THE EFFECTS OF CRICKET VS. BEEF-DERIVED PROTEIN ON POSTPRANDIAL PLASMA AMINO ACID CONCENTRATIONS, SUBJECTIVE APPETITE SENSATIONS, AND AD LIBITUM ENERGY INTAKE IN YOUNG MEN

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List of abbreviations

AEBSF: 2-Aminoethyl benzenesulfonyl fluoride hydrochloride

AMDR: Acceptable macronutrient distribution range

AQC: 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate

ATP: Adenosine triphosphate

AUC: Area under the curve

BCAA: Branched-chain amino acids

CCK: Cholecystokinin

CNS: Central nervous system

C_{max}: Maximum concentration of a substance

DIAAS: Digestible indispensable amino acids score

DPP-IV: Dipeptidyl peptidase-4

EAA: Essential amino acids

En%: Percent of total energy

FAO/WHO: The food and agriculture organization/world health organization

GHG: Greenhouse gas

GLP-1: Glucagon-like peptide-1

HCl: Hydrochloric acid

mTORC1: Mammalian target of rapamycin complex 1

niAUC: Net incremental area under the curve

NEAA: Non-essential amino acids

PDCAAS: Protein digestibility-corrected amino acid score

PYY: Peptide YY

RDA: Recommended dietary allowance

SEM: Standard error of the mean

TAA: Total amino acids

T_{max}: Time the substance is present at the maximum concentration

UNU: United nations university

UPLC-MS: Ultra-performance liquid chromatography mass spectrometry

VAS: Visual analogue scale

Abstract

Background: Dietary protein provides the body with a source of amino acids and plays a key role in regulating appetite, satiety, and subsequent food intake. Sources of dietary protein differ in their amino acid content and digestibility and may therefore have different effects on appetite regulation. Animal-derived proteins such as beef are generally considered high quality sources of protein; however, the production of sufficient amounts of conventional animal-based protein to meet future global food demands represents a challenge. Edible insects such as crickets (*Acheta domesticus*) may represent a novel alternative source of dietary protein that may assist in meeting future global protein demands. However, the ability of insect-derived protein to stimulate postprandial hyperaminoacidemia and regulate appetite, satiety, and subsequent food intake compared to conventional animal-derived proteins has not been investigated.

Objectives: To examine the acute effects of consuming isocaloric, macronutrient, and volume-matched beverages containing either 25 g of cricket or beef-derived protein on postprandial plasma glucose, insulin, and amino acid concentrations, subjective appetite sensations, and *ad libitum* energy intake in healthy young men.

Methods: In a randomized, double-blind, within-subject crossover study, 20 young men (age: 23 ± 1 y; BMI: 23.0 ± 0.6 kg/m^2 [mean ± SEM]) consumed beverages containing 25 g protein derived from crickets or beef. Participants completed two separate 300-min experimental test days involving the ingestion of the respective protein beverage along with repeated blood sampling and questionnaires. Blood sampling and questionnaires were taken at baseline before beverage intake, and 15, 30, 45, 60, 90, 120, 150, 180, 240, and 300 min after beverage intake to assess plasma

glucose, insulin, and amino acid concentrations, and perceived appetite sensations. An *ad libitum* meal was provided at the end of each experimental visit to assess energy intake.

Results: Net incremental area under the curve (niAUC) over the entire 300-min postprandial period was greater for cricket compared to beef-derived protein for plasma leucine ($P = 0.001$), branched chain amino acid (BCAA: $P < 0.001$), and essential amino acid (EAA: $P < 0.001$) concentrations. Over the same time period, niAUC for plasma non-essential amino acid and total amino acids was greater (NEAA: $P < 0.001$; TAA: $P = 0.012$) for beef compared to cricket protein. Postprandial niAUC for perceived sensations of hunger was lower following beef compared to cricket protein ($P = 0.042$), but was not different between protein sources for fullness, desire to eat, or prospective food consumption (all $P > 0.05$). Participants consumed an average of 4466 ± 283 and 4153 ± 264 kJ during the *ad libitum* lunch meals 300-min following the ingestion of cricket and beef protein respectively, with no difference between protein sources ($P = 0.277$).

Conclusion: Postprandial plasma aminoacidemia differs following the ingestion of 25 g cricket vs. beef-derived protein beverages, with a greater niAUC for EAA following the ingestion of cricket protein. VAS-derived niAUC sensations of hunger were lower with beef compared to cricket protein; however, all other appetite sensations as well as *ad libitum* food energy intake were similar between protein sources. Cricket protein may represent a novel alternative source of dietary protein when developing higher-protein meals to support appetite regulation.

Résumé

Contexte: Les protéines alimentaires fournissent au corps une source d'acides aminés et jouent un rôle clé dans la régulation de l'appétit, la satiété et la prise alimentaire subséquent. Les sources de protéines alimentaires diffèrent par leur teneur en acides aminés et leur digestibilité et peuvent donc avoir des effets différents sur la régulation de l'appétit. Les protéines d'origine animale telles que le bœuf sont généralement considérées comme des sources de protéines de haute qualité; cependant, la production de quantités suffisantes de protéines conventionnelles d'origine animales pour répondre aux futures demandes alimentaires mondiales représente un défi. Les insectes comestibles tels que les grillons (*Acheta domestica*) peuvent représenter une nouvelle source alternative de protéines alimentaires qui pourrait aider à répondre aux futures demandes mondiales de protéines. Cependant, la capacité des protéines dérivées d'insectes à stimuler l'hyperaminoacidémie postprandiale et à réguler l'appétit, la satiété et la prise alimentaire subséquent par rapport aux protéines conventionnelles d'origine animale n'a pas été étudiée.

Objectifs: Examiner les effets aigus de la consommation de boissons isocaloriques, égal en volume et macronutriments, contenant 25 g de protéines dérivées du bœuf ou de grillon sur les concentrations plasmatiques de glucose, d'insuline et d'acides aminés postprandiales, les sensations d'appétit subjectif et apport énergétique *ad libitum* chez les jeunes hommes en bonne santé.

Méthodes: Dans une étude croisée, randomisée, intra-sujet, en double aveugle, 20 jeunes hommes (âge: 23 ± 1 an; IMC: 23,0 ± 0,6 kg/m^2 [moyennes ± SEM]) ont consommé des boissons contenant 25 g de protéines dérivées de grillon ou du bœuf. Les participants ont effectué deux jours d'essai expérimentaux distincts de 300 minutes impliquant l'ingestion de la boisson protéinée respective ainsi que des prélèvements sanguins et des questionnaires répétés. Des échantillons de sang et des

questionnaires ont été prélevés au départ avant la consommation de boissons, et 15, 30, 45, 60, 90, 120, 150, 180, 240 et 300 min après la consommation des boissons pour évaluer les concentrations plasmatiques de glucose, d'insuline et d'acides aminés et les sensations perçues de l'appétit. Un repas *ad libitum* a été fourni à la fin de chaque visite expérimentale pour évaluer l'apport énergétique.

Résultats: L'aire incrémentielle nette sous la courbe (niAUC) sur toute la période postprandiale de 300 minutes était plus élevée pour le grillon que pour les protéines dérivées du bœuf pour la leucine plasmatique (P = 0,001), les acides aminés ramifiés (BCAA: P < 0,001), et les concentrations d'acides aminés essentiels (EAA: P < 0,001). Au cours de la même période, le niAUC pour les acides aminés non-essentiels et acides aminés totaux plasmatiques étaient supérieurs (NEAA: P < 0,001; TAA: P = 0,012) pour le bœuf par rapport à la protéine de grillon. La niAUC postprandiale pour la sensation de faim était plus faible après le bœuf par rapport à la protéine de grillon (P = 0,042), mais n'était pas différente entre les sources de protéines pour la satiété, le désir de manger, ou la consommation future de nourriture (tous P > 0,05). Les participants ont consommé en moyenne 4466 ± 283 et 4153 ± 264 kJ pendant les dîners *ad libitum* 300 minutes après l'ingestion de protéines de cricket et de bœuf, respectivement, sans différence entre les sources de protéines (P = 0,277).

Conclusion: L'aminoacidémie plasmatique postprandiale diffère suite à l'ingestion de 25 g de grillon par rapport aux boissons protéinées dérivées du bœuf, avec un niAUC plus élevé pour l'EAA après l'ingestion de protéine de grillon. La niAUC pour la sensation de faim dérivée du VAS étaient plus faibles avec le bœuf que la protéine de grillon; cependant, toutes les autres sensations d'appétit ainsi que l'apport énergétique alimentaire *ad libitum* étaient similaires entre les sources de protéines. La protéine de grillon peut représenter une nouvelle source alternative de protéines

alimentaires lors du développement de repas riches en protéines pour soutenir la régulation de l'appétit.

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Contribution of Authors

Jiaying Dai (first author): conceived and designed the research, conducted the research, analyzed the data, interpreted the results of the experiments, prepared the figures, drafted the thesis, read and approved the final thesis, and holds primary responsibility for the final content along with the principle investigator (Dr. Tyler A. Churchward-Venne).

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Dr. Tyler A. Churchward-Venne (principle investigator): secured financial support for the research, conceived and designed the research, interpreted the results of the experiments, edited and revised the thesis, read and approved the final thesis, and holds primary responsibility for the final content.

Chapter 1. Introduction

Increased bodyweight and obesity are major health problems (1). Studies have shown that relatively high protein (1.2-1.6 g protein · kg^{-1} · d^{-1}), energy-restricted diets can lead to greater fat loss, better maintenance of lean body mass, and greater overall weight loss compared to normal protein diets (2). The effectiveness of higher protein diets in supporting weight loss may due to their effect on appetite regulation (3) including reduced feelings of hunger and increased satiety (2). Indeed, on an energy-matched basis, protein is more satiating than carbohydrate and fat (3). The ingestion of dietary protein elicits a rise in circulating levels of insulin and amino acids that contribute to appetite regulation and the control of food intake. As early as the 1950's, Mellinkoff and colleagues (4) observed that the extent of postprandial excursions in circulating amino acid concentrations influence satiety. The satiating effect of dietary proteins and amino acids may also be mediated by anorexigenic gut-derived hormones such as cholecystokinin (CCK), peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) (2) and possibly the orexigenic hormone ghrelin (5). Additionally, the amino acid leucine can modulate satiety and food intake via actions in the brain (6).

Acute feeding studies have demonstrated that a meal specific protein dose of ~25-30 g protein per meal can improve appetite regulation and satiety (i.e. perceived sensations of fullness and hunger) (7) and maximally stimulate skeletal muscle protein synthesis rates (8). Evidence also suggests that the source of protein may influence its satiating capacity (5, 9–12). Proteins differ in their amino composition and digestive characteristics, which interact to determine postprandial changes in amino acid concentrations after a meal. In a now classic study, Hall and colleagues (5) demonstrated that whey protein was more satiating when appetite sensations were recorded for 180 min, and, in accordance, whey decreased energy intake at an *ad libitum* lunch buffet served

90 min after preload consumption compared with casein protein. Therefore, sources of protein may differ in their satiating capacity and alter food intake at a subsequent meal.

