effect through a network of communication that occurs between the gut and the brain. This line of communication conveys the appetite regulatory messages either neuronally via the vagal afferent nerve or humorally as circulating ligands released from the gut, both kind of messages will stimulate specific receptors in the central nervous system particularly in the hypothalamus (Stanley, Wynne, McGowan, & Bloom, 2005). Our body evolved a crosstalk system between the brain and the gastrointestinal tract to ensure our survival, this conversation between the gut and brain, is initiated by the feeling of hunger or the feeling of fullness. Normally when we feel hungry the stomach starts making a few noises, which are actually stomach contractions that occur after a drop in blood glucose level (Boeree, 2003). Then the gut starts sending peripheral messages to the hypothalamus via different appetite regulating hormones to increase our blood glucose levels through consuming food. On the other hand, thefeeling of satisfaction leads to the cessation of eating, which is the result of certain appetite regulating hormones being secreted from the gastrointestinal tract as a response to the food transit in the gut. These secreted satiety hormones will also be implicated in the conversation between the gut and the brainconveying information to the hypothalamus to inhibit feeding leading to meal termination.

1.2 Hypothalamus

Hormones and neurons are the natural way in which our body tells us when to start or stop eating. The initiation of complex appetite regulating process take place within and by the gut after or before the consuming of food, by launching a cascade of hormonal and neuronal signals that circulate the body until reaching the CNS where they influence the hypothalamus toregulate our appetite and maintain body energy homeostasis.

Located below the thalamus and above the brain stem, is the hypothalamus that is subdivided into discrete nuclei located in various hypothalamic areas performing a range of activities including appetite regulation, food intake, hunger, and thirst (Elmquist, Elias, & Saper, 1999). These hypothalamus interrelating

nuclei includes (Figure 1):

Arcuate nucleus (ARC)

Para-ventricular nucleus (PVN)

Ventromedial nucleus (VMN)

Dorso-medial nucleus (DMN)

Lateral hypothalamic area (LHA).

Food intake and energy expenditure are performed through a network of organized neuronal pathways between these hypothalamus nuclei (Elmquist, Maratos-Flier, Saper, & Flier, 1998) via specific neurotransmitters identified as "orexigenic and anorexigenic", where the first exerts an increase in appetite while the later one decrease it. Implying the critical role that the hypothalamus plays in relying afferent peripheral signals from the gut and processing efferent signals to maintain energy haemostasis.

The appetite gate-keeper "hypothalamus" receives signals from various parts of the body including the periphery, the brain stem and the cortex in order to maintain our body haemostasis. Two areas of the brain, the ARC and the postrema of the brain stem are not completely isolated by the blood brain barrier (Simpson, Martin, & Bloom, 2009). These exposed area provides an alternative pathway for the peripheral satiety signals to communicate with the extensive shared neuronal pathways between the hypothalamus and other parts of the brain.

Moreover, a major line of communication exists between the gastrointestinal tract and the brain that are governed by the vagus nerve. Located in the nodose ganglia and projecting onto the brainstem nucleus tractus solitarii (NTS) are the cell bodies ofafferent fibers of the abdominal vagus nerve (Ter Horst, 1989).

This means that the satiety gut hormones can activate their neuropeptides in the hypothalamus either directly via circulating in the blood stream and crossing the exposed areas of the blood brain barrier or indirectly via activating the afferent vagal nerve which will stimulate a chain of signals starting in the brainstem NTS and projecting to other parts of the hypothalamus.

1.2.1 Arcuate nucleus (ARC)

The arcuate nucleus in the hypothalamus is the main channelof feeding-related signals that contains two unique subpopulation of neurons, functioning antagonistically to maintain homeostasis through regulating feeding behaviour, metabolism and energy expenditure (Chaudhri, Field, & Bloom, 2008). According to Olney (1969) abrasion of the mice arcuate nucleus resulted in obesity and hyperphagia. This study implied the importance of the ARC in regulating appetite as well as being a key hypothalamic nucleus in maintaining the body energy homeostasis.

The hypothalamic arcuate nucleus contains a unique subpopulation of neurons including the anorexigenic population that co-express pro-opiomelanocortin(POMC),cocaine-andamphetamineregulated transcript, and the orexigenic population co-expressing the neurotransmitters neuropeptide Y and agouti-related peptide (Chaudhri, Field, et al., 2008). The ARC unique subpopulation of neurons interconnects with other hypothalamic areas such as the PVN, DMN and LHA through neuronal projections in order to regulate appetite (Bouret, Draper, & Simerly, 2004). Stimulating ARC neurons via peripheral satiety signals will activate asignalling cascade in the hypothalamus nuclei to maintain the body energy homeostasis.

An interesting fact about ARC is that it is not completely isolated from the blood circulation by the blood brain barrier (Simpson et al., 2009).This strategic position allows the peripheral satiety signals to act directly on the ARC by crossing the blood brain barrier and exerting their satiety roles.

1.2.2 Para-ventricular nucleus (PVN)

In a study done on mice, microinjection of the para-ventricular nucleus of the hypothalamus with the orexigenic neuropeptides (NPY & AgRP) stimulated feeding (Legradi & Lechan, 1999). This shows the involvement of PVN in the regulation of food intake and energy expenditure.

Orexigenic neurons from ARC communicate with other secondary neurons through axons projecting from the arcuate nucleus NPY/AGRP neurons to the PVN (Legradi & Lechan, 1999). This permits the PVN to participate in the appetite regulating signal cascade by receiving neuropeptide signals from other parts of the hypothalamus such as the ARC.

1.2.3 Ventromedial nucleus (VMN)

According to Stellar (1954) study that included lesioning of the rats VMN resulted in hyperphagia and a rapid increase in weight to approximately 2 to 3 times normal weight, while electrical stimulation of the VMN resulted in food intake suppression. This suggested that VMN may act as a satiety centre, that when activated it will stimulate the feeling of fullness and the absence of it will induce hunger feeling.

A study in humans by Matsuda et al. (1999) showed VMN neuro-images with an increase signal in that area following an oral glucose load. Analogous to the PVN, a large population ofthe orexigenic neurons (NPY, AgRP) is projected from the ARC. Allowing the VMN to contribute in regulating the body energy homeostasis via controlling the body feeding behaviour.

1.2.4 Dorso-medial nucleus (DMN)

Resembling the PVN/VMN disruption in rats, DMN lesioning cause over-feeding and eventually obesity (Bernardis & Bellinger, 1987). DMN contains a high concentration of the orexigenic neuron NPY originating from the arcuate nucleus (Jacobowitz & O'Donohue, 1978). Therefore, DMN also contributes in regulating the body feeding behaviour and satiety.

1.2.5 Lateral hypothalamic area (LHA)

Opposite to the PVN, LHA lesioning in rats lead to the lack of eating and eventually starvation, while electrical stimulation of the LHA stimulated feeding behaviours (Stellar, 1954). This demonstrated the role of the LHA in transmitting orexigenic signals and the involvement in food seeking behaviours. Arcuate neuronal projections are also located in the LHA, these includes the orexigenic NPY, AgRP and the orexigenic neuropeptides melanin-concentrating hormone (MCH) (Broberger, De Lecea, Sutcliffe, & Hokfelt, 1998). Neurons located in the LHA respond to the signals stimulated in ARC and also send signals to different regions of the brain, participating in the complex network of neuron signals that are responsible in appetite regulation.

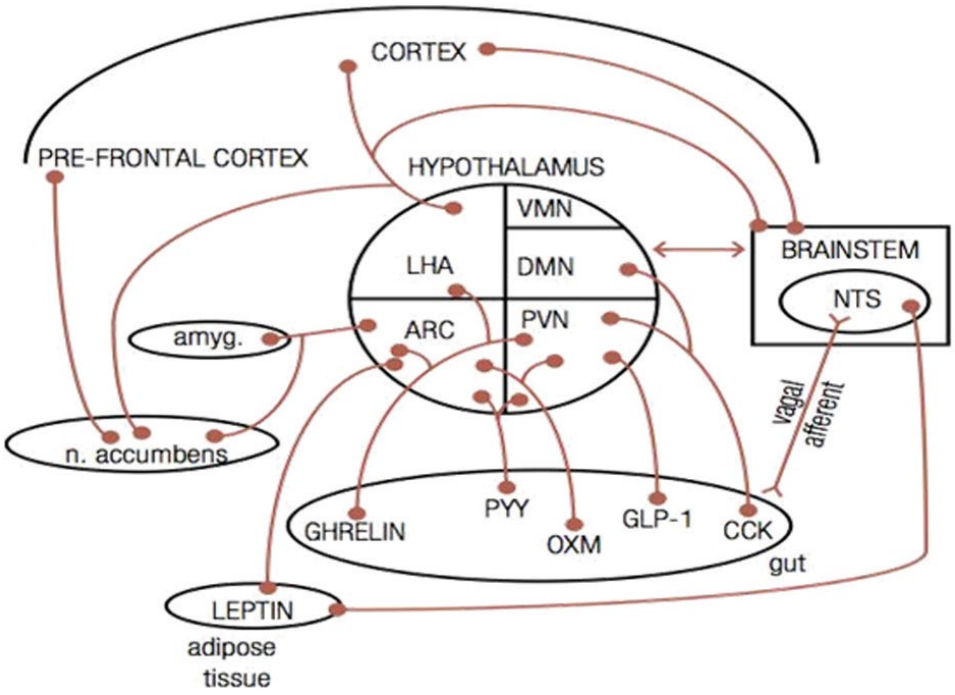


Figure 1 (Broberger et al., 1998; HYPERLINK \l "page79" Simpson et al., 2009).

Summarizes the different pathways the peripheral satiety signals take in order to stimulate different hypothalamus nuclei and regulate homeostasis. It also shows the projection from the different hypothalamic nuclei within each other and across different parts of the brain. Moreover, peripheral satiety hormones can either act directly via the blood circulation crossing the incomplete blood brain barrier or indirectly through the vagal afferent nerve by stimulating the brainstem nuclei (NTS) that will activate a cascade of signals targeting the hypothalamus

1.3 Hypothalamic Neuropeptides

For an effective appetite regulation process, two major players should be considered, hypothalamic neuropeptides and peripheral signals. The hypothalamus contains different neuronal centers including the LHA known as the "hunger" center, VMN known as the "satiety" center, ARC serving as the key hypothalamic nucleus in food intake regulation, NTS serving as the

"gateway" for peripheral neuronal signals from the gut to the hypothalamic feeding centres and finally the PVN.

As mentioned before, the ARC contains two unique subpopulation of neurons that exert opposing actions on the feeding process, these includes:

(a)The hunger center neurons including neuropeptide Y (NPY) and Agouti-Related Peptide (AgRP) that are stimulated via the orexigenic peptides "ghrelin"

(b) The satiety center neurons including the polypeptide proopiomelanocortin (POMC) or the cocaine and amphetamine regulated transcript (CART) that are stimulated via anorexigenic hormones such as leptin.

1.3.1 Neuropeptide Y, NPY

Belonging to a structurally related family known as pancreatic polypeptide (PP), neuropeptide-Y (NPY) is regarded as a hypothalamic neuroendocrine potent orexigenic protein (Neary et al., 2004; Palkovits, 2003). In addition to NPY, the PP family members include pancreatic polypeptide (PP) and peptide tyrosinetyrosine (PYY) gut hormones, a mutual characteristic between the family members is the fact that they are made of 36 amino acids that contain a high amount of tyrosine and α-amidation (Berglund MM, Hipskind PA, & Gehlert DR, 2003) .

In order for the PP family members to exert their physiological actions they are required to bind to specific receptors. A family of G- protein-coupled receptors known as the "NPY receptors" labelled as Y1, Y2, Y4, and Y5 are decisive for the

PP family members roles (Turtzo & Lane, 2006). Activation of Y1 and Y5 receptors by their endogenous ligands will induce 24 Abdallah Mohammed Ayoub, IJPSR 2016, 1:1 International Journal of Pharma Sciences and Scientific Research Volume 1 Issue 1, November 2015 6 an orexigenic response while activation of Y2 will induce an anorexigenic opposite response (Stanley et al., 2005) . Therefore with respect to NPY activity, Y2 receptors are thought to be inhibitory receptors of NPY that are largely expressed in the ARC, and activating them will induce anorexigenic effect. Moreover, NPY binding to the Y1 receptors located in the VMN

will lead to an inhibitory effect of the neuronal function interfering with the VMN satiety role.

According to Seoane (2003) a higher expression of NPY/AgRP mRNA in the ARC was reported after 72 hours of food fasting. Taking into consideration the increase in plasma ghrelin levels in the fasting state, the increase in the NPY/AgRP mRNA expression might be regulated by ghrelin suggesting that these hypothalamic peptides might be the main target for the appetite stimulator hormone "ghrelin". Additional evidence reported by Kohno et al. (2003) showed an increase in calcium ion concentration in the ARC/NPY

containing neurons after ghrelin administration. The elevation in the calcium ion concentration indicated a positive neuronal activity due to the depolarization of the plasma membrane suggesting a direct activation of the ARC NPY neurons via the orexigenic ghrelin hormone, implying the important role neuropeptide Y plays in food intake stimulation and overall energy homeostasis.