In recent years there has been increased attention on the environmental impact of different foods, and a growing recognition that animal-derived products from livestock may contribute more to the production of greenhouse gases and CO_2 emissions than plant-derived products (13). There has also been growing interest in novel, alternative sources of dietary protein that are environmentally sustainable. Insects have received increased interest in recent years as an alternative, environmentally sustainable source of dietary protein (14). Insects represent a protein-dense food source (~40-60% protein) and may meet or exceed indispensable amino acid requirements for humans (14). However, the capacity of insect-derived protein to stimulate postprandial hyperaminoacidemia and regulate appetite, satiety, and subsequent food intake as compared to animal-derived protein has not been explored.

1.1 Purpose and Hypothesis

The purpose of this thesis was to evaluate postprandial plasma glucose, insulin, and amino acid concentrations, subjective appetite sensations (hunger, fullness, desire to eat, and prospective food consumption) and *ad libitum* energy intake following the ingestion of 25 g protein derived from crickets or beef in healthy young men.

It was hypothesized that hyperaminoacidaemia would be more rapid following the ingestion of beef- compared to cricket-derived protein, although total amino acid availability would be similar between protein sources over a 300-minute postprandial period. We further hypothesized there would be no difference between protein sources on postprandial subjective appetite sensations or subsequent *ad libitum* energy intake.

Chapter 2. Literature review

Increased bodyweight (i.e., overweight) and obesity are a major global health concern due to comorbidities such as cancer, type 2 diabetes, and cardiovascular disease (15). Over the last two decades, higher-protein diets have been promoted as a strategy to prevent or treat overweight and obesity via improvements in body composition and weight management (2, 16). Specifically, current evidence supports the consumption of increased amounts of dietary protein at 1.2-1.6 g protein $\cdot$ kg^{-1} $\cdot$ d^{-1} during energy restriction to facilitate fat loss, maintenance of lean mass, and overall weight loss compared to low-protein energy restricted diets (2). The effectiveness of higher-protein diets is thought to be due in part to modulation of appetite, and possibly energy expenditure, leading to reduced energy intake (2).

The ingestion and subsequent metabolism of protein in the body impacts the control of appetite, satiety, and food intake through various mechanisms, not all of which are completely understood. The work presented in this thesis focuses on the impact of different sources of dietary protein, and specifically, novel insect-derived protein, on postprandial glucose, insulin, and amino acid concentrations, subjective appetite sensations, and subsequent *ad libitum* food intake. In the present chapter, a brief overview of dietary protein and amino acids is presented, followed by a discussion of protein requirements and protein quality assessment. Insects as a source of dietary protein are then discussed, followed by an overview of appetite regulation and discussion of common research methods used to assess appetite. Finally, the role of dietary protein in appetite regulation is discussed with an emphasis on the role of protein dose and protein source, followed by an overview of the mechanisms thought to be involved in protein-mediated satiety.

2.1 Overview of protein and amino acids

Dietary protein is fundamental to human health and quality of life and plays an important role in appetite regulation, body composition management (e.g., weight loss), and muscle mass maintenance and/or growth. As a critical component of the human body, proteins are responsible for various chemical reactions. Amongst their numerous functions, proteins regulate gene expression, maintain the general structure and function of all cells, and maintain muscle function (17). Proteins are composed of amino acids which are defined as organic substances containing both amino and carboxy groups (18). There are more than 300 amino acids found in nature, however only 20 of these serve as building blocks of protein. Because of its relatively large mass (~40–45% of body weight), skeletal muscle is the largest reservoir of both protein-bound and free acids in the body. Much effort has been directed toward defining protein/amino acid requirements in humans under divergent developmental, nutritional, pathological, and environmental conditions. On the basis of dietary requirements for nitrogen balance and/or growth, amino acids were traditionally categorized under three groups: indispensable (essential) amino acids (EAA), dispensable (non-essential) amino acids (NEAA) and conditionally indispensable amino acids. The NEAAs are those which can be synthesized *de novo* in adequate amounts by the body to meet optimal requirements. The EAAs are defined as either those amino acids whose carbon skeletons cannot be synthesized or those that are inadequately synthesized *de novo* by the body relative to needs and which must be provided from the diet to meet optimal requirements. Conditionally essential amino acids are those that normally can be synthesized in adequate amounts, but which must be provided from the diet to meet optimal needs under conditions where rates of utilization are greater than rates of synthesis (19). Ingested dietary protein does not provide any nutritional value until it is hydrolyzed by enzymes and broken down into its constituent amino acids (20).

2.2 Dietary protein requirements

Daily consumption of an adequate amount of dietary protein is necessary in order to provide EAA in sufficient amounts to maintain optimal health and regulate body function (21). The Recommended Dietary Allowance (RDA) for protein for healthy adults aged 18-70 years of age is 0.80 g protein · kg^{-1} · d^{-1} (21). Dietary guidelines also include an acceptable macronutrient distribution range (AMDR), which for protein represents 10%-35% of an individual's total daily energy intake. Daily protein requirements and the RDA can be impacted by several factors including the physiological characteristics of the consumer, pathological states, and environmental factors (20). As an example, the RDA for pregnant women, lactating women, and 7-12 month old infants are 1.1, 1.3, 1.2 g protein · kg^{-1} · d^{-1}, respectively (21). There is also growing recognition that protein intakes greater than the RDA (i.e., 1.2-1.6 g protein · kg^{-1} · d^{-1}) may be necessary to achieve optimal health and/or performance outcomes in older adults, athletes, and those at risk for metabolic disease (22). However, recommendations to increase protein consumption to achieve optimal health outcomes are occurring in the face of mounting challenges associated with the production of sufficient amounts of animal-based protein to meet future global food demands.

2.3 Dietary protein quality

Dietary protein quality encompasses the EAA content of a protein and its capacity to meet amino acid requirements in humans, and the digestibility of the protein (23). In 1991, The Food and Agriculture Organization/World Health Organization (FAO/WHO) adopted the Protein Digestibility Corrected Amino Acid Score (PDCAAS) as the preferred method to evaluate protein quality in humans (24). However, in 2011 the FAO/WHO proposed the use of the Digestible Indispensable Amino Acid Score (DIAAS) to replace the PDCAAS as a new and superior approach to evaluate the quality of a protein source, as the latter method often overestimates

protein quality (25). Compared to the PDCAAS, the DIAAS looks at amino acids as individual nutrients and uses true ileal digestibility instead of faecal protein digestibility (24). The calculation for the DIAAS is as follows:

DIAAS % = 100 x [(mg of digestible dietary EAA in 1 g of the dietary protein) / (mg of the same dietary EAA in 1 g of the reference protein)] (24).

For example, the DIAAS of wheat protein is 40.2%, whereas the DIAAS for beef protein is 111.6% (26). Based on contemporary protein quality assessment methods such as the PDCAAS and DIAAS, animal-source proteins (e.g. meat, dairy, poultry) generally represent 'high-quality' sources of dietary protein when compared to plant-based proteins due to their higher EAA content and higher digestibility (27). In general, plant-source proteins are commonly low or absent in certain amino acids, especially EAA (28) including methionine and lysine (29). In a recent study conducted by Gorissen and colleagues (29), it was reported that plant-source proteins are typically 11± 2% lower in EAA compared to animal-source proteins. The EAA content of several plant-source proteins such as oat, lupin and hemp do not meet WHO/FAO/United Nation University (UNU) based guidelines for amino acid requirements. Consequently, to reach the RDA of methionine (10 mg · kg^{-1} · d^{-1}) and cysteine (4 mg · kg^{-1} · d^{-1}) for a 70 kg male, 285 g of wheat flour would need to be consumed compared to only 45 g of meat (20).

Although animal-based proteins generally represent 'high-quality' sources of dietary protein, there are concerns surrounding the environmental sustainability of livestock production and capacity to produce sufficient amounts of animal-based protein to meet future global food demands (30). Therefore, there is a need to identify alternative, sustainable sources of dietary protein that may assist in meeting global demands for dietary protein and help ensure global food security.

2.4 Edible insects as a sustainable source of dietary protein

The global human population is projected to reach ~9.6 billion by 2050 (31). With increases in the population and per capita income, the demand for animal-based, protein-rich food is expected to increase (28). For example, from the year 2013 to 2050, meat consumption per capita has been projected to increase from 40.0 kg to 51.5 kg (28). Accordingly, meat production will need to reach ~494 million tons by 2050, an increase of 206 million tons from 2013 (28). The global demand for other animal-based proteins is also projected to increase, with dairy and egg production expected to reach 1043 and 102 million tons globally by 2050 (32). The inability to meet the projected increase in the demand for protein-rich foods may exacerbate the chronic inadequate protein intake and protein energy malnutrition that currently affect ~1 billion people globally (28).

Edible insects are a trending proposition as a novel alternative source of dietary protein that may assist in meeting the current and projected demand for dietary protein, and at the same time aid in addressing some of the environmental concerns associated with livestock production (14, 33). Although entomophagy, or consumption of insects, is a new concept North American and European countries, it is more common in parts of Africa, Asia, and South America where an estimated ~2 billion people worldwide habitually consume insects as part of their traditional diet (34, 35). Currently, over 2000 species of insects have been reported to be used as food by humans (36), including beetles (*Coleoptera*), caterpillars (*Lepidoptera*), bees, wasps, and ants (*Hymenoptera*), and locusts, grasshoppers, and crickets (*Orthoptera*) (33). Recently there has been increasing interest, particularly in North America and Europe, in the mass production of insects as an environmentally and economically viable protein-dense food source for humans. As a result, edible insects and insect-based protein-rich food products including insect-derived protein

powders, protein bars, cooking flours, pastas, and burgers are becoming increasingly available on the market for consumers (14).

As a food source, insects may possess many environmental, economic, and agricultural advantages when compared with conventional livestock. These advantages have been reviewed by Churchward-Venne and colleagues (14) but include 1) a higher efficiency of converting ingested feed into body mass (i.e., feed conversion efficiency); 2) a higher percent edible weight (~80% for crickets) than conventional livestock (~40% for cattle); 3) a lower contribution to greenhouse gas emissions and ammonia than cattle; 4) a reduced requirement for land and water compared with cattle; 5) and a higher capacity to produce offspring (crickets lay ~1500 eggs over a 1-month period). In corroboration of these advantages, life cycle assessments performed on different species of edible insects indicate that their mass production may offer an environmentally sustainable alternative source of dietary protein (37–39)

2.5 Edible insects as an alternative source of high-quality dietary protein?

Edible insects possess a nutritional profile high in protein, minerals, vitamins, and energy (40). However, the nutritional value of edible insects varies among species (41), and can also vary within the same species depending on factors such as developmental stage, habitat, diet, and processing prior to ingestion (35). Churchward-Venne and colleagues (14) recently carried out a review on the protein content, amino-acid composition, and digestibility of proteins from edible insects. Overall, the average protein content ranged from ~40% for insects belonging to the order *Isoptera* (termites) and *Coleoptera* (beetles) to approximately ~60% dry mater material in insects from the order *Blattodea* (cockroach) and *Orthoptera* (crickets, grasshoppers, locusts) (14). Compared to many staple plant-based proteins, insect protein may contain a more complete amino acid profile (35). Insects in the *Orthoptera* order on average satisfy or surpass the daily EAA

requirements for adults and are comparable to traditional high-quality protein sources like beef, eggs, and milk (14). For example, insects from the order *Orthoptera* possess on average a leucine content of 75.6 (range, 42.5–100) mg/g protein, a content greater than that reported for soy protein isolate (62 mg/g protein) (42) and comparable to that reported for skim milk powder (77 mg/g protein) (42).

Although many species of edible insects represent a protein-dense food source that meet or exceed amino acid requirements in humans, the digestibility of a protein is an important factor when considering protein quality (24) because it influences the postprandial availability of protein-derived amino acids. A number of studies have determined the digestibility of insect-derived proteins using multienzyme *in-vitro* systems (for review see (14)). Ramos-Elorduy and colleagues (43) determined the protein digestibility of several species of edible insects and reported that it ranged from ~77%–98%. One factor that may reduce the digestibility of insect-derived protein is the presence of chitin, a nitrogen containing polysaccharide present in the insect exoskeleton (14). It has been previously shown in rat models that the removal of chitin before ingestion results in higher true protein digestibility as well as amino acid availability (44). Therefore, opting for an edible insect with lower chitin levels or processed insect protein products (e.g., protein isolates) where chitin has been removed may yield better nutritional value with regards to protein (14).

Information on the quality of insect-derived proteins using contemporary approaches such as the DIAAS are currently unavailable; however, some studies have compared different species of edible insects using the PDCAAS (45). Amongst the insects that were investigated, crickets (*Gryllus assimilis)* were found to have the highest PDCAAS (0.73) along with the highest protein efficiency ratio (45). This PDCAAS is superior to that of plant-derived proteins such as wheat, oats, and pea (PDCAAS: 0.45, 0.57, and 0.67, respectively) but inferior to animal proteins such as

milk, eggs, and beef (PDCAAS: 1.0, 1.0, and 0.92, respectively) (46). Although insects may represent a protein-dense food source, with certain species able to meet or exceed EAA requirements in humans, studies are required in order to evaluate the DIAAS of various insect-derived proteins in humans. Furthermore, there is a need to evaluate various functional outcomes known to be regulated by dietary protein in response the ingestion of insect-derived protein. For example, protein ingestion modulates appetitive signaling and can lead to reduced energy intake (47). However, proteins may differ in their effects on appetite depending on the type or source of ingested protein, and no studies to date have evaluated the capacity of insect-derived protein to regulate appetite, satiety, and/or food intake in humans.

2.6 Overview of appetite regulation

Appetite can be defined as 'the internal driving force for the search, choice, and ingestion of food' (48), but is also used as a general term to describe overall sensations related to food intake (49). Satiation and satiety are part of the body's appetite control system and play a key role in controlling energy intake (50). The ability to control food/energy intake and balance it with energy expenditure is vital to control bodyweight, which is particularly relevant given the rising prevalence of obesity. Satiation is the process that causes a person to stop eating whereas satiety is the sensation or feeling of fullness that persists after food intake, supressing further intake until one feels the sensation of hunger (50). Hunger can be described as a conscious sensation that may be irritating or unpleasant; it reflects the urge to eat and signals that the next eating episode should take place (51, 52).

Blundell and colleagues (53) proposed a 'Satiety Cascade' characterizing the factors affecting satiation and satiety over time following food intake and includes sensory, cognitive, post-ingestive, and post-absorptive stages (53) (**Figure 1**). In the Satiety Cascade, satiation and

satiety are initially influenced by sensory and cognitive factors related to food intake (e.g. smell, texture, taste, and/or associations with previous experiences). Subsequently, post-ingestive factors come into play once the ingested food reaches the stomach. Food causes stomach distension, sending signals to the brain that initiate satiety. As the digestive process proceeds, gut-derived appetite regulatory hormones that promote satiation and satiety are released. In the late post-absorptive stage, nutrients are detected by specific receptors located throughout the body, including the brain, that provide information about nutrient status that also influences satiety (53)

Overall, the body has a complex array of signals and networks that promote satiation and satiety following food intake. In daily life, food intake (including the amount and type of food to be eaten) is affected by internal appetite signals such as satiation and satiety, but also other factors including but not limited to palatability, texture, portion size, motivational state, and time of day (50). For example, external factors such as the presence of other people during a meal and distractions such as television viewing are factors that can influence satiation and satiety (50). Overall, meals consumed during high cognitive load conditions have been shown to lead to smaller reductions in desire to eat and fullness compared to conditions where meals are consumed in silence (54). With regards to palatability, an increase in satiety has actually been shown to follow the lesser palatable meal (50). Therefore, the study of appetite including satiation and satiety encompasses both physiological and behavioural components. For a detailed overview of the factors that influence satiety and eating behaviour, the interested reader is referred to the following reviews (48, 50). Below, common methods to assess appetite in human research studies are discussed.

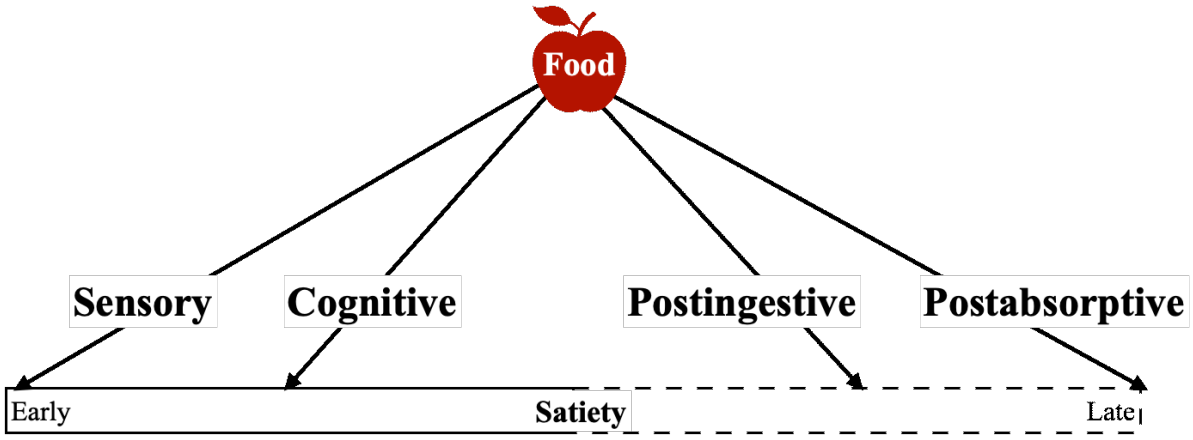

Figure 1*. The Satiety Cascade as proposed by Blundell and colleagues. Adapted from Blundell et al.,(53).*

2.7 Methods to assess appetite in humans

Appetite in humans can be measured in two ways: 1) using rating scales of subjective appetite sensations, and 2) measuring actual food intake. A common study design when assessing appetite over a short (i.e., hours) time-frame is the use of a test 'preload' in which variables of interest (e.g., the amount or source of protein in a meal) are tightly controlled (50). Before, and at selected time-intervals after ingestion of the preload, research participants subjectively rate various sensations/feelings associated with appetite. Subsequently, a test meal is provided to measure food/energy intake (50). Visual analogue scale (VAS) questionnaire's and an *ad libitum* test meal to assess appetite were applied within the current thesis and are discussed below.

2.7.1 Visual analogue scales (VAS) and appetite research

VAS questionnaires are a common method used capture perceived somatic (i.e., bodily) sensations such as hunger, fullness, satiety, desire to eat, and prospective food consumption (55). A typical VAS is 100 mm in length with words located at either end of the scale that express the most positive and most negative rating in response to a specific question. For example, the question

"How hungry do you feel?" is anchored by responses that range from "Not hungry at all" to "As hungry as I have ever felt" (55). In practice, VAS questionnaires to assess components of appetite are completed before a given test meal, and at various time-points (e.g., every 30 min) following the ingestion of a test meal for ~3-6 hours into the postprandial period (50). At each time-point, participants make a mark on the line to indicate how they feel at that moment in response to a given question, and this is quantified by measuring the distance from the left end of the line to the mark using a ruler. As reviewed by Stubbs and colleagues (56), benefits of VAS questionnaires in appetite research are that 1) they are easy and quick to use, 2) easy to interpret, 3) do not require research participants to invoke their own descriptive terms, 4) allow considerable discrimination, and 5) are presented in a standardized format that can be compared under different experimental conditions. Flint and colleagues (57) determined the reproductivity, power, and validity of VAS questionnaires in the assessment of appetite sensations following a single test-meal and concluded that VAS scores are reliable for appetite research. However, as there may be inter-individual differences in the way VAS questionnaires to assess appetite are interpreted, a 'within subject' study design is preferred over an 'independent groups' design when implementing VAS questionnaires (57).

2.7.2 Ad libitum test meals and appetite research

In addition to VAS questionnaires, appetite can be assessed by measuring actual food intake. This is typically performed in a laboratory environment under standardized conditions and consists of an *ad libitum* meal that participants are instructed to consume until they are comfortably full. The *ad libitum* meal often contains different food items varying in macronutrient composition provided in excess of what individuals would be expected to eat (50). In a research setting, the *ad libitum* meal is typically provided several hours following the ingestion of a test-meal (e.g., that

varies in macronutrient composition, physical state, volume and/or energy density depending on the research question). The meal is weighed before and after consumption in order to determine energy intake and ultimately obtain a measure of appetite in response to a test meal. A benefit of the *ad libitum* meal is that food intake is directly observed and measured, not derived from dietary intake records in which subjects record their own food intake. A number of studies have found that the *ad libitum* single test meal used to measure spontaneous energy intake is reproducible (57–59).

2.8 Dietary protein and appetite regulation

The consumption of increased quantities of dietary protein (i.e., 1.2-1.6 $g \cdot kg^{-1} \cdot d^{-1}$) represents an effective strategy to support body composition management through reductions in fat mass concomitant with a preservation of lean mass (2, 60). The effectiveness of higher protein diets is thought to be due in part to appetitive signaling leading to a reduction in *ad libitum* energy intake (2, 60). For example, on a kJ per kJ basis, dietary protein is more satiating than dietary carbohydrate or fat (3, 61, 62). The increased satiety that occurs in response to protein ingestion is observed in a single meal (63) and over an entire day (64). However, factors such as the amount and type (i.e., source) of ingested protein may influence protein-mediated appetite responses (3).

2.8.1 Dietary protein source and appetite regulation

When evaluating consumer perceptions about satiating foods, research has shown that when presented with a series of pictures depicting common protein-rich foods, red meat was one of the items most associated with the term ‘satiation’ (65). This demonstrates that red meats such as beef are considered one of the most satiating protein sources based solely on consumer perceptions (65). At the same time, fish was given the lowest expected satiety score amongst the protein foods available (65). However, existing studies have come to various conclusions on how dietary protein source may impact appetite regulation. In an early study comparing the satiating

effects of ingested beef, chicken, and fish protein over 180 min, Uhe and colleagues (66) reported that VAS-derived measures of satiety were lower with beef and chicken compared to fish protein; however, energy intake was not assessed. Lang and colleagues (67) reported similar effects of meals enriched with egg albumin, gelatin, casein, soy, pea, and wheat gluten on appetite scores and energy intake; however, doses used in this study were very high (70 g) which may have limited the capacity to detect protein source-dependent differences. When comparing animal to plant protein, Kristensen and colleagues found that a high protein legume meal induced greater fullness and lower hunger compared to a high protein meat-based meal and a low protein legume meal (12). The *ad libitum* meal intake was also lower in the high protein legume meal group (12). Noticeably, with the same protein content, the high protein legume group had 19 more grams of fiber compared to the high protein meat group and 15 more grams compared to the low protein legume group (12). Thus, the high fiber content in legumes may have played an important role in inducing a greater satiety response when compared to animal meat (12). When comparing milk-derived proteins, Hall and colleagues reported that 48 g of ingested whey protein resulted in significantly less *ad libitum* meal intake compared to same amount of casein protein (5). It was also found that the desire to eat was significantly less while fullness was higher compared to the casein preload (5). The plasma CCK and GLP-1 concentration were also reported to be 60%-65% higher in the whey group (5). Similar results have been shown by Veldhorst et al., as they found that whey reduced subjective hunger to a greater extent compared to casein and soy protein but with no difference in *ad libitum* meal intake (68). Overall, while some studies have found differences between sources of dietary protein on appetite sensations and/or subsequent energy intake, many have not. Differences between studies may relate to differences in the amount of protein provided, subject population studied, co-ingestion of protein with other macronutrients, and/or timing of *ad libitum* meal intake.