1.3.2 Agouti-related peptide, AgRP

Agouti-related peptide (AgRP) is an orexigenic peptides composed of 132 amino acids with cell bodies expressing predominantly in the arcuate nucleus with its fibers projecting to different hypothalamic areas including PVN, DMN and the LHA (Shutter et al., 1997). AgRP belongs to the melanocortin family that in addition to AgRP stimulates α-melanocyte hormone "α-MSH" and 2 melanocortin receptors known as melanocortin receptor-3 (MC3R) and melanocortin receptor-4 (MC4R) (Morton & Schwartz, 2001). α-MSH is an agonist endogenous ligands of both MC3R/ MC4R receptors, while AgRP acts as an antagonist to the α-MSH activity at these same receptors (Ollmann et al., 1997). Therefore AgRP orexigenic function is due to blocking and inhibiting of the anorexigenic agonist α-MSH.

AgRP expressions are found in the same area that produces NPY; hence the sharing of similar physiological roles. Backed up with

reported evidences, in case of negative energy balance such as the one seen in food deprivation, both AgRP and NPY expression levels increases, this elevation in their mRNA expressions are due to adecrease in leptin/insulin levels and an increase in ghrelin levels (Ahima & Hileman, 2000; Jequier, 2002). This parallel pattern between NPY/AgRP and ghrelin indicates the roles NPY/AgRP shares in regulating feeding behaviors particularly in stimulating food-intake.

Leptin and ghrelin are the two peripheral hormones that act on the ARC and regulate energy homeostasis through their influence on the action of AgRP and NPY (Kalra et al., 1999; Nakazato et al., 2001). On one hand, leptin binding to its receptor located on the NPY/AgRP hypothalamic neurons, reducing the expression of these neurons is one of the mechanisms by which leptin stimulates satiety feelings. While on the other hand, ghrelin binding to its NPY/AgRP hypothalamic neurons increases the expression of these neurons resulting in feeding behavior stimulation which can be considered as the mechanism behind ghrelin's orexigenic role.

1.3.3 Melanocortin system

The melanocortin system is a peptide family that is composed of POMC-derived melanocortin peptides. Post- translational cleavage of the precursor protein "POMC" will produces biologically active peptides, includeing melanocyte-stimulating hormones (α-MSH, β-MSH, γ-MSH), corticotrophin (ACTH1-24, ACTH1-13–NH2) and β-endorphin (Millington, 2007) .This melanocortin system has a critical role in regulating our food intake and energy homeostasis through binding to an extracellular G-protein coupled melanocortin receptor (MCRs) family labeled MC1R through MC5R (Kask, Rago, Wikberg, & Schioth, 2000).

In the CNS, POMC neurons cell bodies are primarily located in the hypothalamus arcuate nucleus and the brainstem nucleus tractus solitaries, and projecting from these POMC neurons to different areas of the hypothalamus (PVN/DMN) are the melanocortin receptors (MCRs) (Appleyard et al., 2005; Bouret et al., 2004). The ARC and NTS are areas known to play a critical role in regulating our appetite and food intake, and the presence of POMC neurons and their receptors in these hypothalamic areas suggests a major role for the melanocortin system in such a mechanism.

Through all the melanocortin system members, alpha-MSH and AgRP and their agonist and antagonist actions on the CNS widespread expression of the MC3/MC4 receptors indicates a fundamental role for these two neurons in mediating the energy balance (Tucci, Kobelis, & Kirkham., 2009). Alpha-MSH and AgRP acting as a non-selective endogenous agonist (inhibiting food intake) and antagonist (stimulating food intake) for MC3/ MC4 receptors respectively, gives the melanocortin family a unique characteristic in having both endogenous agonist/antagonist ligands for the target receptors acting as a dynamic system to reduce food intake and regulate our body weight.

1.3.4 Cocaine-and Amphetamine-Regulated Transcript, CART

Douglass et al. (1995) reported that an acute administration of cocaine and amphetamine increased the expression of specific mRNA in the brain, hence they names this transcript 'cocaine- and amphetamine-regulated transcript'

(CART). These neuro-endocrine peptides play a physiological role in appetite regulation, cardiovascular functions, drug reward system and bone remodelling (Hunter & Kuhar, 2003). Satiety peripheral hormones such as leptin, cholecystokinin and ghrelin may either increase or decrease the expression of this neuropeptide in order to control our appetite and regulate the energy homeostasis.

According to Couceyro et al. (1998), CART gene expressions were found in several hypothalamic regions including the arcuate nucleus, lateral hypothalamus (LH), PVN, VMN and the nucleus of the solitary tract (NTS). This finding supported the CART gene expressions role in regulating feeding behaviour and in our reward system because most of these hypothalamic

regions are involved in regulating feeding behaviour and in the limbic system circuits that are associated with the body reward system. In addition to its distribution in such areas it was observed by Vrang et al. (1999) that CART peptides are co-expressed with 25 Abdallah Mohammed Ayoub, IJPSR 2016, 1:1 International Journal of Pharma Sciences and Scientific Research Volume 1

Issue 1, November 2015 7 melanin-concentrating hormone that also act as neuropeptides associated with appetite regulation within the DMN, LH and ARC.

An interesting fact observed by Broberger et al. (1999) is the considerable concentration of the cholecystokinin A (CCK-A) receptor mRNA that are expressed outside the hypothalamus by the NTS CART neurons. The Link between CCK-A and satiety may suggest one of the ways by which CART's peptide mediates appetite, an increase in postprandial CCK-A binds with the overexpressed CCK-A receptors in NTS that are generated through CART neurons and thus activating a cascade of neuro-signals in different regions in the hypothalamus.

1.4 Gastrointestinal Tract/Gut Hormones

Our brain have approximately 100 billions neurons transmitting information throughout our body, the gastrointestinal tract on the other hand has nearly 500 million nerve cells and approximately 100 million neurons with a relatively smaller size than our brain (Mastone, 2011; Rajvanshi, 2010). Thus, the gut can be considered as an organ that has its own mind with an independent autonomous nervous system that allows it to digest food and absorb nutrients without the help of the brain.

The gastrointestinal tract has developed a surveillance system that controls our feeding behavior; this system employs unique entero-endocrine cells that will secrete hormonal products in response to the presence or the absence of food in the gut lumen (Dockray, 2009). Once these gut peptides are released they either circulate in the blood until they reach the hypothalamus or activate the major pathway in the brain- gut axis, the vagal nerve. This way of communication between the complex gut machine and the brain can either use chemicals or electrical signals to relay their messages to the hypothalamus.

The vagal nerve acts like a cable that receives information from the gut-hormones as electrical impulses and conducts them to the hypothalamus for further interpretations (Sobocki, Krolczyk, Herman, Matyja, & Thor, 2005). A tremendous amount of information flows from the gut to the brain in almost a one-sided pattern through one of the longest nerves within the body "the vagus nerve", from the gut to the brainstem to the hypothalamus and the rest of the brain regions involved in regulating our appetite. Gut hormones are released from special enteroendocrine cells lining the gastric and the intestinal lumen containing chemoreceptors on their microvilli which release peptides in the response to different ingested nutrients and chyme constituents (Strader & Woods, 2005). The role of gut hormones in regulating appetite is to initiate or terminate a meal, either by enhancing the digestion process or by boosting nutrient adsorption through controlling nutrient transit time in the various gastrointestinal tract compartments (Chaudhri, Wynne, & Bloom, 2008). As summarized in figure 2, the body secretes peripheral signals composed of a variety of gut hormones or adipokines in response to our feeding behavior; these hormones can be categorized into two subgroups, the orexigenic hormones released in response to food ingestion including leptin, Peptide-YY (PYY), Cholecystokinin (CCK), amylin, glucagon-like peptide-1 (GLP-1) and oxyntomodulin and the orexigenic hormone "ghrelin"

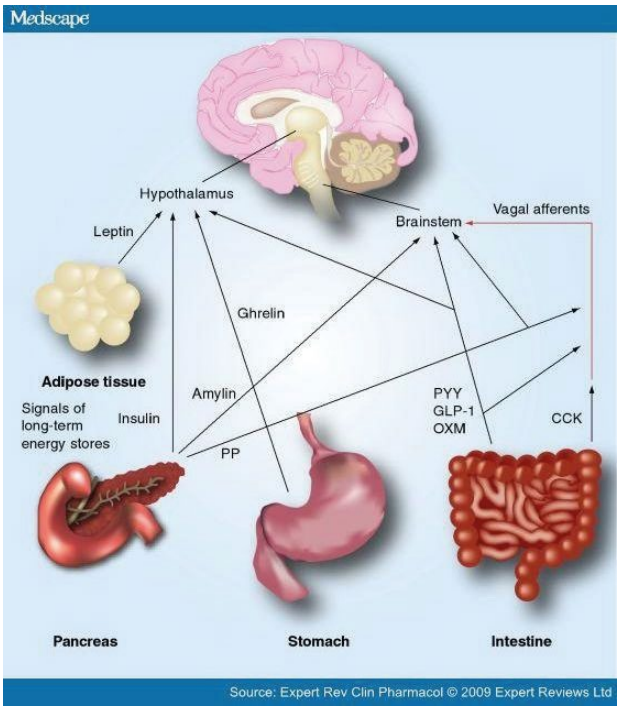


Figure 2 Different gut hormones are secreted via different enteroendocrine organs including adipose tissue that mainly secretes leptin, stomach mainly secreting ghrelin and the intestine releasing CCK, PYY, GLP-1 and OXM hormones.

1.4.1 Ghrelin

1.4.1.1 Tissue Distribution

The only known appetite-stimulating hormone ghrelin was first discovered as an endogenous ligand for the growth hormone secretagouge receptor (GHS-R) (Kojima et al., 1999) but succeeding reports shifted the focus of this peptide to its role in energy homeostasis. Alongside the potent neuropeptide Y, ghrelin is considered to be the second most potent stimulator of short-term food intake that can exert its effect whether injected peripherally or centrally by initiating our feeding behavior.

Stomach, small intestine, placenta, pituitary, kidney, pancreas and the hypothalamus all express ghrelin-mRNA in different concentrations (Mori et al., 2000); (Date et al., 2000). The widespread expression of ghrelin in a variety of tissues suggests its multifunctional nature. Beside its role in energy homeostasis, ghrelin exert diverse biological action, including effects on hormone secretion, glucose homeostasis, pancreatic function, gastrointestinal motility, cardiovascular function, immunity, inflammation, cell proliferation and survival, bone metabolism, reproduction, memory and our sleeping pattern (van der Lely, Tschop, Heiman, & Ghigo, 2004).

Gastric fundus is the principal site of ghrelin synthesize, producing approximately 10 times more ghrelin than the duodenum, which is considered to be the second richest ghrelin site (Gnanapavan et al., 2002),(Tanaka-Shintani & Watanabe, 2005) with a pattern of expression decreasing as the distance increases from the pylorus (Sakata et al., 2002). The effect of gastrostomy on plasma ghrelin levels indicates that 3/4 of circulating peptides are produced from the stomach, leaving the remaining 1/4 to the small intestine (Ariyasu, Takaya K, & Tagami T, 2001),(Jeon et al., 2004). Inside the stomach, enteroendocrine cells in the oxyntic mucosa called "X/A-like cells" are responsible for ghrelin secretion. These cells are considered to be closed cells, opposing the basolateral membrane adjacent to the bloodstream and not in direct contact with gastric lumen (Date et al., 2000),(Sakata et al., 2002). However as we move more distally toward the intestine, "X/A-like cells" become more open and come in contact with the blood vessels and the intestinal lumen (Sakata et al., 2002). This may indicate that food interaction in the stomach could have no effect on ghrelin suppression due to its closed cells nature.

1.4.1.2 Chemistry

Cleaved from a larger precursor pre-proghrelin, the 28 amino acid ghrelin, undergoes further modification to exert its role in energy balance in which the third amino acid (serine) covalently binds to a medium chain fatty acid through an ester bond (Kojima et al., 1999). Our stomach can only acylate ghrelin with a medium chain fatty acid, usually octanoic acid, and not with long-or short-chain fatty acids (Nishi et al., 2005). And since our body is not capable of synthesizing medium chain fatty acids, the octanoic acid is obtained through dietary sources (Nishi et al., 2005).

The further posttranslational process of the mature 28 amino acid ghrelin is required to form the biologically active ghrelin form, in which the 3rd-serine residue of the 28-amino acid is acylated with n-octanoic acid or n-decanoic acid (Figure 3). This process is done via a member of the Membrane-Bound O-Acyltransferases (MBOAT) family that is responsible for forming the active acylated ghrelin form via n-octanoylation of the serine 3 hydroxyl group; this enzyme is called Ghrelin O-Acyltransferase (GOAT) (Yang, 2008.). This enzyme gene expression is largely found in the stomach and the intestine, which are the predominant sources of the peptide ghrelin.