Because of the variability between study design/utilized methodology, it is difficult to make accurate global statements regarding the satiating effect of different sources of dietary protein.

2.8.2 Dietary protein dose and appetite regulation

When comparing differing amounts of protein from the same source, results seem to favor higher doses of protein in order to increase satiety (2, 68–70). VAS-derived ratings for fullness and satiety were increased when casein protein was provided at 25% of the total energy (En%) of a breakfast meal compared to 10 En% (68). Moreover, the 25 En% condition resulted in higher levels of plasma branch chain amino acids (BCAA) and total amino acids (TAA) during the postprandial period (68). When comparing the same percentages of total energy but with soy protein, the 25 En% condition yielded higher VAS ratings for satiety compared to the 10 En% condition (70). The AUC postprandial insulin response was also increased in the 25 En% condition (70). With whey protein, the 25 En% condition saw greater increases in insulin and GLP-1 in addition to a reduction in ghrelin concentrations compared to the 10 En% condition (69). Despite these hormonal differences, the results could not be supported by increased VAS ratings (69). Overall, amongst these three different sources (casein, soy and whey), results seem to favor the higher protein condition despite no differences being seen in any of the *ad libitum* energy intake measurements (69). These results generally align with the findings of a recent summary of 24 acute feeding trials comparing lower- with high-protein meals by Leidy and colleagues (2). Of the 24 included studies, the majority (55%) showed greater increases in postprandial VAS-derived fullness with the high- vs. lower-protein meals. Less than half of the studies (35%) reported greater reductions in postprandial hunger with the high- vs. lower-protein meals. In addition, some but not all of the included studies reported lower postprandial ghrelin and/or greater increases in GLP-1 or PYY with higher- vs. lower-protein meals (2). Overall, it appears that higher amounts of

ingested protein result in greater increases in fullness than comparatively lower doses; however, their effects on hunger and appetite regulatory hormone concentrations are less clear. Finally, an important point highlighted by Leidy and colleagues (2) is that higher-protein meals have been shown to either improve appetite control compared to lower-protein meals or show no difference. There is currently no evidence that higher-protein meals lead to a weakening in appetite control or lead to increased energy intake during a subsequent *ad libitum* meal when compared to a lower-protein meal (2).

2.9 Mechanisms of protein-mediated satiety

The mechanisms that contribute to protein-mediated satiety are not completely understood but are thought to relate to increases in: 1) concentrations of select circulating satiety hormones, 2) energy expenditure, 3) the process of gluconeogenesis, and 4) postprandial amino acid concentrations (47).

2.9.1 Dietary protein and appetite regulatory hormones

The ingestion of food, including protein, leads to an increase in a number of circulating hormones including GLP-1, CCK, and PYY that have been shown to induce feelings of satiety via direct or indirect actions in specific areas of the brain (47). Because they reduce appetite, these hormones are known as 'anorexigenic' hormones. Alternatively, ghrelin is an 'orexigenic' gut hormone that acts to cause hunger (71). Collectively, these hormones serve as episodic signals of satiety since changes in their concentration coincide with episodes of food intake (72).

GLP-1 is secreted by the mucosal endocrine L cells of the intestine where its release into the circulation is driven by the presence of nutrients in the gut lumen (73). It has also been shown that GLP-1 release can be stimulated during the cephalic stage of digestion where food has not yet entered the stomach (74). GLP-1 can change appetite levels by altering gastrointestinal (GI)

function via a reduction in intestinal motility and a delay in gastric emptying (75). In doing so, the process of digestion is slowed down which prolongs the feeling of satiety. GLP-1 can also impact satiety by exerting effects on the central nervous system (CNS) which itself regulates appetite and satiety (76). This is achieved in several ways such as directly by crossing the blood-brain barrier, or indirectly through neural afferents (76). GLP-1 producing neurons found in the nucleus of the solitary tract of the brainstem can project to areas of the brain that have been shown to influence the control of food intake, namely the ventral tegmental area, the nucleus accumbens, and the hypothalamus (77). This is substantiated by studies that have observed increased activity in brain areas involved in the regulation of feeding as a result of peripherally administered GLP-1 (78, 79). As a result of GLP-1 activity in the brain, efferent signals can be sent down to peripheral organs to downregulate the food intake loop. This is partially linked with GLP-1's function as an incretin, lowering blood glucose levels by promoting the secretion and production of pancreatic insulin. Although, various studies have found links between GLP-1 and satiety, the latter is not known to be its primary function. Instead, postprandial GLP-1 concentrations are primarily modulated as a result of the nutrients consumed. One study found no change in GLP-1 response following the consumption of two meals with differing amounts of protein despite the high protein meal inducing a greater satiety response (80). Nevertheless, the body's release of GLP-1 following food intake has been shown to reduce meal size in addition to increasing the time to the next meal (81). Therefore, GLP-1 is impactful on both satiation (i.e., the point during a meal when one stops eating) and satiety (i.e., the feeling of fullness that lasts until the next meal).

The actions of the hormone CCK are quite similar to that of GLP-1. Both hormones have the ability to influence satiety via actions in the brain and the GI tract. CCK is released by enteroendocrine cells following the entrance of food into the duodenum, the proximal section of

the small intestine where the majority of CCK-producing cells are located (82). Specifically, the secretion of CCK is stimulated by the ingestion of fats, proteins, and amino acids, with only brief and transient increases following the ingestion of carbohydrates (83). Within the GI tract, CCK has various physiological functions which contribute to the feeling of satiety. Within the gallbladder, CCK stimulates its contraction while also relaxing the sphincter of Oddi (hepatopancreatic sphincter) in order to promote the release of bile into the intestine. Within the pancreas, the release of exocrine pancreatic secretions is stimulated by CCK, which represents one of the most important stimulants of this process (84). Similarly to GLP-1, CCK has also been shown to delay the rate of gastric emptying (85). Within the CNS, the activation of CCK receptors on vagal afferent nerves results in the provision of negative feedback to areas of the brain that control food intake (82). The role of CCK in promoting satiety is supported by numerous studies that have observed reduced food intake, meal size, and frequency as a result of the administration of exogenous CCK (86, 87).

PYY is another circulating hormone released by enteroendocrine cells in the distal part of the GI tract. Similar to the postprandial concentrations of GLP-1, the release of PYY from the gut occurs in a nutrient-dependent manner (88). PYY also delays gastric emptying which has been shown in studies that have used peripheral administration of PYY (89). Overall, an increase in circulating PYY has been shown to increase satiety, reduce gastrointestinal motility, and decrease food intake (90). The stimulation of PYY release is particularly high immediately following meals with high fat content while protein content has been found to influence prolonged increases in PYY concentrations (91). There seems to be a consensus that the ingestion of fat or protein can induce the highest PYY response while carbohydrates have the lowest impact (90).

The ingestion of protein has also been associated with reductions in the concentration of the orexigenic hormone ghrelin, which acts to supress the hunger response (47). Ghrelin is secreted by enteroendocrine cells in the stomach as well as by neurons in certain areas of the brain. Its secretion is largely dependent on the nutritional state of the individual. Increases in circulating levels of ghrelin are seen most before the intake of a meal, with a subsequent decrease during the postprandial period (71). Ghrelin has also been shown to have diurnal properties in that there is an observable decrease after midnight due to the inhibitory effect of sleep in addition to a gradual rise in levels upon awakening (92). Because of this, the timing of meals would be an important factor to consider in research studies investigating levels of ghrelin following food intake. Previous studies have shown that the ingestion of carbohydrates and protein reduce ghrelin secretion levels more than fat (93, 94). In addition, circulating amino acids can influence the reduction in ghrelin secretion (95).

2.9.2 Dietary protein and energy expenditure

The ingestion of relatively high protein meals or diets increases energy expenditure through an increase in postprandial thermogenesis (i.e., diet-induced thermogenesis) and basal metabolic rate (96). Diet-induced energy expenditure is related to the stimulation of energy-requiring (i.e., ATP requiring) processes during the postprandial period including metabolism, storage, and/or oxidation of nutrients (96). Previous studies have shown that the satiating effect of different macronutrients can partially be attributed to their effect on metabolic rate after consumption (i.e., postprandial thermogenesis) (97). Dietary protein requires ~20%-30% of its usable energy be directed towards metabolism and/or storage, whereas dietary carbohydrates require ~5%-10%, and dietary fats require only 0-3% (98). The theoretical basis underpinning the relationship behind protein-induced satiety and energy expenditure is that increased resting energy expenditure that

occurs following protein ingestion indicates increases in oxygen consumption and body temperature and thus increases in satiety (99). The heat produced during post-absorptive metabolic processes may activate temperature receptors in the brain that discourage feeding (99). The link between oxygen deprivation and satiety ratings has been shown before (96). It has been suggested that the perception of satiety may be the result of limited oxygen availability due to increases in metabolic rate (96). Indeed, correlational relationships have been found between satiety and diet-induced thermogenesis following the consumption of meals with different macronutrient compositions (96). Protein and carbohydrate-rich diets resulted in relatively higher diet-induced thermogenesis compared to fat-rich diets (62, 97, 100).

Differences in protein source can impact the resulting postprandial thermogenic effect after protein intake (47). Rapidly digested proteins induce greater increases in protein synthesis and amino acid oxidation during the postprandial period (101), hence a greater increase in ATP consumption and higher diet-induced thermogenesis (101). The amino acid profile of a protein is another factor that influences the resulting thermogenesis as there is variability that exists in terms of how they are oxidised (47, 102). Therefore, more attention should be given to the amino acid composition of a protein source when looking to induce satiety through the ingestion of dietary protein.

2.9.3 Dietary protein, gluconeogenesis and satiety

Although mostly studied in animal models, the process of gluconeogenesis may be another factor involved in protein-mediated satiety (47). Gluconeogenesis refers to the process of glucose synthesis from non-carbohydrate sources including amino acids. In rodents placed on a higher-protein diet, the expression of gluconeogenic enzymes in the liver are upregulated to support increased gluconeogenesis (103). Gluconeogenesis prevents a decline in blood glucose

concentration, and modulation of glucose homeostasis and signaling to the brain may contribute to the satiating effect of protein (104, 105). In addition, gluconeogenesis is an energetically expensive process; the removal of nitrogen from amino acids and conversion of the carbon skeleton to glucose results in ~20% of the energy content of glucose being expended to produce it via gluconeogenesis (106). Therefore, the increase in energy expenditure observed with higher protein meals and/or diets may partly be explained by protein-mediated gluconeogenesis and its associated energy costs (107).

2.9.4 The role of amino acids in appetite regulation

It has been suggested that reduced food intake may be the result of elevated plasma amino acid concentrations following a protein-containing meal (104). This is based on *Mellinkoff's Foundational Amino Static Theory* which stems from the observation that a larger increase in plasma amino acid concentrations coincides with increased satiety (4). In 1956, Mellinkoff and colleagues (4) suggested that an increase in the concentration of circulating amino acids which are not used for protein synthesis serve as a satiety signal for a food intake regulating mechanism in the brain, and thereby results in reduced food intake. In support of this theory, it was previously shown that satiety and fullness ratings were improved following a breakfast that contained a higher concentration of casein compared to an isoenergetic meal containing a lower concentration of the same protein (68). The difference was attributed to the prolonged elevation in plasma amino acid concentrations with the consumption of the higher vs. lower casein breakfast (68). Alternatively, the digestibility of a protein source plays a large role in its ability to cause a rise in plasma amino acid concentrations. For example, digestion and absorption of casein protein progresses at a much slower rate when compared to whey protein, resulting in a relatively reduced postprandial rise in plasma amino acid concentrations (5). In alignment with Mellinkoff's work (4), greater subjective

satiety ratings and reduced *ad libitum* meal intake were reported following whey vs. casein protein and this was attributed to differences in postprandial hyperaminoacidemia (5).

Energy expenditure during the process of ATP synthesis from amino acids also varies between each individual amino acid, ranging from 99.2 kJ/ATP for glutamate to 153.2 kJ/ATP for cysteine (102). Thus, the amino acid composition of a protein source may potentially dictate the degree of thermogenesis and the resulting feeling of satiety. In support of this notion, Acheson and colleagues (108) found that whey protein increased diet-induced thermogenesis to a greater extent than casein, while Karst et al. (109) demonstrated a higher diet-induced thermogenesis after casein compared to egg and gelatin protein. Individual amino acids have also been found to influence satiety when present in high concentrations. With regards to food intake, the BCAA leucine has been investigated on multiple occasions because it resists first-pass splanchnic metabolism (110) and is one of the fastest to cross the blood brain barrier (100). Enhancing low-protein foods with leucine has been found to elicit greater feelings of fullness as well as decrease prospective food consumption (111). Many of the regions in the brain that contain cells that are capable of sensing leucine are also responsible for the modulation of feeding behaviour (112). As such, most studies have utilized intracerebroventricular injection of leucine as the method of delivery and have found reductions in food intake as a result (6). In rodent models, an increase in the hypothalamic availability of leucine has been shown to reduce food intake via the activation of mTOR signalling (113). Past studies have utilized different methods of leucine administration making it difficult to ascertain whether the results can be replicated through traditional oral ingestion. Nevertheless, leucine is one of the only EAA that has been shown to have an anorectic response (6). Other specific amino acids implicated in protein-mediated satiety include taurine (70) and tryptophan (114). Overall, the postprandial increase in circulating amino acids following protein intake serves

as an important signal for protein-mediated satiety. Given that protein sources differ in their respective amino acid content and digestibility, the capacity of different proteins to induce hyperaminoacidemia and regulate satiety warrants further research.

2.10 Postprandial hyperaminoacidemia following insect-derived protein

There is surprisingly limited information on the capacity of various non-animal derived protein sources to elicit a postprandial rise in circulating amino acid concentrations. To date, only a single study has evaluated the impact of insect-derived protein on changes in postprandial circulating amino acid concentrations in humans (115). Vangsoe and colleagues (115) compared changes in postprandial amino acid concentrations following the ingestion of 25 g protein from whey, soy, and insect protein (lesser mealworm; *Alphitobius diaperinus*) in 6 young men. All proteins increased plasma concentrations of EAA, BCAA, and leucine over a 120 min postprandial period. Insect protein induced a postprandial increase in amino acid concentrations similar to soy protein (based on AUC); however, the response of insect and soy protein was reduced compared to whey. However, appetite sensations and *ad libitum* food intake were not assessed in this study. Furthermore, a limitation of this study was that the postprandial period was evaluated for only 120 min. Whereas plasma amino acid concentrations (EAA, BCAA, and leucine) following the ingestion of whey and soy protein peaked at 60 min after intake, peak amino acid concentrations following insect protein occurred at 120 min (115). This suggests that insect protein may be a more slowly digested protein source compared to whey and soy. Therefore, a longer period (> 120 min) of evaluation is necessary to fully capture changes in postprandial amino acid concentrations following the ingestion of insect-derived protein and characterize its bioavailability as a protein source in humans. With a longer testing period, insect-derived protein may demonstrate a

comparable total amino acid availability despite showing a delayed temporal response with respect to peak concentrations (115).

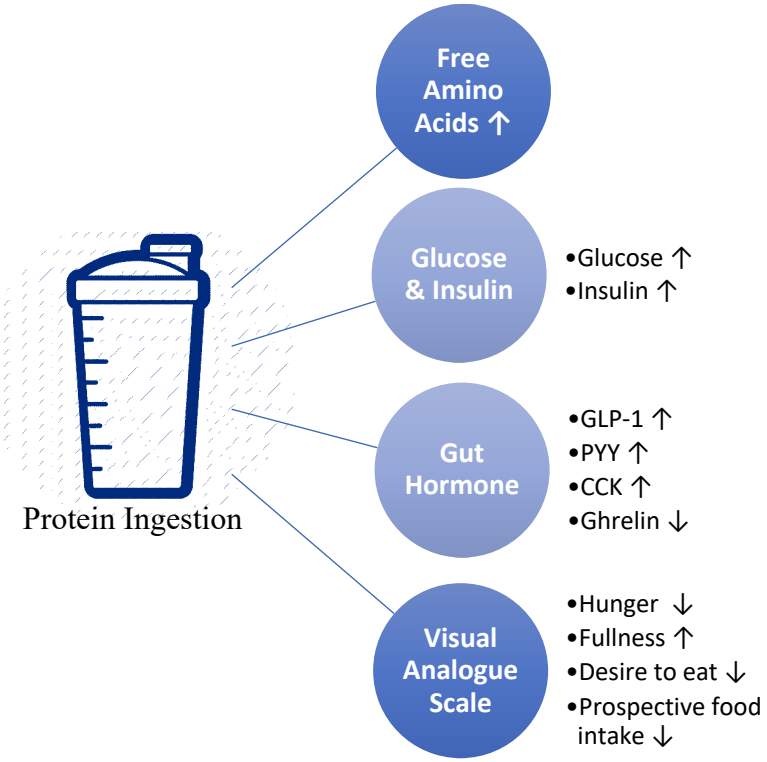

Figure 2. *Postprandial response following protein ingestion. Following the ingestion of protein there is an increase in amino acid concentrations in the in the blood also concurrent with the rise in glucose and insulin. The ingestion of amino acids can trigger the release of gut hormones such as GLP-1, PYY, and CCK to increase whereas ghrelin will typically decrease. Hunger hormones are thought to then impact subjective appetite sensations including hunger, fullness, desire to eat and prospective food intake.*

Chapter 3. MANUSCRIPT

The effects of cricket vs. beef-derived protein on postprandial plasma amino acid concentrations, subjective appetite sensations, and ad libitum energy intake in young men

3.1 Introduction

Dietary protein plays an important role in body composition/weight management. Relatively high protein, energy-restricted diets containing 1.2-1.6 g protein $\cdot$ kg^{-1} $\cdot$ d^{-1}, lead to greater fat loss, better maintenance of lean body mass, and greater overall weight loss compared to normal protein diets (2). The effectiveness of higher protein diets to support high quality weight loss may be due in part to their effect on appetite (3). Dietary protein increases feelings of fullness, which are accompanied by postprandial increases in the satiety hormones PYY and GLP-1 (2). There is also evidence of reduced sensations of hunger and a decline in the hunger hormone ghrelin in response to the ingestion of higher vs. lower protein meals (68). The ingestion of dietary protein also stimulates increased rates of whole-body and skeletal muscle protein synthesis leading to a positive net protein balance (116), which may contribute to the maintenance of lean body mass during energy restriction. Acute studies have demonstrated that meal specific quantities of ~25-30 g protein/meal can improve appetite regulation and satiety (i.e. perceived sensations of fullness and hunger) (7), and maximally stimulate skeletal muscle protein synthesis rates (8).

In addition to protein quantity, the source or type of ingested protein (e.g. animal vs. plant-derived proteins) may influence subsequent effects on appetite regulation and satiety. For example, Hall and colleagues (5) reported that whey protein was more satiating than casein. However, Lang and colleagues (67) reported similar effects of egg albumin, gelatin, casein, soy, pea, and wheat gluten on appetite scores and energy intake. Therefore, the effect of different sources of dietary protein on appetite regulation and satiety is unclear.

Conventional animal-derived proteins (e.g. beef, pork, lamb, poultry, eggs, and dairy) are generally considered high-quality sources of dietary protein because they meet all of the indispensable amino-acid requirements for humans and are highly digestible. However, the

production of sufficient amounts of conventional animal-based protein to meet future global food demands represents a challenge (14). Crickets (*Acheta domesticus*) are edible insects that may represent an alternative source of dietary protein for human consumption. Compared with more conventional sources of high-quality animal-derived proteins, insects require less land and have a lower environmental impact (117). From a nutritional standpoint, the average protein content of edible insects from the order *Orthoptera* (of which crickets are a member) is relatively high (~60% dry matter) and may exceed EAA requirements for humans (14). However, the capacity of insect-derived protein from crickets to stimulate postprandial hyperaminoacidemia and regulate appetite and satiety as compared to a more conventional animal-derived protein has not been explored.

The purpose of this study was to compare postprandial plasma glucose, insulin, and amino acid concentrations, subjective appetite sensations (hunger, fullness, desire to eat, and prospective food consumption) and *ad libitum* energy intake following the ingestion of 25 g protein from crickets or beef in healthy young men. We hypothesized that hyperaminoacidaemia would be more rapid following the ingestion of beef compared to cricket protein, although total amino acid availability would be similar between protein sources over a 300-minute postprandial period. We further hypothesized there would be no difference between protein sources on postprandial subjective appetite sensations or subsequent *ad libitum* energy intake.

3.2 Subjects & Methods

3.2.1 Participants

Twenty healthy recreationally active young men (mean ± SEM: age 23 ± 1 y, weight 72.3 ± 2.1 kg, body mass index 23.1 ±0.6 $kg \cdot m^{-2}$) volunteered to participate in this randomized, double-blind, crossover study. Participants were excluded if they met any of the following criteria: identified metabolic or intestinal disorders, use of tobacco products, adhere to a strict vegetarian or vegan diet, use of certain medications (i.e. corticosteroids, non-steroidal anti-inflammatories, or prescription strength acne medications), and allergies to shellfish or crustaceans. Participants' characteristics are presented in **Table 2**. All participants were informed about the purpose of the study, the experimental procedures, and possible risks prior to providing informed written consent to participate. The study was conducted in accordance with the ethical standards of the Faculty of Medicine Institutional Review Board at McGill University on human experimentation and in accordance with the Helsinki Declaration of 1975 as revised in October 2013.