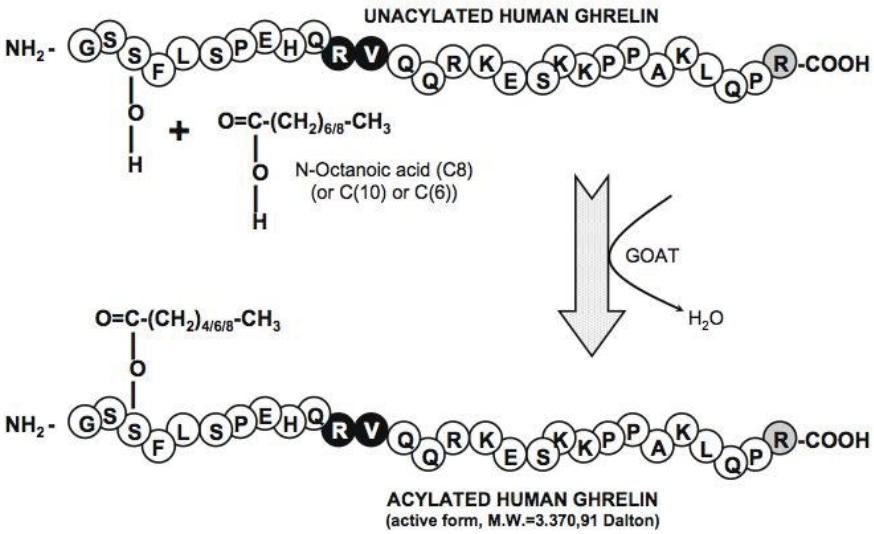


Figure 3 (Castaneda, Tong, Datta, Culler, & Tschop, 2010)

The two forms of ghrelin found in the body, the un-acylated and the acylated ghrelin molecules. The acylation of the mature ghrelin molecule on the 3rd serine position by the action of the enzyme ghrelin O-Acyltransferase (GOAT) forms the active acylated ghrelin.

This acylated ghrelin post-translational modification is essential for the hormone to bind and activate ghrelin receptor "GHS-R1a", consequently exerting its endocrine and anabolic effects (van der Lely et al., 2004),(Kojima et al., 1999). The octanoylation of Ser3 is an important step for the binding of ghrelin to its receptors (GHSR-1a) and triggering both GH secretion and food intake. Binding and activation of the receptors are achieved by the first seven amino acids at the N- terminus with the octanoic fatty acid moiety (Bednarek et al., 2000),(Matsumoto et al., 2001) forming the receptor-binding pharmacophore of ghrelin.

It has been estimated that 20% of the circulating ghrelin are acylated, with the n-octanoyl group being the critical part for both receptor binding and activation Espelund et al. (2003). But when compared to the acylated form of ghrelin, the non-acylated form circulates our systems in higher concentrations than that of the acylated form, suggesting a biologically active role for this form of ghrelin to.

The problem with the acylated form of ghrelin is that it's highly unstable, due to the ester bond that connects the medium chain fatty acid to the 3rd-serine. This ester bond can be degraded by either enzymatic cleavage or spontaneous hydrolysis, transforming "active" ghrelin into "non-active" des-acyl ghrelin (Kanamoto et al., 2001). This degradation of the ester bond of the acylated ghrelin can happen during storage, handling, and/or dissolution in the culture medium. Furthermore, the quantity of the circulating active acylated ghrelin form is 20% of the total ghrelin form in the human plasma (Espelund et al., 2003) implying that circulating des-acyl ghrelin inactive form is proportionally higher than the active acylated form, making the later harder to detect.

Therefore certain measures should be taken when dealing with ghrelin blood samples in order to obtain maximum effective reliable acylated ghrelin concentrations. According to (Hosoda & Kangawa, 2012) to protect the active acylated form of ghrelin, blood samples should be collected in ethylenediaminetetraacetic acid (EDTA) tubes under chilled conditions followed by immediate centrifugation within 30 minutes after collection, and for maximum affectivity acidify the plasma with 1 mol/l HCl (10% of volume).

1.4.1.3 Mechanism of Action

Energy homeostasis, is a complex multi- centered system composed of a network of neurons, regulated by neuropeptides through binding to special receptors (Kalra et al., 1999). Recent studies in rodents reported that the hypothalamus is the crucial intermediate between peripheral afferent signals, CNS wiring and efferent neuroendocrine (Spiegelman & Flier, 2001). A classic G-protein-coupled receptor known as GHSR-1a is considered to be the major receptor which ghrelin peptide acts on (Howard, Feighner, Cully, Arena, & P.A. Liberator, 1996). GHSR-1a is present in bulk concentrations in the hypothalamus and the pituitary gland (Harrold, Dovey, Cai, Halford, & Pinkney, 2008), where they are expressed mainly by neurons co- expressing NPY and AGRP (Hahn, Breininger, Baskin, & Schwartz, 1998). Stimulation of these receptors will activate a cascade of signals particularly in the hypothalamus regulating our appetite. Based on previous studies, there aretwo main hypothalamic pathways of ghrelin's impact on energy homeostasis, either by stimulating the orexigenic neuropeptideY neurons and their co-expresses neuropeptides AGRP or by inhibiting the anorexigenic melanocortin receptors and their coexpressed pro-opiomelanocortin-derived melanocyte-stimulating hormone (a-MSH) (Gehlert, 1999).

Consistent with almost all studies, ghrelin central targets are neurons located in the hypothalamic arcuate nucleus (ARC). The ARC co-express NPY/AgRP anabolic neuropeptide that stimulate positive energy balance (Schwartz, Woods, Porte, Seeley, & Baskin, 2000), and express ghrelin receptor, and the activation of ghrelin to these neurons can be confirmed by an increase in their firing rate and intracellular Ca2+ concentration.(Nakazato et al., 2001),(Kohno et al., 2003).

Beside the hypothalamic NPY/AgRP neurons as a clear target for the hormone ghrelin, it might also exerts its action indirectly via the vagal nerve and signaling the hindbrain, as well as acting in the mesolimbic dopaminergic reward system (Faulconbridge, Cummings, Kaplan, & Grill, 2003). Ghrelin is either released into the bloodstream where it crosses the blood brain barrier and binds to its receptors located in the hypothalamus or the brainstem, or may reach the brain through the vagal nerve signals activation, or by locally production in the hypothalamus in order to regulate the complex body energy hemostasis.

Ghrelin as a meal initiator characteristic should be reflected by a rise in its circulating plasma levels before a meal, and fall after a meal, with a peak concentration that is enough to stimulate appetite and initiate our feeding behavior. This idea that ghrelin hormone acts as a hunger signal that plays a major role in our feeding process can be backed up with the pre-meal rise of circulating ghrelin levels (Cummings et al., 2001). Plasma ghrelin levels increased nearly twofold immediately before each meal and fell to trough levels within 1 h after eating, a pattern that is compatible with the hypothesis that ghrelin is a major player in human's meal initiation process (Figure 4).

Coherent with this, previous studies reported a rapid suppression of ghrelin levels after nutrient ingestion, with a 24-h plasma profile showing marked pre-prandial increases and postprandial decreases associated with every meal (Cummings et al., 2001). This suppression might be due to many factors that are not yet clear. Studies have reported that prandial suppression of ghrelin is does-dependent to the number of ingested calories (Callaha et al., 2004), were lipids being the least potent suppressor of ghrelin compared to carbohydrates or proteins (Overduin, Frayo, Grill, Kaplan, & Cummings, 2005), which might

reflect the mechanism behind a high fat diet stimulating weight gain. Some studies suggests that ghrelin prandial suppression is

not due to nutrient exposure in the stomach or duodenum, but might be due to signals originating downstream in the intestine and from post-absorptive events (Williams, Cummings, Grill, & Kaplan, 2003).

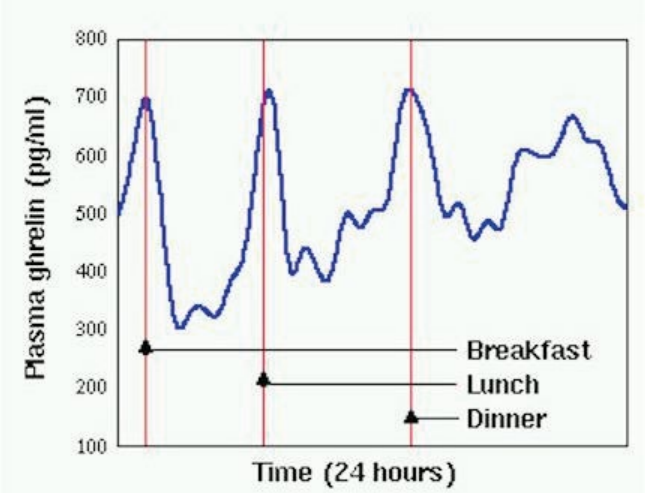


Figure 4 (Cummings et al., 2001)

shows the effect of feeding on the levels of serum ghrelin, and how food suppress the level of ghrelin within one hour of feeding reflecting the role of ghrelin in initiating our feeding behavior.

1.4.2 Leptin

Three theories for the regulation of body weight have been put forward by Pospiech (2010) "a thermoregulation theory, where maintenance of a basal body temperature through energy expenditure influences weight; a glucostatic theory, where plasma glucose regulates all energy stores; and a lipostatic theory, where a product of fat metabolism circulates in the blood and interacts with certain receptors to either burn or maintain fat stores". Leptin can be categorized in the third theory, as the mechanism of its action is similar to this theory.

From the eleven genes that are responsible for weight gain, the obesity gene (Ob) that is located on chromosome 7, codes for the satiety hormone leptin (Friedman & Halaas, 1998). This protein hormone is secreted by different tissues but mostly by whiteadipose tissue and to a lesser extent by the gastric epithelium and the placenta (Pospiech, 2010). Composed of 167 amino acids, circulating levels of leptin are directly proportional to the amount of body fat (Kelesidis, Kelesidis, Chou, & Mantzoros, 2010). Leptin acts as a feedback mechanism that inhibit food intake and regulates energy homeostasis by activation of arcuate POMC/CART neurons in the hypothalamus, where it gives information concerning the status of the bodies energy stores (Kelesidis et al., 2010) & (Klok, Jakobsdottir, & Drent, 2007). Leptin is a protein released by fat cells that reports to our brain regarding our body energy thermostat, if we have enough energy stored in our fat cells, leptin will tell the brain to engage the body in a normal metabolic process and burn energy at a normal pace. But in the case of high energies stored as fats, leptin levels will increase to report to the brain and advise the brain to burn more energy and stop energy consumption.

After being produced inside an adipose cell, activated leptin is secreted into the plasma while the remainder is stored in the endoplasmic reticulum of these cells (Pospiech, 2010). In order for leptin to reach the hypothalamus it must first cross the blood brain barrier (BBB) via Ob-Ra, once leptin has crossed the BBB it binds to Ob-Rb (Pospiech, 2010). After binding to its receptor in the hypothalamus, phosphorylation of the Ob-Rb activates a cascade of signal transduction pathways, such as activation the hypothalamic anorexigenic neuropeptides POMC/CART that mediates energy homeostasis, and regulating food intake and glucose homeostasis (Figure 5).

The action of leptin energy homeostasis can be divided into an immediate and long-term balance between food intake and energy expenditure. The instantaneous response of leptin occurs by the activation of a complex neural action circuit that involves a synergistic interaction with CCK peptide on the vagal afferent pathway (Kelesidis et al., 2010). Moreover, outside the hypothalamus the satiety effect of leptin might also arise by interacting with the mesolimbic dopamine reward system resulting in a pleasant consequences after consuming a palatable food, which will introduce a learning process connecting the palatablefood experience with the pleasant rewarding feedback (Scott A. Robertson, Gina M. Leinninger, & Martin G. Myers Jr, 2008) & (Arias-Carrion, Stamelou, Murillo-Rodriguez, Menendez-Gonzalez, & Poppel, 2010). This suggests another pathway in which leptin induces the feeling of fullness, "satiety", through making us enjoy eating via stimulating the brain's reward circuitry.

In the case of obese patients, it has been reported that circulating leptin levels increase while circulating ghrelin levels decrease (Klok et al., 2007). This might be due to the saturable transport

system of leptin to the brain, lowering the capacity of its transport to the hypothalamus causing leptinresistance condition (Yang, Brown, Liang, Grishin, & Goldstein, 2008)& (Klok et al., 2007). These elevated circulating levels of leptin over-stimulate the transportation receptor system leading to the satiety cells insensitivity to leptin triggering a leptin resistance condition. From the name, leptin resistance can be of a similar characteristic to insulin resistance conditions where the pancreas secretes high levels of insulin but the body doesn't respond to it. Here the body secretes high level of leptin reflecting that the person is fat, but the problem is the brain does not capture these reports, so the brain is still in a starving status while the body is obese. On the other hand, low levels of ghrelin result in making obese patients hypersensitive to ghrelin (Klok et al., 2007), and hence increase their feeding habits.