3.2.2 Research ethics approval

The study was approved by the Faculty of Medicine Institutional Review Board at McGill University on October 31, 2018 (IRB Study Number: A08-M28-18B). All participants provided written informed consent prior to study participation.

3.2.3 Preliminary testing

Participants 18-35 years of age with a BMI > 18.5 and < 30.0 underwent an initial screening visit to assess height, weight, blood pressure, and body composition (by dual-energy X-ray absorptiometry; GE Healthcare, Madison, WI, USA). Participants were deemed healthy based on their responses to a medical questionnaire and screening results. Questions regarding physical activity and exercise preferences were also asked in order to determine daily habitual activity status.

Participants with an exercise frequency between 3-5 times per week were considered recreationally active and were included in the study.

3.2.4 Diet and physical activity

Study participants were asked to refrain from strenuous physical activity and alcohol consumption for 2 days immediately prior to each experimental trial. In addition, all participants filled out food intake and physical activity logs for 2 days immediately prior to each experimental trial. Each participant was provided with a dietary log, instructed to maintain their regular diet, and record their dietary intakes for 2 d before the first experimental trial. On completion of the first trial, a copy of the dietary log was returned to participants who were instructed to maintain their previously logged dietary habits in the 2 days immediately preceding the second experimental trial. Dietary intake prior to the experimental trials is shown in **Table 3** and was analyzed using commercially available software (Food Processor version 11.7; ESHA Research; Salem OR, USA). On the evening before the experimental trials, all participants were instructed to stop consuming food or beverages (except water) by 20:00 h, after which they remained fasted until testing the following morning.

3.2.5 Study design

The current study was a randomized, double-blind, within-subject crossover study in which research participants reported to the laboratory on two occasions (not including the visit for preliminary testing) that were separated by at least one week. On each experimental test day, participants ingested iso-caloric, volume and macronutrient-matched nutritional treatment beverages containing 25 g protein derived from either crickets or beef. Appetite ratings and arterialized venous blood samples were obtained before, and at select time intervals over a 300-minute postprandial period following beverage intake. Following the final appetite rating and

blood sample, participants received an *ad libitum* meal to assess energy intake. The randomization procedure to allocate treatment beverage order was determined via a random-number generator (http://www.randomization.com/). An independent person was responsible for the randomization and preparation of the study beverages. The beverages were prepared in non-transparent plastic containers. To limit diurnal and intrasubject variation, all measures were carried out according to a standardized time schedule at the same time of day on each experimental visit.

3.2.6 Experimental protocol

Study participants were asked to come to the laboratory at ~0800 after a 12-hour overnight fast. Upon arrival, participants rested comfortably on a bed in the laboratory for ~10 minutes before completing a validated visual analogue scale (VAS) questionnaire (described below) to assess subjective appetite sensations in the overnight postabsorptive (i.e. basal) state. After the completion of the first VAS questionnaire, a Teflon catheter was inserted into a dorsal hand vein, connected to a 3-way stop-cock, and placed under a heated (60°C) blanket for 10 minutes prior to obtaining a baseline arterialized venous blood sample. A saline drip was connected to the stop-cock to keep the catheter patent for repeated blood sampling. Following the baseline blood draw, participants received a 400 mL beverage corresponding to their randomized treatment allocation that they were asked to consume within 5 minutes. Participants then drank another 250 mL of water that was used to rinse the beverage container in order to ensure that they ingested any residual protein. Immediately upon the initiation of consumption of the protein beverage, a timer was started and VAS questionnaires to assess subjective appetite sensations were completed at t = 15, 30, 45, 60, 90, 120, 150, 180, 240, and 300 minutes in the postprandial period. Arterialized venous blood samples (total 10 mL each; two 3 mL tubes and one 4 mL tube) were drawn immediately after completion of each VAS using prechilled blood collection tubes (BD

Vacutainer®; New Jersey, USA) coated with K_2EDTA. The 4 mL collection tube was used for analysis glucose, insulin, and amino acids. All tubes were inverted 10 times and centrifuged at 3,000 x g for 15 min at 4°C. After centrifugation, the plasma samples were aliquoted out into microtubes. All plasma samples were frozen in liquid nitrogen and transferred into a -80 °C freezer until further analysis. After the last blood draw, an *ad libitum* meal (described below) was provided to the participants. The consumption of food and water were recorded and used for the calculation of energy intake.

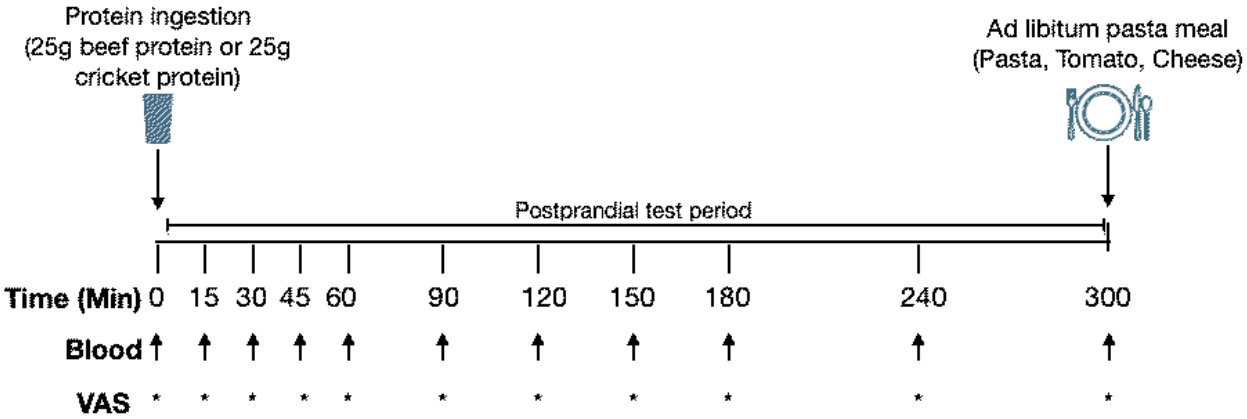

Figure 3. *Schematic representation of the experimental design.*

3.2.7 Nutritional treatments

The nutritional treatment beverages used in this study were iso-caloric, volume and macronutrient-matched. Cricket (*Acheta domesticus*) powder/flour was obtained from Entomo Farms (Cricket Protein Powder 2050; Entomo Farms, Ontario, Canada). Beef (*Bos taurus*) protein powder was obtained from ATP Labs (Swedish Beef Protein; ATP Labs, Quebec, Canada). Samples of each product were sent to Eurofins Scientific (Ontario, Canada), a certified third-party testing laboratory for analysis of ash, moisture, protein, fat, carbohydrate, amino acid, and fiber content. Details of the analysis are shown in **Table 1**. A small amount of cream (35% Lactose Free

Whipping Cream, Natrel, Quebec, Canada) was added to the beef protein beverage in order to match the fat and carbohydrate content of the cricket beverage. Stevia (Stevia Drops, Crave Stevia) was added to the cricket protein beverage to match the flavouring of the beef protein beverage. The final volume of each beverage was 400 mL. In order to ensure that the participants remained blinded, they were instructed to put on a nose clip before receiving the beverage in order to mask the smell and taste of the beverages. Both beverages were served in identical black bottles to blind the appearance of the drink with the participant only able to consume the drink from a small opening on the lid. The participants were instructed to consume the beverage within five minutes. To rinse the container following beverage intake, 250 mL of water was added to the shaker to ensure the participants consumed all content.

Table 1. *Amino acid, carbohydrate, fat, and protein contents of nutritional treatments consisting of 25 g cricket or beef protein ingested by male study participants*

	Nutritional treatment group	
	BEEF	CRICKET
Amino Acid content		
Alanine, g	2.56	2.20
Arginine, g	2.21	1.75
Aspartic acid, g	1.69	2.56
Glutamic acid, g	3.00	3.18
Glycine, g	6.09	1.34
Histidine, g	0.27	0.68
Isoleucine, g	0.46	1.17
Leucine, g	1.04	2.07
Lysine, g	1.13	1.67
Phenylalanine, g	0.65	0.99
Proline, g	3.36	1.54
Serine, g	0.93	1.42
Threonine, g	0.60	1.07
Tyrosine, g	0.26	1.43
Valine, g	0.76	1.93
Totals		
Carbohydrate, g	1.8	3.7
Fat, g	9.1	9.5
Protein, g	25.0	25.0
ΣEAA, g	4.91	9.58
ΣNEAA, g	20.10	15.42
Energy (kcal)	189	200

Total protein was calculated as sum total of amino acids. Cysteine, methionine, tryptophan asparagine, and glutamine were not measured. ΣEAA, sum total essential amino acids; ΣNEAA, sum total non-essential amino acids.

3.2.8 VAS questionnaires for appetite profile

Validated VAS questionnaires assessing hunger, fullness, desire to eat, and prospective food consumption (57) were completed during each experimental trial. The VAS questions consisted of a 100-mm horizontal line rating scale. The most positive and most negative ratings were anchored at each end of the line. The VAS questions on appetite included: (1) How hungry do you feel? Responses could range from 'not hungry at all' to 'as hungry as I have ever felt'; (2) How full do you feel? Responses could range from 'not full at all' to 'very full'; (3) How strong is your desire to eat? Responses could range from 'very weak to 'very strong'; (4) How much do you think you could eat? Responses could range from 'nothing at all' to 'a large amount'. During each visit, participants received thorough instructions from one of the study investigators on the meaning of each appetite sensation and how to rate their appetite sensations using a VAS. Participants were instructed to rate themselves by marking the VAS at the point that was most appropriate to their feeling at that time. The distance from the point marked on the VAS to the left end of the scale was measured in mm. The change from baseline was calculated by subtracting the baseline score from the score at each postprandial time-point.

3.2.9 Ad libitum meal

The *ad libitum* meal was prepared on site during each experimental visit by one of the study team members. The *ad libitum* meal consisted of pasta (Selection; Quebec, Canada) with marinara sauce (President's Choice; Quebec, Canada) and lactose free marble cheddar cheese (Black Diamond Cheese Limited; Ontario, Canada). The *ad libitum* meal contained 547.7 kJ per 100 g with 15 % of energy from protein, 60 % of energy from carbohydrate, and 25 % of energy from fat. The participants were instructed to "eat and/or drink as much or as little as desired until feeling

comfortably full" within 30 minutes. The meal was weighed before consumption and remaining contents were weighed after the meal to calculate the energy intake.

3.2.10 Blood plasma analysis

Plasma glucose and insulin concentration were measured by the Clinical Biochemistry Laboratory of McGill University Health Centre (Montreal, Quebec). Plasma amino acid concentrations were assessed in collaboration with the Proteomics and Clinical Mass Spectrometry platform at the Research Institute of the McGill University Health Centre (Montreal, Quebec). Amino acids were extracted from plasma using protein precipitation and derivatized with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC; Toronto Research Chemicals, Ontario, Canada) for analysis using reversed phase ultra-performance liquid chromatography mass spectrometry (UPLC-MS). Plasma samples were extracted alongside a calibration curve of amino acids in 0.1N HCl with norvaline as an internal standard (all amino acids and norvaline purchased from Sigma-Aldrich, St. Louis, Missouri, USA). A calibration curve of 1 to 1000 µM was used for all amino acids except cysteine (0.5 to 500 µM). An internal standard working solution (ISWS) containing 50 µM norvaline in 5% 5-sulfosalicylic acid was used to extract plasma and calibration samples. ISWS aliquots (25 µL) were added to sample aliquots (25 µL) in microcentrifuge tubes, vortexed and centrifuged at 10,000 x g at 10°C for 10 mins. Supernatant aliquots (10 µL) were transferred into glass tubes containing 70 µL buffer solution (0.2M sodium borate pH 8.8) along with 20 µL derivatization solution (10mM AQC in acetonitrile), mixed and incubated for 10 min at 55°C. After cooling to room temperature, aliquots (10 µL) were transferred to autosampler vials containing 990 µL Type-1 water for UPLC-MS analysis.