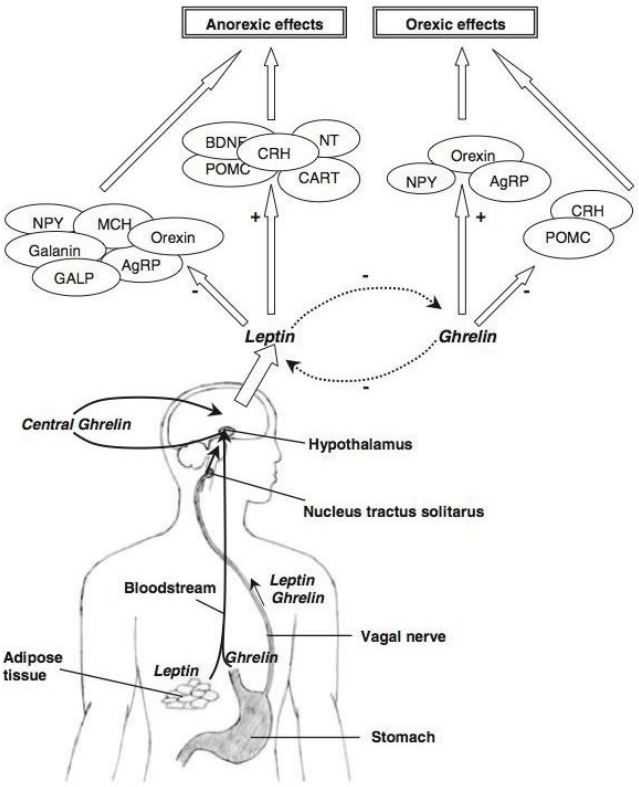


Figure 5 Klok et al .2006

this diagram summarize both pathways by which the anorexigenic peptide leptin induces satiety and the orexigenic peptide ghrelin in meal initiation. Leptin being secreted by adipose tissue travels through the bloodstream and crosses the BBB to reach the ARC where it stimulates the anorexigenic neuropeptides. On the other hand, stomach released ghrelin might activate the vagal nerve and stimulate NTS to induce a cascade of signals projecting to the hypothalamus where it induces the orexigenic effect via activating the orexigenic neuropeptide

1.4.3 Cholecystokinin

Cholecystokinin (CCK) the first gut-hormone discovered to affect appetite that has dual signaling effects of working as a central neurotransmitter in the brain and as a peripheral signal that activates the vagal nerve after being released from the endocrine I cells of the duodenum and the jejunum (Moran, 2000). Under physiological conditions, CCK performs a sequence of functions that combine together to reduce food intake in humans. These functions includes: stimulation of pancreatic enzyme secretions, contraction of the gall bladder, stimulation of neural activity in the gastric vagal afferents, pylorus constriction and inhibition of gastric emptying when food is in the stomach thus increasing gastric distention (Kissileff, Carretta, Geliebter, & Pi-Sunyer, 2003). In humans, CCK is known to suppress appetite through a series of actions. When food leaves the stomach and enters the duodenum, the duodenal mucosa start responding to the digested food by releasing CCK. CCK binds to its receptor and slows down gastric emptying as well as stimulating the release of bile salt and pancreatic enzymes promoting a feeling of fullness. Cholecystokinin's role as a gatekeeper for the GIT food surveillance system is mainly due to its peripherally mediating effect that generates satiety signals in the vagal afferent pathway. CCK signaling action is controlled by two different G protein-coupled receptors, CCK1 and CCK2 receptors that have different binding

affinities to CCK (Moran, 2000). Anorectic actions of CCK depend mainly on activation of the CCK1R (CCK-A) on the afferent vagus nerve, which conveys the signals to the hypothalamus through the brain stem (Moran, 2000). Activation of the CCK1R is a crucial step in CCK short-term food intake inhibition; this is due to the inability of the CCK to cross the blood brain barrier and therefore uses the vagal afferent nerve as a mean of signals transportation to the CNS (Owyang & Heldsinger, 2011).

In addition to satiety stimulation, CCK can regulate the expression of other neurotransmitters. In a fasting state, a low plasma concentration of CCK is associated with vagal nerve expression of food stimulating neurons, the melanin concentrating hormone (MCH)-1 receptors (Dockray, 2009). With CCK plasma concentration rising within 15 minutes in response to meal initiation, an immediate decrease in the expression of receptors and stimulation of the expression of Y2 receptors in neurons projecting to the stomach, mainly the PYY3-36 receptors which are responsible for delaying gastric emptying and hence influencing satiety stimulation (Dockray, 2009) & (Perry & Wang, 2012).

Another important role played by CCK in controlling the overall energy balance, is its involvement in mediating certain satiety actions of leptin adiposity signals. A combined CCK and leptin mixture has been demonstrated to reduce body weight over a 24-hour period compared to leptin alone, this synergic interaction

between the two hormones serves as an important modulator in short-term feeding inhibition with the limitation of it being a short half-life peptide (Perry & Wang, 2012) & (Moran, 2000). This synergistic combined action between CCK and leptin might be due to the location of their receptors on the same vagal afferent neurons, and hence inducing satiety through the same pathway.

1.4.4 Peptide YY

Named due to the presence of two tyrosine residues both at its N- and C-terminals, the full length thirty-six amino acid peptide YY (PYY) is synthesized and secreted in a nutrient-sensitive fashion from the enteroendocrine L-cells of the ileum and the colon (Yang et al., 2008) & (le Roux & Bloom, 2005).PYY is secreted into the circulation postprandially, generally peaking within one hour of meal consumption and with levels being related to meal energy content (Karra, Chandarana, & Batterham, 2009). PYY acts as a satiety hormone by delaying the gastric emptying process and therefore hindering gastrointestinal nutrient transit (le Roux & Bloom, 2005). The secretion of the peptide PYY induces an anorexigenic effect in our body through the slowing down of gastric emptying as well as being associated with the ileal brake.

Polypeptide YY belongs to a family of peptides having the sametertiary structure of a beta-turn connecting the two alpha and polyproline-helix resulting in a PP-fold peptide characterized by its U-shape (Berglund MM et al., 2003). This PP-fold family includes peptide YY (PYY) and neuropeptide Y (NPY). Two forms of PYY are available; they are first released as PYY1–36 form, then cleaved by dipeptidyl peptidase- 4 (DPP4) into the thirty-four amino acid shortened form known as PYY3–36 which is considered to be the predominant circulating PYY form (Yang et al., 2008) & (le Roux & Bloom, 2005).

PYY uses the Y receptor (G-protein-coupled receptor) as a potential site of action to mediate its effects (Karra et al., 2009). The five Y-receptor families differ in their distribution and function and have diverse affinities to the PP-fold peptides, except for the PYY1–36 form that has affinity for almost all the Y-receptors, while PYY3–36 has a higher affinity for the Y2-receptor subtype. (Karra et al., 2009; Koegler et al., 2005). PYY1–36 is considered to be a potent agonist of both types of Y-receptors, the Y1 and Y2, while PYY3–36 has a higher affinity for the Y2 receptor and is considered to be a Y2-specific agonist.

Like almost all gut hormones, PYY3–36 exerts its effect on feedingbehavior via the hypothalamus. To reach the hypothalamus, it can either act centrally via activation of Y2-receptors located on the arcuate nucleus of the hypothalamus (ARH) and vagal afferent nerves, or crossing the BBB through a non-saturable transport mechanism (Karra et al., 2009). The PYY exerts its satiety role via an action on the hypothalamic ARC, which is the area that is not completely covered by the BBB, and hence allowing this area to respond to the peripherally circulating PYY hormone.

1.4.5 Glucagon-like peptide-1

Released from the intestinal and colonic L-cells as a cleavage product of pro-glucagon in a directly proportional fashion toingested calories, Glucagon-like peptide-1 (GLP-1) acts as an anorectic peptide hormone by inhibiting gastrointestinal motility (Strader & Woods, 2005) & (Gutzwillerb et al., 1999). Moreover, GLP-1 is considered to be function as an incretin that stimulate the secretion of insulin from the b-cells upon food intake.thus acts as blood glucose regulator (Gutzwillerb et al., 1999) & (Chaudhri, Wynne, et al., 2008). GLP-1's main physiological reaction after food intake is glucose-dependent insulin secretion followed by slowing down gastric emptying and inhibition of gastric acid secretion, which results in the feeling of fullness and the reduction of food intake.

One of the major limitations of the GLP-1 peptide to be used in treatment of obesity is its short circulating half-life (Perry & Wang, 2012). This is due to the

inactivation and clearance of the circulating GLP-1 via dipeptidyl peptidase-IV enzyme (DPP-IV) resulting in a half-life of less than five minutes (Chaudhri, Field, et al., 2008) & (Perry & Wang, 2012).

1.4.6 Amylin

Co-secreted with insulin via pancreatic b-cells during a meal, Amylin is a peptide hormone that induces satiety by inhibiting

gastric emptying and gastric acid secretions causing a dosedependent reduction in meal size (Chaudhri, Wynne, et al., 2008) & (Strader & Woods, 2005). This inhibitory satiety effect is predominantly mediated by directly acting on an area that is rich in amylin receptors found in the area postrema of the brain (Lutz, 2010). Amylin peptide hormone has a dual role as a satiety hormone like CCK and as an adiposity signal resembling leptin and insulin actions (Lutz, 2010).

Amylin's mechanism of action is unclear to date; it's rapid release

after meal consumption does not depend on the nutrient contact with the entero-endocrine L-cells, its action might be either due to directly binding to its peripheral or central receptors or indirectly by influencing the release of other satiety hormones (Strader & Woods, 2005)& (Gutzwillerb et al., 1999).

1.4.7 Oxyntomodulin

Sharing the same production pathway as GLP-1, the thirty-seven amino acid peptide oxyntomodulin (OXM) is a result of proglucagon cleavage, co-secreted with GLP-1 and PYY3–36 from the intestinal L-cells in response to nutrient ingestion with a direct proportion to caloric content (Cohen et al., 2003) & (Chaudhri, Wynne, et al., 2008).

OXM plasma circulating levels increase within five to ten minutes after food consumption (Cohen et al., 2003). This increase influences satiety, decreasing food intake as well as stimulating an increase in energy expenditure. Although, the mechanism of this OXM anorexigenic action is unclear, it has been suggested that it might be due to binding via the GLP-1 receptors, with a lower affinity toward these receptors compared to GLP-1 (Perry & Wang, 2012)& (Chaudhri, Wynne, et al., 2008). On the other hand, a novel finding suggested that the OXM mechanism of action in reduction of appetite is a result of an inhibitory role that OXM exerts on pre-prandial rise in ghrelin levels (Cohen et al., 2003). This suggests that the anorexigenic effect of OXM in suppressing our appetite might be due to reducing ghrelin secretion from the stomach. Moreover, OXM and GLP-1 share the same inactivation and degradation pathway via DPP-IV enzymes (Chaudhri, Wynne, et al., 2008). Figure 6 summarizes the satiety hormones and their routes to the hypothalamus to influence the body energy hemostasis.

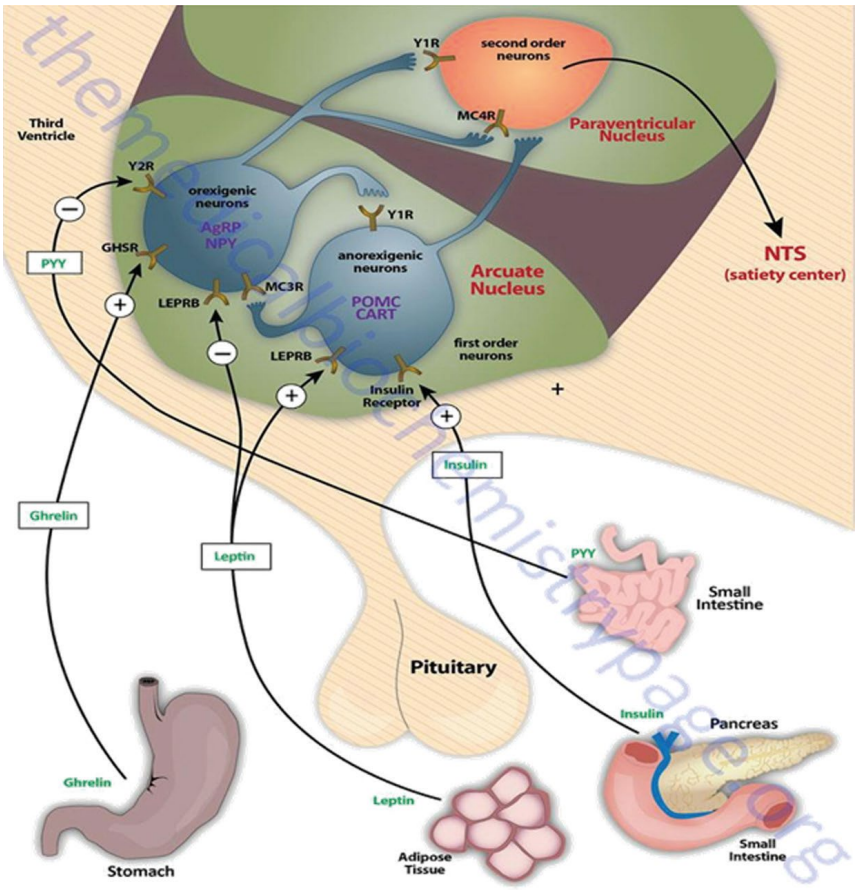


Figure 6 sums up the complex hemostatic system

"Gut-Brain Alliance" that is responsible for controlling our body weight in both of its scenarios, weight loss and weight gain. Influenced by the food we eat, the gut secretes anorexigenic and orexigenic signals either immediately or after a period of time. These signals travel from their source of secretion the gut or the fat cells to the brain either via the bloodstream or the afferent vagal nerve, to either inhibit or stimulate the hypothalamic neuropeptides. In addition to signalling the hypothalamic nuclei, peripheral signalsmay also stimulate neuropeptide receptors found in the brainstem particularly the NTS, which in turn projects signals to other parts of the brain and regulates our appetite.

1.5 Gut Hormones Stimuli

Gut hormones have to be first stimulated in order to exert their satiety/hunger roles. Their stimulation can either be generated by several mechanical (volume-dependent) or chemical (nutrient dependent) stimuli. Mechanical stimuli involves distention of the luminal wall by a volume load while chemical stimuli are triggered by the presence of nutrients in the gastrointestinal tract, both forms of stimuli result in the activation of the vagal afferent nerve or the secretion of gut peptides into the bloodstream (Maljaars & Masclee 2010). To induce satiety/hunger the hormones secreted have to be stimulated or inhibited first in response to ingested food, this stimulation might be either mechanical or chemical, in this study we will be focusing on the chemical stimulation of the gut hormone "ghrelin" and the hypothalamic feedback mechanism that regulates satiety.

People often stop eating as a result of satiation that might be due to an increase in gastric viscosity or a decrease in gastric emptying speed that can cause a higher gut transit time (Van Kleef, Van Trijp, Van Den Borne, & Zondervan, 2012). These gut actions are the fruits of satiety hormone feedback mechanism response to food ingestion, therefore, we can conclude that food is the chemical stimuli that our body needs to induce satiety or stimulate hunger. Carbohydrates, fats and proteins are the three predominant macronutrients that are considered to be the body's source of energy, with a variable energy provided from each source. Fats provide the body with 38 kilojoules (9 kilo calories) per gram while carbohydrates and protein provide 16-17 kilojoules (4 kilo calories) per gram (Van Kleef et al., 2012). An indirect relationship exists between the energy generated from each macronutrient and their satiety effect. For example although it only generates 4 kilo calories/gram, the highest satiety extent is delivered after a high protein diet compared to a high carbohydrate or a high fat diet; to a certain degree this is due to proteins ability to induce higher levels of thermogenesis compared to other macronutrients (Westerterp-Plantenga, 2003) & (Van Kleef et al., 2012).

Another important feature of food that has a profound impact on satiety is energy density. According to Whybrow (2005), energy density is described as

'the energy per unit weight of a ready to eat food'. Three main factors affect food density: water, air and fibers. The higher the content of these elements in the food, the lower the energy density the food is, characterized by low calories and higher satiety effect (Van Kleef et al., 2012). This suggests that consuming low energy density foods in large volume are actually less palatable but more satiating than consuming a small quantity of high energy density food. characterized by low calories and higher satiety effect (Van Kleef et al., 2012). This suggests that consuming low energy density foods in large volume are actually less palatable but more satiating than consuming a small quantity of high energy density food.

The exposure of different regions of the gut wall to special macronutrients leads to the release of different gut peptides (Maljaars & Masclee 2010). In the case of ghrelin, carbohydrates are thought to be the most potent suppressor compared to fats and proteins (Patterson, Bloom, & Gardiner, 2011). Leptin on the other hand exhibited increased in its levels after the consumption of a high carbohydrate-low fat meal compared to a high fat-low carbohydrate meal with no noticeable effect seen after a highprotein meal, but a high fatty meal would have a longer reduction on leptin levels (more satisfying) compared to a high carbohydrate meal (Klok et al., 2007). Moreover, long chain fatty acids (greater than C10) and proteins are crucially effective in stimulating the release of CCK from the duodenum and jejunum, whereas in the distal small intestine, mainly fat followed by carbohydrates and protein stimulates the release of PYY hormone (Maljaars & Masclee 2010 ; Matzinger et al., 1999).

Moreover, according to Maljaars et al. (2008), the presence of undigested fat in the distal part of the small intestine activate what's called an 'ileal break', that if stimulated will produce a dose-dependent slowing down of gastric emptying accompanied by increased transit time of the meal in the small intestine. Animal studies demonstrated that all three macronutrients activated the 'ileal brake', however similar data in humans are limited According to Spiller et al. (1988) study that compared the perfusion of the GIT with low protein, carbohydrates and fats, they showed that only the infusion of the hydrolyzed free fatty acid activates an intracellular signaling cascade that slowed the jejunal motility and induced satiety, whereas a higher concentration of carbohydrates was required to induce a similar effect to that of fat.

From the literature review and the previous studies, we know that the macronutrients affect the gut hormones secretion in different degrees, but herein we would like to introduce a new dimension in the food-gut hormone relationship and hypothesize that it is the nature of the food matrix of the macronutrients, fats in particular, that determines the feeling of satisfaction, not the amount or the type of fats that we consume.

1.5.1 Food Matrix

As mentioned above, in order for macronutrients to trigger the gut hormone response, they must be present in different regions of the gastrointestinal tract; hence they have to be bio-accessible.

According to Hedren et al. (2002), bio-accessibility is defined as"the amount of an ingested nutrient that is available for absorption in the gut after digestion". In other words, it is the amount of nutrients available for absorption after being released from its food matrix. In addition to the food caloric content, food structure and its breakdown is an important determinant in nutrient postprandial actions that arises during the process of digestion and its following metabolic responses (Robertson, 2006).

Through evolution, plants have developed defense mechanisms that protects their nutrients against degradation by predators either by attaching them to the membranes and sealing them inside cell organelles or by binding them to cell walls (Parada & Aguilera, 2007). Foods can be considered as a nutrient delivery systems that is made of structural elements such as water, air cell, oil droplets, polymer strands, fat crystals, granules and micelles that interact

and arrange themselves in a spatial manner varying from the submicron level to those seen by the naked eyes microstructure (J. Aguilera, M.,, 2000) & (Lundin, Golding, & Wooster, 2008). Thus it is an interaction between similar or dissimilar elements that bind together to form an organized structure that plays a major role in the physicochemical properties and nutritional value of the food we consume.

There are two existing kinds of food structures; natural and processed food structures. According to Parada & Aguilera (2007), natural food structures can be categorized into four differentclasses:

1. Fibrous structure analogous to muscle structure.
2. Fleshy material bonded together at the plant cell wall (fruits and vegetables).
3. Encapsulated embryos of plants (grains and pulses).
4. Milk the complex fluid.

On the other hand, processed foods are made of multicomponent matrices that are composed of individual macronutrients. These are resembled as colloidal dispersion, amorphous, crystalline phases, emulsions or acquire networks either by heating, cooling or by shear application (Parada & Aguilera, 2007). Consequently, nutrients can be either contained in a natural cellular vehicle or in a microstructure produced by processing.

Applicable to nutrients too, bioavailability is defined as "the rate and extent to which the active substances or therapeutic moieties contained in a drug are absorbed and become available at the site of action" (John Shi & Le Maguer, 2000). The bioavailability of an ingested nutrient is more important than the amount present in the original food for its functionality. The release, transformation and the absorption of the nutrients are greatly influenced by the degradation of the food matrix (Parada & Aguilera, 2007).

Therefore when natural and processed foods are ingested, some of the constituents get digested and distributed in the human system while others remain embedded in the intact matrix and then excreted out of the body.

Food structure manipulation may either increase or decrease the bioavailability of the nutrients (Zúñiga & Troncoso, 2011). This structure manipulation can either occur during food digestion or through the cooking process. Food processing either through several digestive stages or through heating and cooking enhances the nutrient bioavailability as a result of plant cell wall disruptions that leads to the detachment of the nutrient-matrix complex (Parada & Aguilera, 2007). Mashing, cutting or cooking carotenoid rich food releases more β-carotene compared to raw, uncooked vegetables due to the release of the cell content after the cell wall disruption (J. M. Aguilera, 2005). For example, lycopene which is an effective antioxidant found in tomatoes has improved bioavailability after cooking due to the extensive break down of the cell wall and weakening of the bond between the nutrients and their structure (J. M. Aguilera, 2005). These findings provide a clear picture on the role of the food matrix that can be a key parameter in controlling the amount of macronutrients released into the GIT, which will ultimately affect satiety signals by activating specific food sensors.

This prompted the exploration of food from a different perspective to consider the whole food and not just the nutrients as the primary component in the diet. This gave rise to the food synergy perspective that implies that more information can be obtained if we examine the food as a whole rather than isolated individual components (Jacobs & Tapsell 2007).This concept suggests that the distinctive interaction between the food matrix and the human physiological system is unique compared to the action of isolated food components. Thus isolated nutrients may not be the important element in the diet but rather the release of those nutrients from their microstructure as well as the rearrangement of the food components during the GIT transit is a crucial aspect in regulating energy homeostasis.

This synergistic relationship between dietary nutrients and their food matrix has shown better health outcomes over the isolated food constituents apart from cases of particular nutrient insufficiencies (Jacobs & Tapsell 2007).

Dietary intervention studies supported the food synergy concept reporting that isolated nutrient supplementation lacked their biological efficacy when administrated to healthy participants (Jacobs, Gross, & Tapsell, 2009). Another advantage of havingthe whole food rather than the individual supplement is that food acts as a buffer during absorption. When the food constituents are isolated from their biological environment (cell wall) to the GIT, their biological activity is enhanced by the presence of whole food like in the case of lipid soluble vitamins (Jacobs et al., 2009).

Moreover, the presence of the food matrix will delay the release of nutrients and hence delay their absorption decreasing the possibility of a bolus effect (Jacobs et al., 2009). This reflects the importance of the food matrix to our body and how that the presence of the food matrix acts like a nutrition absorption regulator.

We can now appreciate the effect of the food matrix on the bio-accessibility and the bioavailability of the nutrients and subsequently how the release of nutrients from their matrix will influence a cascade of actions and ultimately its effects on gut hormones. Using almond and almond products as an example we examined how the food matrix of almond might affect satiety by comparing intact-structures (raw almonds and almond extract), a processed almond product (almond oil emulsion) and purified almond fat (almond oil) with no matrix on gut acylated-ghrelin levels.

The cell wall (structure) physicochemical properties influence the bio-accessibility of the food nutrients by acting as a physical barrier between the food contents and the GI lumen and therefore hindering the process of digestion (Ellis et al., 2004). Disruption of the non-starch polysaccharides cell wall during mastication and transit along the GIT or during food processing will affect the amount of the intracellular lipid released from the almondseed (Ellis et al., 2004; Waldron, Smith, Parr, & Parker, 1997).

According to a study by Elias et al. (2004), fecal material collected from healthy participants consuming an almond-rich diet showed some intact almond seed cotyledon and testa (seed coat) in the fecal samples (Figure 7 & 8). These findings showed that the bio accessible fraction of lipids in the GIT was the result of just the

disruption of the first layer of the fractured cell wall while the other layers of intracellular oil bodies remained encapsulated in the intact cell wall resisting mastication and digestion. Another study by Berry et al. (2008) showed that consuming almond seeds lowered postprandial lipemia compared to a free lipid source, due to the encapsulation of the intracellular lipids within the cell walls hence hindering their release. Thus the stable structure of the almond seed slowed down the metabolism of their interacted nutrients through slowing down the release of their nutrients.

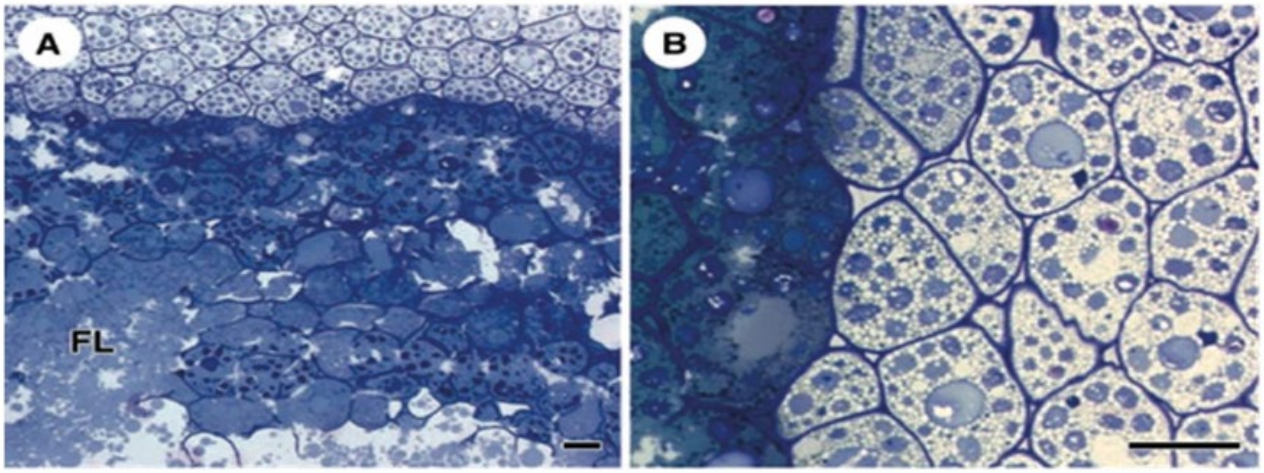


Figure 7.