Extracts were analyzed by UPLC-MS using an Agilent 6460 triple quadrupole mass spectrometer coupled with an Agilent 1290 UPLC system (Agilent, Santa Clara, California, USA).

Extracts (10µL) were injected onto an Agilent Eclipse Plus C18 100 x 2.1 mm (1.8 µm) column and chromatographed with a reverse phase gradient at 0.200 mL/min using 0.1% formic acid in water and 0.1% formic acid in acetonitrile. The derivatized amino acids were detected using electrospray positive mode ionization followed by MS/MS fragmentation. Data acquisition was performed using Agilent MassHunter Data Acquisition (version B.04.01) software. Peak area measurements from selected product ions, calibration curve regression analysis and resulting sample quantification were performed using Agilent MassHunter Quantitative Analysis (version B.05.00) software.

3.2.11 Statistical analysis

A within-subject crossover design was used for this study. Time-dependent differences in plasma glucose, insulin, and amino acid concentrations, as well as VAS-derived data were tested via a 2-factor (treatment × time) repeated-measures ANOVA. Non time-dependent measures (AUC and *ad libitum* energy intake) were evaluated using a paired-sample *t* test. Maximum (C_{max}) and time to maximum (T_{max}) amino acid concentrations were also evaluated using a paired-sample *t* test. When a statistically significant interaction effect was observed following ANOVA testing, a Bonferroni post hoc test was performed to locate differences. Because of the lack of data comparing postprandial plasma amino acid concentrations following the ingestion of cricket vs. beef-derived protein sources, the current study was powered based on differences in peak plasma leucine concentration following the ingestion of 30 g protein from beef and milk (118) to determine sample size. Based on an effect size of 3.996225, a power of 80%, and type I error of 0.05, we determined that only 6 subjects were required to detect differences in peak plasma leucine concentration between protein sources. However, as the present study also aimed to evaluate differences in appetite, satiety, and food intake between protein sources as secondary outcome

measures, 20 subjects were recruited for the present study. This was based on data from Veldhorst et al. (70) who reported a 20% difference in postprandial perceived hunger with whey vs. soy protein. This difference led to an effect size of 0.8, indicating a sample size of 20 would provide 80% power to detect differences between protein sources. Statistical analysis was performed with use of the Statistical Package for the Social Sciences (SPSS, version 24. IBM Corp., Armonk, NY, USA). For all analyses, differences were considered statistically significant at $P < 0.05$. All data are expressed as means ± SEM.

3.3. Results

3.3.1: Subject characteristics

Participants' characteristics are shown in **Table 2**. Subjects had a normal BMI, normal blood pressure, and normal body fat percentage.

Table 2. *Characteristics of male study participants who ingested nutritional treatments consisting of 25 g protein from cricket and beef protein.*

Age (y)	23	±	1
Height (m)	1.77	±	0.01
Weight (kg)	72.3	±	2.1
BMI (kg/m^2)	23.0	±	0.6
Systolic Blood Pressure (mmHg)	112	±	2
Diastolic Blood Pressure (mmHg)	69	±	2
Body Fat (%)	19.5	±	1
Bone- and Fat-Free Mass (kg)	55.5	±	1.3

Values are means ± SEM.

3.3.2: Dietary intake

Dietary intake data before the first experimental trial is shown in **Table 3**. The average energy intake aligns with the daily energy intake for Canadian males aged between 19-30 y in 2015 (119). The macronutrient distribution follows the Health Canada AMDR recommendation (21).

***Table 3**. Average 2-d dietary intake of male study participants prior to their first experimental trial who ingested nutritional treatments consisting of 25 g protein from cricket and beef protein.*

Energy, kJ· d^{-1}	9102	±	683
Carbohydrate, g	269	±	22
Fat, g	74	±	8
Protein, g	111	±	12
Protein, g · kg^{-1} · d^{-1}	1.6	±	0.2
Carbohydrate, % total energy	50	±	3
Fat, % total energy	30	±	2
Protein, % total energy	20	±	2

Values are means ± SEM.

3.3.3: Plasma glucose, insulin, and amino acid concentrations

Plasms glucose **(Figure 4, panel A)** concentrations (mmol•L^{-1}) averaged 5.2 ± 0.09 and 5.2 ± 0.11 under basal overnight postabsorptive conditions (t = 0 min) in the beef and cricket trial respectively. Glucose concentrations showed a main effect for time (P = 0.039) but did not differ between treatments (P = 0.628).

Plasms insulin **(Figure 4, panel B)** concentrations (pmol•L^{-1}) averaged 28.1 ± 3.7 and 28.1 ± 3.0 under basal overnight postabsorptive conditions (t = 0 min) in the beef and cricket trial respectively. Insulin concentrations increased in response to beverage ingestion (Interaction, P =

0.001) and were greater with beef compared to cricket protein from 30-60 min in the postprandial period.

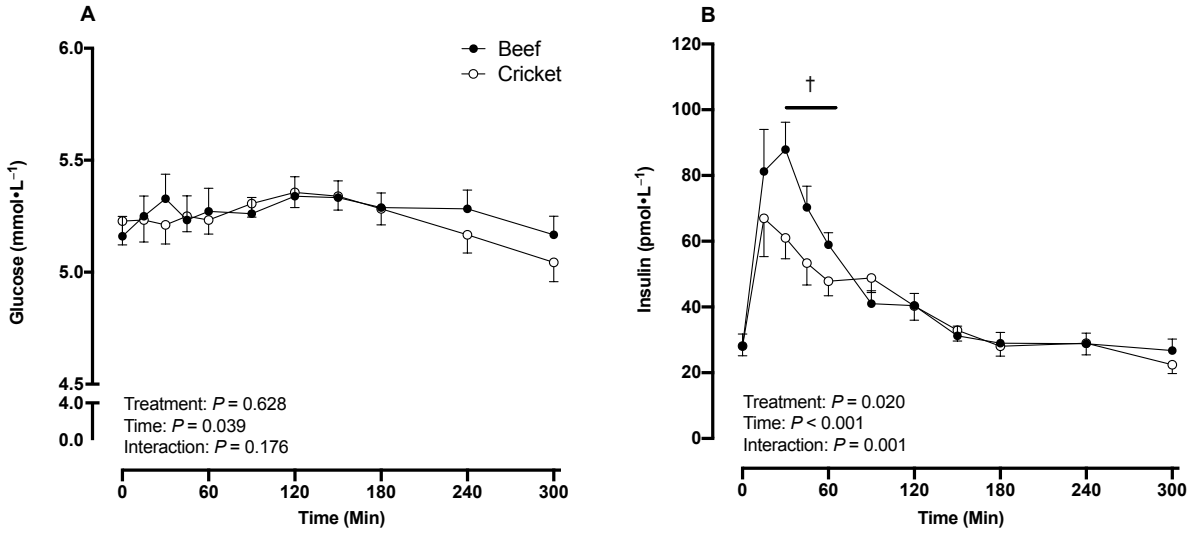

Figure 4*. Plasma glucose (A) and insulin (B) concentrations during postabsorptive conditions (t = 0 min) and during postprandial conditions (t = 15-300 min) after protein beverage intake in young males. Values represent means ± SEMs, n = 20 study participants. Data for glucose and insulin were analyzed by a 2-factor repeated-measures ANOVA. When a significant interaction effect was identified in the ANOVA, Bonferroni post hoc tests were performed to determine the difference between means within each time-point.* † *indicates a difference between treatments, P < 0.05. Beef, beverage containing 25 g protein derived from beef; Cricket, beverage containing 25 g protein derived from crickets.*

Plasma leucine **(panel A and B)**, branched-chain amino acids **(panel C and D)** are shown in **Figure 5**. Plasma leucine concentrations (μmol•L^{-1}) increased in response to beverage ingestion (Interaction, $P < 0.001$) and was greater with cricket compared to beef protein from 90-180 min and at 300 min in the postprandial period (panel A). Similarly, net incremental area under the curve (niAUC) for leucine (panel B) over the entire 300 min postprandial period was greater for cricket

compared to beef protein (P = 0.001). Plasma BCAA concentrations (μmol•L^{-1}) increased in response to beverage ingestion (Interaction, $P < 0.001$) and were greater with cricket compared to beef protein from 90-180 min and at 300 min in the postprandial period (panel C). Similarly, niAUC for BCAA (panel D) over the entire 300 min postprandial period was greater for cricket compared to beef protein ($P < 0.001$).

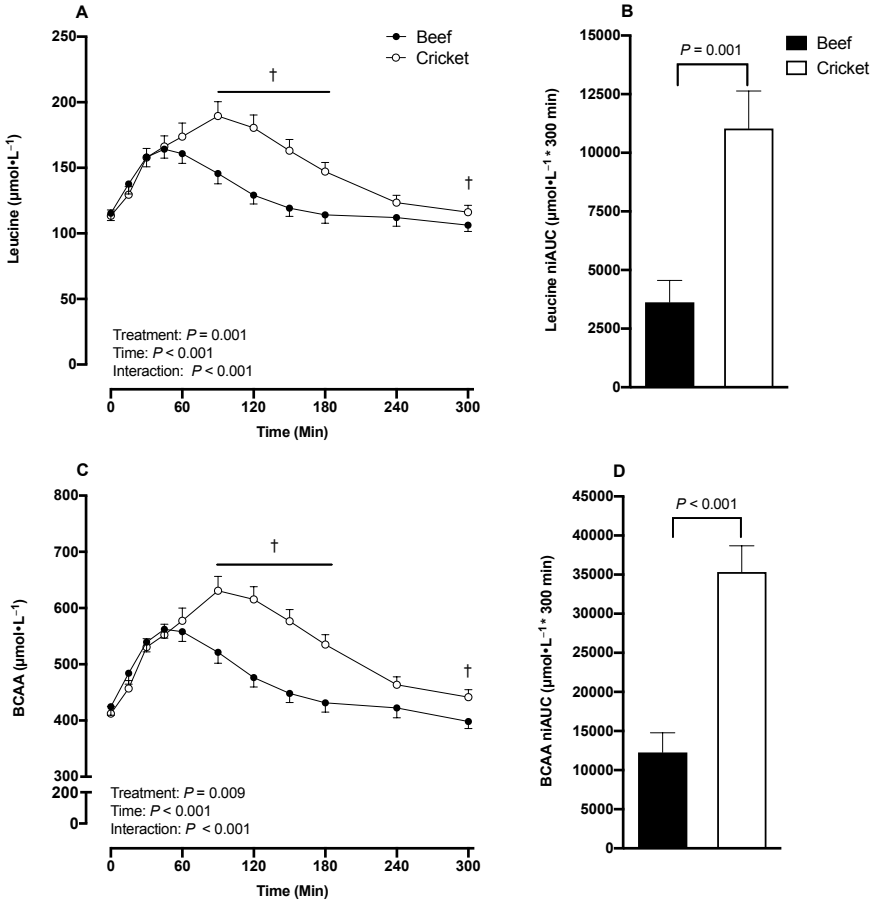

Figure 5. *Plasma leucine (A) and branched-chain amino acid (C) concentrations during postabsorptive conditions (t = 0 min) and during postprandial conditions (t = 15-300 min), and corresponding net incremental area-under-the curve for leucine (B) and branched-chain amino acids (D) after protein beverage intake in young males. Values represent means ± SEMs, n = 20 study participants. Data for leucine and branched-chain amino acids were analyzed by a 2-factor*

repeated-measures ANOVA and paired-sample t tests (for net incremental area-under-the curve). When a significant interaction effect was identified in the ANOVA, Bonferroni post hoc tests were performed to determine the difference between means within each time-point. † *indicates a difference between treatments, P < 0.05. BCAA, branched-chain amino acids; Beef, beverage containing 25 g protein derived from beef; Cricket, beverage containing 25 g protein derived from crickets; niAUC, net incremental area-under-the-curve.*