Part of the almond tissues physically ruptured by chewing showing some lipids released from the cells. (B) Shows the same but magnifying the intact cell bodies under the ruptured surface where lipids are not released from the oil bodies (Elias et al. 2004).

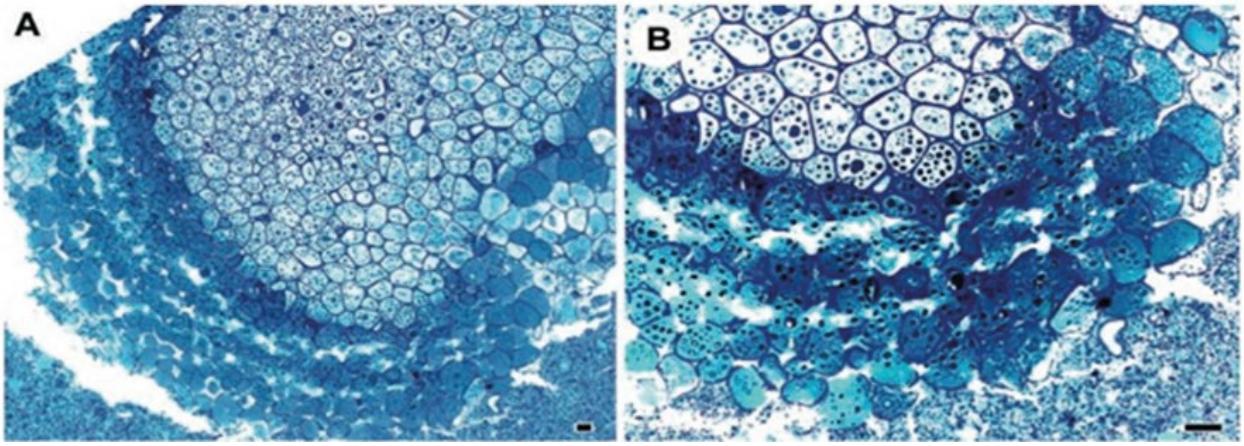


Figure 8.

Fecal almond tissues under light microscopy showed intact almond tissue encapsulating its nutrients. (B) Shows a magnified image of (A) Elias et al. 2004

1.6 Lipids

Given that a high percentage of almonds are made up of fatty acids, we need to consider the fate of almonds from the moment they are consumed, their transit time in the GIT where they trigger gut hormones until their absorption into the blood. In mono-gastric animals and humans, fat digestion requires several consecutive physiochemical and enzymatic activities before being utilized by the body. Unlike most of the fundamental dietary nutrients that are transported via aqueous medium in the human body, lipid insolubility in water requires an emulsification process that rearranges dietary lipid structure into droplets that can be transported in the aqueous GIT medium (Bauer, Jakob, & Mosenthin, 2005; Patton & Carey, 1979). The triglyceride (TGs) molecule is the basic unit of most natural occurring fats and oils, with monoglycerides (MGs), diglycerides (DGs), and phospholipids (PLs) as minor fat components (Zúñiga & Troncoso, 2011).

The digestive process starts in the complex environment of the mouth with the first physical transformation of the food matrix occurring via the grinding of the food into small pieces and saturating them with saliva containing an acid-stable lipase enzyme forming a swallow-able bolus (Norton, Moore, & Fryer, 2007; Parada & Aguilera, 2007). This mastication process enhances the digestion process and nutrient absorption by decreasing the particle size increasing the surface area of the food providing asuitable circumstance for the further enzymatic attacks (Parada & Aguilera, 2007).

Once the bolus reaches the stomach, the physical motility of the luminal wall of the stomach mixes the food with the gastric secretions creating an auxiliary particle size reduction, re-shufflingand phase separation of the chyme (Zúñiga & Troncoso, 2011).

Meal size, physical state and food structure, plays a role in stomach retention time ranging from a few minutes to a few hours prior to entering the small intestine (Weisbrodt, 2001). From the stomach fundus, the actions of acid-stable lipases on the surface of the lipid droplets are responsible for hydrolyzing 10% of the dietary triacylglycerol's, particularly on the sn-3 position to DGs, MGs and short/medium chain fatty acids (Marciani et al., 2009; Zúñiga & Troncoso, 2011). The physical state of the fat within the gastric lumen is a crucial factor that may determine the rate of delivery of fat to the small intestine, consequently affecting the rate of absorption, metabolism

and the gut-brain signaling pathway (Marciani et al., 2009). Relying solely on their surfactant properties, the polar lipolysis products FA and DG accumulate at the interface of the emulsion forming a solid or crystalline phases preventing the enzymes from further lipolysis (Wilde & Chu, 2011). This product inhibition is the mainreason for the limited lipolysis effect of these enzymes on dietarylipids. Moreover, if the concentration of FFA's released from lipid droplets reaches 10-30% in the stomach, the process of lipolysis will seize (Armand, 2007).

After the stomach phase, the chyme travels to the small intestine, particularly the duodenum where they are mixed with the pancreatic juice containing sodium bicarbonate, bile salts, phospholipids and enzymes. Duodenum lipolysis is a more complex process than the previous lipolysis. Unlike the lingual and gastric lipase, pancreatic lipase (P-LIP) can hydrolyse both the sn-1 and sn-3 positions of TG forming two fatty acids and one monoacylglycerol that will accumulate at the interface due to their amphiphilic properties, andthus responsible for hydrolyzing 50 -70% of dietary TG (Bauer et al., 2005; Patton & Carey, 1979) Lipolysis in the duodenum is regulated by a lipase/colipase complex involving pancreatic enzymes that require a higher pH than the stomach to be active, therefore sodium bicarbonatebuffers the pH to neutral in the duodenum enhancing the enzymecomplex activity (Bauer et al., 2005). Lipolysis product inhibition is prevented by the action of bio-surfactants that aid in the removal of these products from the interface, allowing the P-LIP to gain access to the TG (Wilde & Chu, 2011) & (Reis P, Holmberg K, Watzke H, Leser ME, & Miller R., 2009). Produced in the liver and secreted through the bile duct, bile salt aids in solubilizing lipolysis products from the interface by replacing the surface active material and also helping in the formation of mixed micelles shifting the lipase reaction towards that of lipolysis (Reis P et al., 2009). However, due to their high surfactant property, bile salt accumulates at the interface forming a detergent monolayer on the substrate surface preventing the P-LIP enzymes from exerting their actions. Co-lipase, a small protein acting as P-LIP cofactor due to an electrostatic interaction with the bile salt is able to form a bridge between bile salt dominated interface micelles and the P-LIP enzymes, consequently improving lipid hydrolysis (Pignol et al., 1998; Wilde & Chu, 2011).

The products of this complicated lipolysis are then removed from the water-oil interface by incorporation into mixed micelles that are formed spontaneously by the interaction of bile salts (Bauer et al., 2005). Monoacylglycerols and phospholipids enhance theability of bile salts to form mixed micelles. Formation of mixed micelles is essential in transporting non-polar lipids from the water-oil interface in the lumen across the unstirred water layer of the enterocyte brush border membrane, where approximately 90% of the dietary fats are absorbed through this membrane (Zúñiga & Troncoso, 2011).

1.7 Rationale

Based on previous studies, different complicated mechanisms exist in order to control our energy intake and regulate our satiety/hunger feelings. A variety of factors might influence our feeding behaviour as well as our body satiety/hunger signals that are the major contributor in mediating feeding process. These factors includes meal portion size; the bigger the meal the more full you will feel, the energy density; where the lower the food energy density means eating more and feeling fuller without gaining weight, the different macronutrients that we consume; a meal containing high-protein content will keep us full for a longer period of time, our social situation in a special eating occasions as

well as our emotional state where different people respond totheir mood in a different pattern to their eating behaviour.

Our body responds to the food we ingest by releasing gut hormone as a feedback mechanism to induce either fullness or hunger feelings, therefore satiating effects of dietary foods are well established, however, it is not known if the rate of entry of the food nutrients into the circulation, due to the presence of food matrix, can influence satiety. This gives the bio-accessibility and the bioavailability of nutrients a crucial importance in the satiety/ hunger process. But before these nutrients are bioavailable or bioaccessible in our gut, they have to detach from their cell walls to be released from their food structure "food-matrix" in order to be able to stimulate our gut hormones. Therefore these findings provided a clear picture on the role of the food matrix that can be a key parameter in controlling the amount of macronutrients released into the GIT, which will ultimately affect our satiety hormones and induce a cascade of actions to control our feeding behaviour.

1.8 Aims

Using almond fats as an example, the aim of this study was to explore the importance of the physical structure of food as a determinant of digestion and nutrient absorption examining the effects of almond matrix in which almond lipids are complexed (oil bodies) on postprandial satiety hormone particularly the peptide, ghrelin. The aims of the studies conducted included

•Trial A: Compare the effects of consuming almond extract (oils in natural oil-bodies covered by oleosin) with almond oil emulsion (oils in artificial oil-bodies covered by casein, the milk protein) on blood lipid profile and acylated ghrelin levels. • Trial B: Compare the effects of consuming raw almonds (oil in matrix composed of a cell wall and oleosin) with almond oil (no matrix) on blood lipid profile and acylated ghrelin levels. Elucidation of the role of food matrix (structure) on ghrelin hormone might unravel the mysterious black box behind the healthfulness of natural foods and the adverse health effects of processed food, which will result in a potential solution for the obesity epidemic and related chronic diseases. With the knowledge of food structure and its function, designer foods with modified structures can be produced to improved public health benefits and slow down the obesity pandemic, diabetes and cardiovascular disease.

1.9 Hypothesize

We hypothesized that it's the food matrix (structure) not the amount or type of fat in foods that determines satiety- the feeling of fullness or satisfaction. According to this we suggested that natural oil bodies would lower acylated ghrelin levels more than artificial oil bodies. Moreover an intact food matrix (raw almond kernels) will not only empty slower than processed food (almond oil) from the GIT, but also will influence a faster suppression of ghrelin levels, and that this would be related to a faster feeling of satisfaction after consuming food with an intact matrix.

Chapter 2 - Material and Methods

2.1 Subjects

Two randomized controlled dietary interventions were conducted in healthy volunteers, aged between 18-65 years with a body mass index (BMI) < 30kg/m2 that were recruited from the Newcastle region, NSW, AUSTRALIA via advertisement and flyers posted in the university and the surrounding area. For trial A, power calculations showed that we need 16 participants in order to perform this study, but considering a dropout rate of 20% we recruited 24 participants as per the approved ethics protocol (H-2012-0119). On the other hand, we manage to recruit eight participants for the pilot trial B. Participants exclusion criteria included, those who are on cholesterol lowering drugs, on nonsteroidal anti-inflammatory drugs, on weight loss programs, have a body mass index (BMI) higher than 30, diagnosed with any gastrointestinal disorder, diabetic, pregnant and allergic to nuts, particularly almonds. In addition, participant's eligibility was confirmed using a medical checklist upon enrolment, and they were also required to complete a medical history questionnaire and undergo weight and BMI measurements.

2.2 Study Design

The study design was randomized controlled dietary interventionsthat were divided into two distinct parts. The experiments participants were divided into

2 trials: • Trial A participants consumed two iso-caloric meals, meal I(Almond Extracts) Vs. meal II (Almond Emulsion) separated by at least one week as a washout period; comparing the oil bodies found in the meals were made from the naturally existing protein "oleosins" while the almond emulsion oil bodies covered by a synthetic made protein "casein".

• Trial B participants consumed the other iso-caloric meals, meal III (Raw almond) Vs. meal IV (Almond oil) separated by at least one week as a washout period; comparing the naturally intact cell wall found in the almond seed against the almond oil which completely lacked a food matrix All meals were equal in fat content (30%) but with the only difference

being in the meal food matrix. The study was undertaken at the Nutraceuticals Research unit in the Medical Sciences Building, University of Newcastle, Australia. Following an overnight fast, subjects were requested to attend the research unit on two individual morning sessions to receive one of the two test meals on a random order using computer-based randomization. On the subject's first treatment, they had to complete a medical questionnaire and undertook body composition analysis (InBody 230; Biospace Co., Ltd. Seoul, Korea). On the day of the experiment, subjects donated a fasting blood sample, the test meal was then consumed and blood collections were taken at 1 and 3 hours post-consumption. No additional food or fluid was allowed during this time period.

This study was carried out in accordance with the guidelines laid down in the Declaration of Helsinki and the Human Research Ethics Committee of the University of Newcastle (H-2012-0119) that approves all procedures involving human subjects.

2.3 Diet Interventions

Separated by at least one week, two different experimental meals were performed in a randomized order in each of the trial's subjects. According to the Treatment Preparation Protocols, meals were either prepared in our laboratory or purchased fresh. Meals consisted of:

I. 150g of mashed potato and approx. 300mL of almond extract

II. 150g of mashed potato and 300mL of emulsified almond oil

III. 150g of mashed potato and 60g of raw almonds and 300mL of water

IV. 150g of mashed potato, 30g (1.5 tablespoons) of almond oil and 300mL of water.

Besides meals (III, IV), which are made respectively of 60g raw almond kernels and 30ml of almond oil respectively, meal (I & II) had to be prepared in the laboratory. We prepared 300mL of almond extract (milk) by mixing the ingredients for 1 minute in a commercial blender (Multi Blender Pro, PB7600) prior to consumption. Almond extract (milk) ingredients include 125g of raw almonds kernels, 500ml of water, 1 tsp. of Equal sweetener Then the resulting mixture was sieved using cheesecloth into jug before serving 300ml of the milk to the subject. For the almond emulsion meal (II), ingredients were mixed at a maximum speed for approximately 2 minutes in a commercial blender (Multi Blender Pro, PB7600) prior to consumption. Ingredients include 12g of sodium caseinate (Riddett Institute, NZ), 30mL of almond oil (Beaufor AOB50), 258 mL of water and 1 tsp. Equal sweetener.

All test meals were served with a 150g of prepared plain mashed potato that served as a vehicle (delivery system) for the almond oil meal and as a source of energy following the subjects overnight fasting. Moreover following the almond extract and almond oil emulsion meals, water was served at 1 and 3 hours.

All test meals were developed with a protocol to provide an identical fat content (30g) in each meal but with a structurally diverse complex. The raw almond kernels serving as the naturally intact food matrix followed by the almond milk that is made of intact oil bodies with their phospholipid monolayer surrounded entirely by oleosins, then the almond emulsion which instead of having a natural oil body it had a free oil structure stabilized by a manufactured casein and finally the almond oil which entirely lack a food structure.

2.4 Blood Collection

For each subject in both trials, 8mL venous blood samples were collected at 0, 1 and 3 hours following each treatment. The total blood volume that was collected (24mL) is less than 1% total blood volume and thus represents no hemodynamic risk to subjects Blood was drawn from the antecubital vein into a 4mL Vacutainer tube containing anticoagulants (EDTA). Whole blood was centrifuged at 3000g for 10 min at room temperature. Plasma was separated and transferred into Eppendorf Safe-Lock Tubes™ 1.5 m and stored at -80°C until assayed. Whole blood sample analysis was

performed using a standardized-automated analyser in Hunter New England Area Health Pathology Services (NSW, Australia).

2.5 Plasma Hormone Assay

Plasma Ghrelin concentration was measured using a commercially available enzyme immune-metric assay (EIA) test kit based on a ouble-antibody sandwich technique. All samples were assayed in duplicate in order to obtain more dependable results. The C-terminal and the N-terminal of each standard or sample ghrelin binds to the monoclonal antibody and the acetyl-cholinesterase (AChE) – Fab' conjugate that coats the 96 wells of the plate forming a sandwich. In order to enhance the assay sensitivity we incubated the plate mixture for 20 hours at 4°C; 0.3pg/ml versus 0.8pg/ml for the shorter incubation period. After the incubation period, Ellman's reagent is added causing an enzymatic activity with the immobilized AChE forming a yellow colour that determines the acylated ghrelin concentration. Using spectrophotometry, the plates were read between 405 and 414nm; the yellow colour intensity being proportional to the amount of human acylated ghrelin found in the wells during the immunological incubation period.

2.6 Statistical Analysis

Data analysis was completed using IBM Statistics software for windows (version 20.0, SPSS Inc., Chicago). Advised by (Berry et al., 2008) Power analysis for sample size: Using a two-tailed t-test, an alpha of 0.05 and a power of 0.90, a sample size of 24 (including 20% dropouts) was calculated as necessary to detect significant differences in variables in trial A. The Shapiro-Wilk test of normality was utilized to detect if data were skewed or normally distributed. To determine the changes in the variables within the same group over time, after meal consumption we performed paired sample Student t tests. All data are presented as mean ± SEM, and a P value of less than 0.05 was considered statistically significant.

2.7 Results

Table 1 displays the physical and the anthropometric characteristics of the 24 participants that completed the two trials. At the time of enrolment, all subjects were healthy, non-obese, age-sex matched with a mean ± SEM age of 25.7 ± 0.72 y. All female subjects were of pre-menopausal age. There was no significant difference that can be noticed within groups' anthropometric and fasting baseline variables.

All the four meals were satisfactorily tolerated by the 24 participants and there were no complaints about the size or the palatability of the meals.

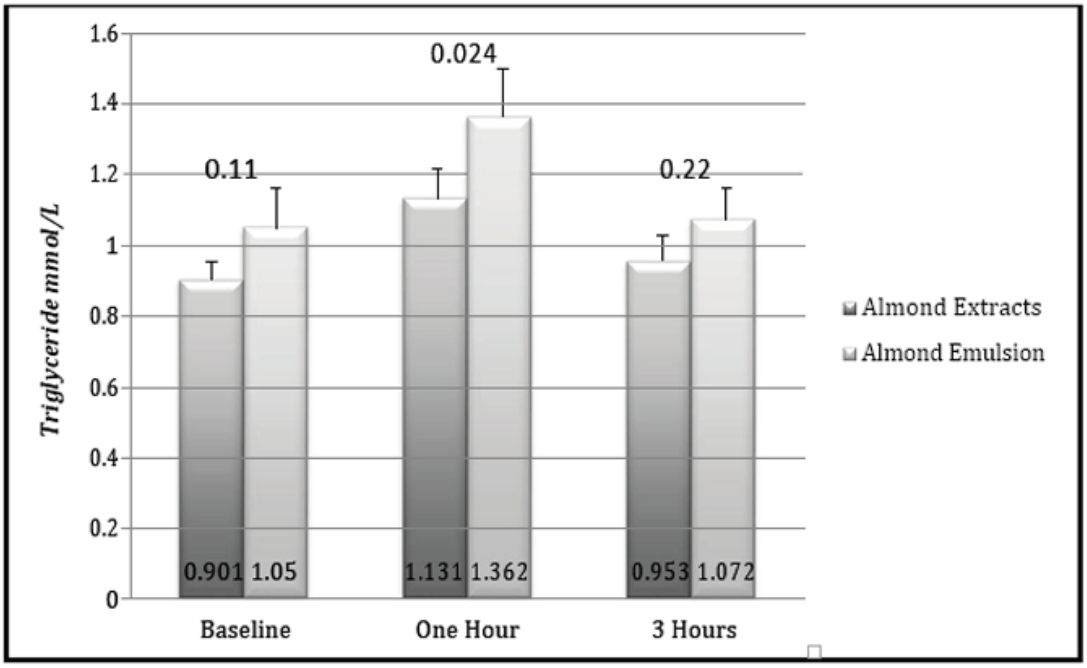
Participant's blood withdrawal went with no particular discomfort during the process. Figure 9 shows the plasma triglyceride levels for trial A participants who consumed the almond extract and almond emulsion meals at baseline, one hour and 3 hours after food intake. Within this group of participants, there was a significant difference observed in plasma triglycerides levels after one hour following consumption of almond extract and emulsion (1.131 mmol/L at baseline versus 1.362 mmol/L respectively; p=0.024). There was no significant difference in total cholesterol, LDL, HDL or glucose levels (see appendix table A).

Figure 10 shows the plasma triglyceride levels for trial B participants who consumed raw almonds and almond oil meals at baseline, one hour and 3 hours after food intake. Within this group, the only significant difference after comparing both meals was the plasma triglycerides levels at 1 hour following meal consumption (1.188 mmol/L after raw almond consumption compared with a 1.364 mmol/L after almond oil consumption with a 0.034 significance difference. While we found no significant difference in total cholesterol, LDL, HDL or glucose levels (see appendix table B)

Table 1: Anthropometric and fasting baseline variables.

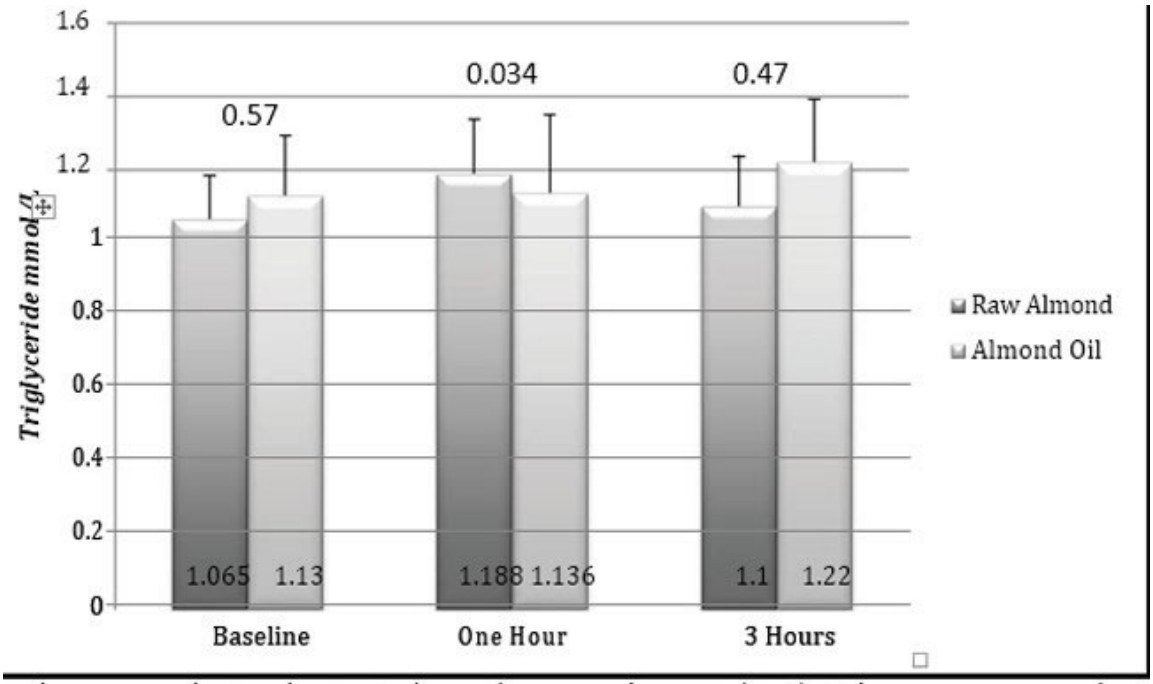
	Trial A (n=16)	Trial B (n=8)
Age (y)	25.7±0.93	29.12±3.81
BMI (kg/m^2)	23.3±0.54	25.08±1.06
Body fat (%)	21.9±1.41	21.5±2.84
Cholesterol (mmol/L)	4.6±0.21	4.9±0.37
Triglyceride (mmol/L)	0.91±0.09	1.24±0.13
LDL-C (mmol/L)	2.76±0.25	2.94±0.42
HDL-C (mmol/L)	1.49±0.09	1.39±0.19

Values are presented as mean ±SEM.



P-values were obtained using independent sample t-test (n=16). Values are presented as mean ±SEM.

Figure 9: Lipid Profile after consuming Almond Extracts and emulsion meals (Trial



-values were obtained using independent sample t-test (n=8). Values are presented as ıean ±SEM.

Figure 10: Lipid Profile after consuming Raw Almond and Almond Oil meals (Trial B)

2.7.1 Acylated plasma ghrelin

For both trials, acylated plasma ghrelin concentration was measured on three different time intervals; first at baseline then at one hour and three hours after consuming the meals. Within all participants in both trials, plasma acylated ghrelin concentrations were suppressed following one hour of the meal consumption and then increased at 3 hours after meal consumption. In trial A (figure 11), plasma acylated ghrelin levels dropped at 60 min after consuming the meals and rose again after 3 hours of food intake.

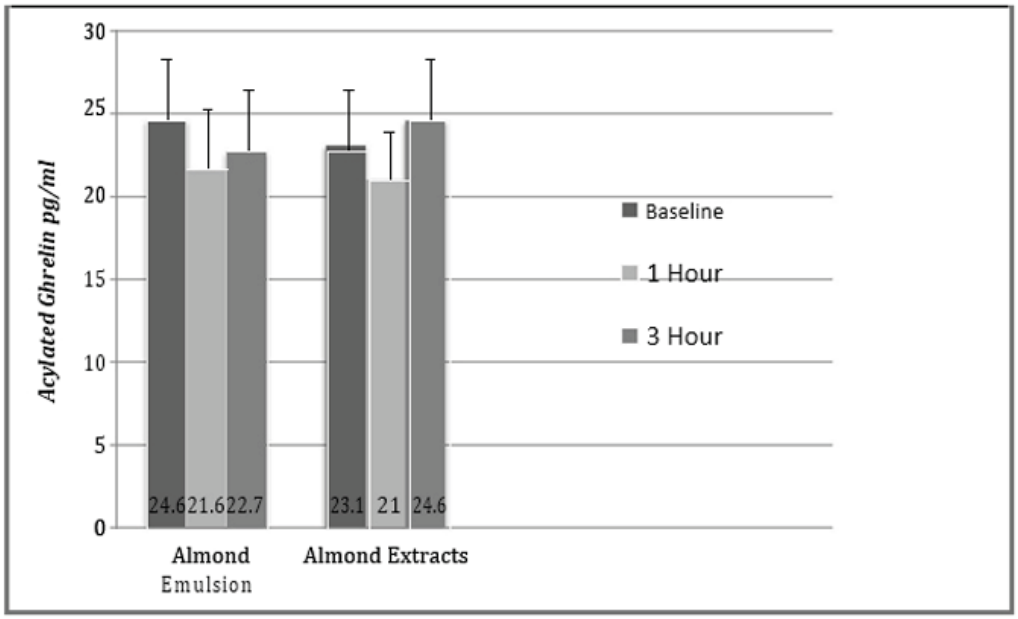


Figure 11 Acylated ghrelin levels after consuming almond emulsion vs. extracts meals (Trial A). Following food intake, P-values were obtained using independent sample t-test (n=16). Values are presented as mean ±SEM. For the pilot trial B of the experiment where we examined the impact of the cell wall against the lacking of a food matrix on postprandial concentration of acylated ghrelin levels. Acylated ghrelin concentrations were significantly higher in the participant consuming the meal containing raw almonds compared with the almond oil (figure 12).

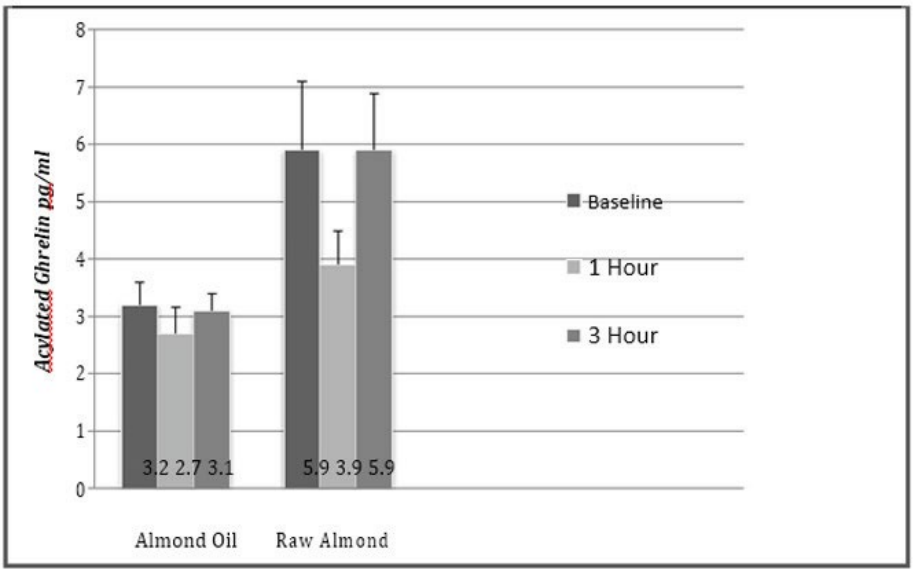


Figure 12 : Acylated ghrelin levels after consuming Raw Almond vs. Almond oil meals (Trial B). P-values were obtained using independent sample t-test (n=8). Values are presented as mean ±SEM.

We did investigate if there is any gender difference within the same groups and the post-prandial acylated ghrelin levels after consuming the meals and found that there is no significant difference (table 2).

Table 2: P-values for gender differences in acylated ghrelin levels

	Baseline	1 hour	3 Hours
Raw Almond	0.53	0.39	0.19
Almond Oil	0.89	0.13	0.17
Almond Emulsion	0.07	0.06	0.07
Almond Extracts	0.08	0.10	0.08

P-values were obtained using independent sample t-test for Male vs. Female within the same groups.

P-values were obtained using independent sample t-test for Male vs. Female within the same groups.

Chapter 3 - Discussion and Conclusion

3.1 Discussion

This is the first study to report the acylated ghrelin levels response to food matrix, as no previous study has investigated whether themacronutrient matrix has an impact on post-prandial acylated ghrelin levels. Therefore our novel approach aimed to investigate the effect of an iso-energetic lipid load encapsulated in different kind of food structures "food matrix" varying from natural to processed food structures and their impact on suppressing the hunger feeling by lowering ghrelin levels. We predicted that the difference in the food matrix would have a greater impact on suppressing postprandial ghrelin concentrations.

In trial A we compared acylated ghrelin levels in 16 subjects consuming two iso-caloric meals, one meal "almond extracts" having naturally intact oil bodies covered by the naturally found protein "oleosin", while the other meal "almond emulsion" instead of containing natural oil bodies had a free oil structure stabilized by milk protein, casein. The finding from this part of the study showed no significant difference in the postprandial acylated ghrelin levels between the two meals, showing that the oil bodies in its natural or artificial form had no influence in the postprandial acylated ghrelin levels. This led us to perform the pilot trial B to see the impact of the whole structure of the raw almond kernals rather than its impounded oil bodies.

In the pilot trial B of the study where we compared the acylated levels of ghrelin within eight subjects consuming two iso-caloric meals, one meal contained an intact natural food structure "raw almond" while the other contained no food structure "almond oil". The finding from this part of the study showed no significant difference in the postprandial acylated ghrelin levels between the two meals, but it showed that there is more tendency for
raw almond to suppress ghrelin more than the almond oil which can be shown by the larger drop in acylated ghrelin levels after consuming the raw almond - 2.0 pg/ml compared to -0.5 pg/ml drop in the post-prandial acylated ghrelin levels after consumingthe almond oil. Unlike trial A, there was a greater tendency for the naturally intact raw almond structure in suppressing the acylated ghrelin levels. This can be explained by the mutual weaker structure in the two iso-caloric meals forms (trial A) compared to the naturally intact raw almond, where they both are emulsions but one having oleosin covering their oil bodies and the other having processed casein covering their oil bodies. These results suggest that the raw almonds which have a cell wall covering its oil bodies have more effect on acylated ghrelin levels than the natural or artificial oil bodies alone, reflecting a role for the cell wall in reducing the acylated ghrelin levels.

In both parts of experiments A and B, a significant increase in post prandial triglyceride levels, specifically one hour after the meals consumption was noticed, +0.024 and +0.034 respectively. According to (Berry et al., 2008), the structure of the almond kernal cell wall plays a major role in the lipid bio-accessibility as well as bioavailability that influences the postprandial lipemic response. This suggests that the structure of the almond seeds played an important role in the rate and extent of lipid release by acting as a physical barrier protecting and encapsulating their nutrients from our digestive system. This reflects the significant difference in our study which might be explained by the food structure, the stronger the structure the harder the lipase enzyme to penetrate and digest the structure to release the lipids.

Ingested nutrients are the most likely mediator of the postprandial fall in circulating ghrelin, although it is not yet clear whether the presence of nutrients in the stomach and in the gut or the metabolic response to food
ingestion is the major determinant of ghrelin's level drop after eating .By saying that, when we compared the difference in triglycerides and the difference in acylated ghrelin levels at one hour after meals consumption, we didn't find any correlation between them in both trials. This is in agreement of previous (Foster-Schubert et al., 2008) study in which they reported that lipids are least potent suppressor of acylated ghrelin levels when they compared three equal total volume, caloric content, and energy density meals and found out that high-protein meals suppressed ghrelin (acyl and total ghrelin) greater than high-carbohydrates meal, that was found to have a greater impact than the high lipidmeals. Moreover, this finding might also support our hypothesis that it is not the type or amount of fat that suppress hunger or induce satiety, it's the food matrix and herein the almond cell wall that might be playing a role in suppressing the acylated ghrelin levels and hence suppressing our hunger.

Ghrelin levels are known to increase in circulation just prior to consumption a meal, and fall after a meal, with a peak concentration that is enough to stimulate appetite. Although the study produced no significant evidence that relates acylated ghrelin levels to food matrix but consistent with other studies (Cummings et al., 2001),(Monteleone, Bencivenga, Longobardi, Serritella, & Maj, 2003) a pattern was noticed in our study trials (Figure 11, 12) where plasma ghrelin levels were higher before each meal and dropped to minimum levels after 1 hour of feeding. This observation reflects the physiological role that ghrelin plays in initiating individual meals and controlling our hunger feelings.

Although the different levels of pre-prandial acylated ghrelin levels within the same subjects are still yet inexplicable but might be due to the effect of sham feeding on ghrelin levels. According to (Arosio et al., 2004) meal effects on
circulating ghrelin levels, including both pre-prandial rise and post-prandial drop can be influenced by sham feeding without the food ingestion process. Smell, sight and oropharyngeal stimulation of food are considered to be human pre-absorptive cephalic phase responses, which might play a part in the pre-prandial rise ofghrelin in our subjects. These responses are due primarily to activation of the efferent vagal nerve that might have an impact on gastrointestinal response (Strubbe, 1992). This refers to the importance of the vagally mediated cephalic phase in initiating

ghrelin response to feeding, a rise followed by a suppression, which is later, maintained suppressed via either gastrointestinal or post-absorptive mechanisms.

Moreover, we did investigate the difference in gender effect on acylated ghrelin within the different groups of the trials and found out that there is no significant difference between gender and postprandial-acylated ghrelin levels. This finding is consistent with the study done by (Akamizu et al., 2006) but inconsistent with (Greenman, Golani, Gilad, & Yaron, 2004) were they reported that female participants had higher ghrelin levels compared to male participants after both glucose and lipids loads. Which might be due to the higher appetite hormonal levels in females compared to males, in addition to higher levels of ghrelin regulated ho rmones like growth hormone and prolactin in females (Maccario et al., 2000).

3.2 Limitation

The present study is not without limitations. The relatively small sample size, particularly in trial B, might not be a reflection ofthe general population. With reference to our power calculations, adequate participant numbers were not able to be recruited within the study period, therefore the study may have had insufficient power to detect significant results and future studies may require a larger sample size. In addition to sample size, we used the acylated ghrelin form as a measurement of ghrelin levels, although the stability of the des-acylated ghrelin form is more than the stability of the acylated ghrelin form, we used the later one due to the more reliable data reported after using the acylated ghrelin form.

3.3 Conclusion

In conclusion, the results of our study suggested that food structure "Matrix" have no significant impact on postprandial-acylatedghrelin levels. Although in trial B, we noticed that the cell wall of the almond seed had a higher tendency to lowerpostprandialacylated ghrelin than trial A natural and artificial oil bodies. This may be a reflection of stronger cell walls, that contains and protects the oil bodies within its structure, having a greaterimpact on acylated ghrelin levels than the oil bodies alone found in both emulsion forms. Moreover if we look at it from a different perspective, from the bioavailability and bio-accessibility of triglycerides, the cell wall structure has a larger effect on acylated ghrelin levels than the affect of lipids "triglyceride" in particular, which can be reflected by the significant difference noticed in thelevels of triglyceride that didn't have any correlation with the postprandial-suppressed levels of acylated ghrelin. This mightshed a new light upon the involvement of intact food structures onthe acylated ghrelin levels and the regulation of feeding behavior and our complex energy homeostasis system.

Our findings might add to the reported data regarding the underlining cause of the hunger control meal-induced ghrelinsuppression, whether it's the metabolic response, the presence offood in the gut, mechanical factors, food-induced taste stimulation,or the effect of other gut satiety hormones. Further studies involvinga larger number of participants are warranted to elucidate the roleof food matrix in energy homeostasis and human meal-hungerresponse by looking at the food as whole structure rather thanfood individual nutrients.

3.4 Acknowledgments

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Appendix

Table A: Lipid Profile after consuming Almond Extracts and emulsion meals

	Total Cholesterol (mmol/L)		
	Baseline	**One Hour**	**3 Hours**
Almond Extracts	4.74±0.29	4.71±0.29	4.67±0.27
Almond Emulsion	4.80±0.29	4.85±0.29	4.8±0.28
P-Value	0.82	0.57	0.54

P-values were obtained using independent sample t-test (n=16). Values are presented as mean ±SEM.

	LDL-Cholesterol(mmol/L)		
	Baseline	**One Hour**	**3 Hours**
Almond Extracts	2.74±1.28	2.72±1.17	2.75±1.12
Almond Emulsion	2.75±1.31	2.71±1.26	2.70±1.28
P-Value	0.97	0.96	0.88

P-values were obtained using independent sample t-test (n=16). Values are presented as mean ±SEM.

HDL-Cholesterol (mmol/L)

Almond Emulsion	2.75±1.31	2.71±1.26	2.70±1.28
P-Value	0.97	0.96	0.88

P-values were obtained using independent sample t-test (n=16). Values are presented as mean ±SEM.

	HDL-Cholesterol (mmol/L)		
	Baseline	**One Hour**	**3 Hours**
Almond Extracts	1.51±0.09	1.49±0.09	1.47±0.09
Almond Emulsion	1.49±0.08	1.48±0.08	1.44±0.07
P-Value	0.65	0.78	0.44

P-values were obtained using independent sample t-test (n=16). Values are presented as mean ±SEM.

	HDL-Cholesterol (mmol/L)		
	Baseline	**One Hour**	**3 Hours**
Raw Almond	1.52±0.21	1.51±0.20	1.42±0.16
Almond Oil	1.44±0.18	1.45±0.19	1.44±0.14
P-Value	0.58	0.81	0.64

P-values were obtained using independent sample t-test (n=16). Values are presented as mean ±SEM.

	Fasting Blood Glucose (mmol/L)		
	Baseline	**One Hour**	**3 Hours**
Raw Almond	5.1±0.2	4.0±0.26	5.0±0.1
Almond Oil	5.3±0.2	4.8±0.70	4.9±0.2
P-Value	0.34	0.19	0.22

P-values were obtained using independent sample t-test (n=16). Values are presented as mean ±SEM.