Essential amino acids **(panel A and B)**, non-essential amino acids **(panel C and D)** and total amino acids **(panel E and F)** are shown in **Figure 6**. Plasma EAA concentrations (μmol•L^{-1}) increased in response to beverage ingestion (Interaction, $P < 0.001$) and were greater with cricket compared to beef protein from 90-300 min in the postprandial period (panel A). Similarly, niAUC for EAA (panel B) over the entire 300 min postprandial period was greater for cricket compared to beef protein ($P < 0.001$). Plasma NEAA concentrations (μmol•L^{-1}) increased in response to beverage ingestion (Interaction, $P < 0.001$) and were greater with beef compared to cricket protein from 15-180 min and 300 min in the postprandial period (panel C). In alignment, niAUC for NEAA (panel D) over the entire 300 min postprandial period was greater for beef compared to cricket protein ($P < 0.001$). Plasma TAA concentrations (μmol•L^{-1}) increased following beverage ingestion (Interaction, $P = 0.013$) and was greater with beef compared to cricket protein from 15-90 min as well as from 150-180 min in the postprandial period (panel E). In alignment, niAUC for TAA (panel F) over the entire 300 min postprandial period was also greater for beef compared to cricket protein ($P = 0.012$).

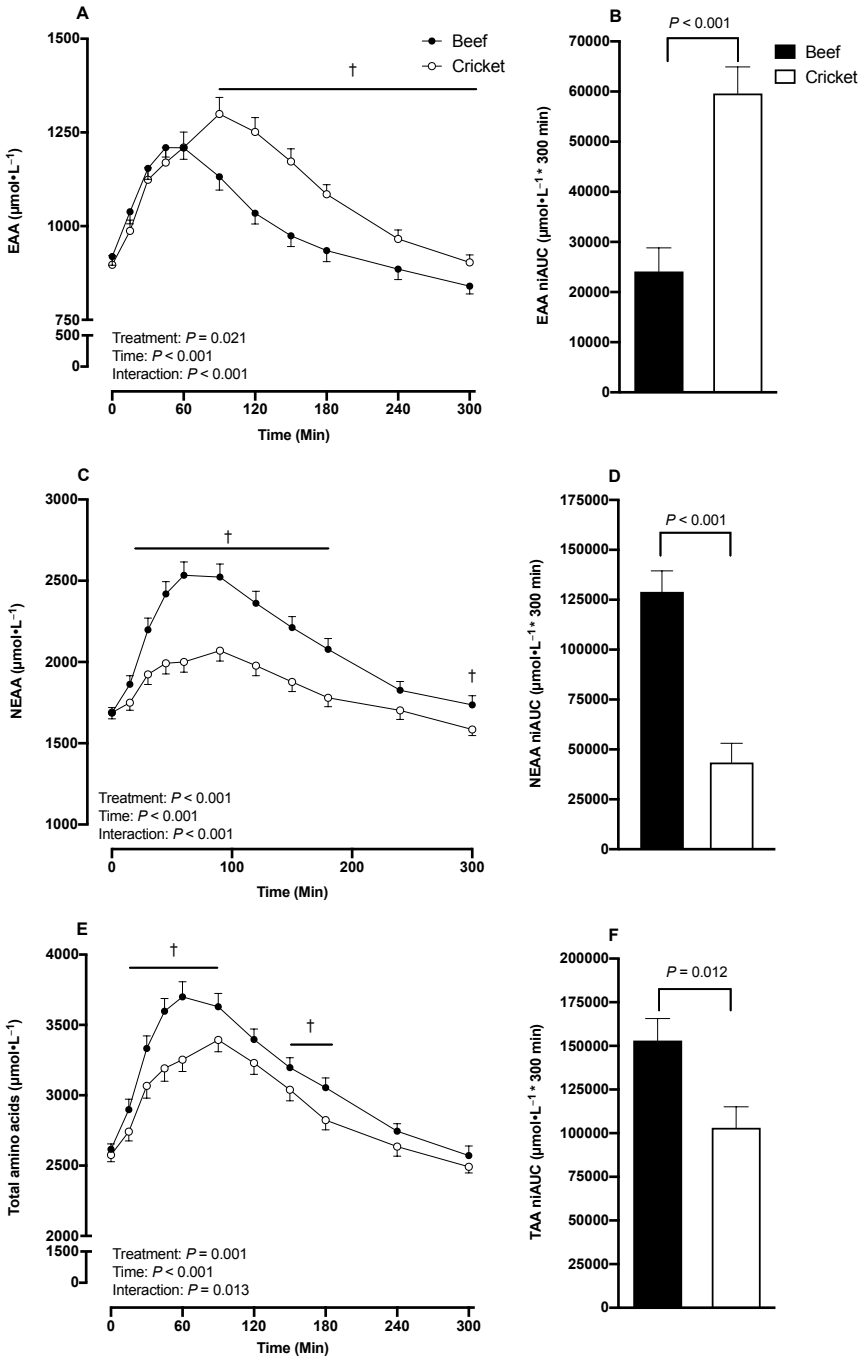

Figure 6. *Essential amino acid (A), non-essential amino acid (C) and total amino acid (E) concentrations during postabsorptive conditions (t = 0 min) and during postprandial conditions (t = 15-300 min), and corresponding net incremental area-under-the curve for essential amino acid (B), non-essential amino acid (D) and total amino acid (F) after protein beverage intake in young males. Values represent means ± SEMs, n = 20 study participants. Data for essential amino*

acids, non-essential amino acid and total amino acid were analyzed by a 2-factor repeated-measures ANOVA and paired-sample t tests (for net incremental area-under-the curve). When a significant interaction effect was identified in the ANOVA, Bonferroni post hoc tests were performed to determine the difference between means within each time-point. † *indicates a difference between treatments, P < 0.05. Beef, beverage containing 25 g protein derived from beef; Cricket, beverage containing 25 g protein derived from crickets; EAA, essential amino acids; NEAA, non-essential amino acids; niAUC, net incremental area-under-the-curve; TAA, total amino acids.*

The peak concentration (C_{max}) and time of peak concentration (T_{max}) for plasma leucine, branched-chain amino acids, essential amino acids, non-essential amino acids, and total amino acids are shown in **Table 4**. Plasma C_{max} for leucine (μmol•L^{-1}) was greater with cricket compared to beef protein ($P = 0.001$). T_{max} for plasma leucine (min) was earlier for beef compared to cricket protein ($P = 0.029$). Plasma C_{max} for BCAA (μmol•L^{-1}) was greater with cricket compared to beef protein ($P = 0.002$). T_{max} for plasma BCAA (min) was earlier for beef compared to cricket protein ($P = 0.027$). Plasma C_{max} for EAA (μmol•L^{-1}) was greater with cricket compared to beef protein ($P = 0.007$). T_{max} for plasma EAA (min) was earlier for beef compared to cricket protein ($P < 0.001$). Plasma C_{max} for NEAA (μmol•L^{-1}) was greater with beef compared to cricket protein ($P < 0.001$). T_{max} for plasma NEAA (min) was earlier for beef compared to cricket protein ($P = 0.018$). Plasma C_{max} for TAA (μmol•L^{-1}) was greater with cricket compared to beef protein ($P = 0.004$). T_{max} for plasma TAA (min) showed no difference between cricket and beef protein ($P = 0.278$).

Table 4. *Maximum and time to maximum concentrations for plasma leucine, BCAA, EAA, NEAA and TAA in male study participants who ingested nutritional treatments consisting of 25 g protein from cricket and beef protein.*

	Beef		Cricket		*P* – Value	
	C_{max} (μmol•L^{-1})	T_{max} (Min)	C_{max} (μmol•L^{-1})	T_{max} (Min)	C_{max}	T_{max}
Leucine	170 ± 8	59 ± 10	205 ± 9	89 ± 9	*P* = 0.001	*P* = 0.029
BCAA	582 ± 19	65 ± 10	670 ± 20	96 ± 9	*P* = 0.002	*P* = 0.027
EAA	1242 ± 32	56 ± 4	1373 ± 37	98 ± 9	*P* = 0.007	*P* < 0.001
NEAA	2611 ± 80	74 ± 24	2195 ± 61	96 ± 28	*P* < 0.001	*P* = 0.018
TAA	3817 ± 92	77 ± 8	3546 ± 78	89 ± 7	*P* = 0.004	*P* = 0.278

Maximum plasma leucine, BCAA, EAA, NEAA, and TAA concentrations and time of maximum concentrations during postabsorptive conditions (t = 0 min) and during postprandial conditions (t = 15-300 min) after protein beverage intake in young males. Values represent means ± SEMs, n = 20 study participants. Data for C_{max} and T_{max} for leucine, BCAA, EAA, NEAA, and TAA were analyzed by a paired-sample t test. BCAA, branched-chain amino acids; Beef, beverage containing 25 g protein derived from beef; Cricket, beverage containing 25 g protein derived from crickets; C_{max}, maximum concentration; EAA, essential amino acids; NEAA, non-essential amino acids; TAA, total amino acids; T_{max}, time of maximum concentration.

3.3.4: VAS-derived appetite sensations

VAS-derived sensations of hunger **(panel A and B)**, fullness **(panel C and D),** desire to eat **(panel E and F),** and prospective food consumption **(panel G and H)** are shown in **Figure 7**. Hunger showed a main effect for time ($P < 0.001$), but no difference ($P = 0.119$) between treatments (panel A). niAUC for hunger (panel B) over the early (0-150 min) and late (150-300 min) postprandial period was lower for beef compared to cricket protein (Treatment, $P = 0.042$).

Furthermore, niAUC for hunger was lower over the early (0-150 min) compared to late (150-300 min) postprandial period (Time, $P < 0.001$). Fullness (panel C) showed a main effect for time ($P < 0.001$), but there was no difference between treatments ($P = 0.358$). Similarly, niAUC for fullness (panel D) over the early and late postprandial period was not different between cricket and beef (Treatment, $P = 0.137$), but was greater over the early (0-150 min) compared to late (150-300 min) postprandial period (Time, $P < 0.001$). Desire to eat (panel E) showed a main effect for time ($P < 0.001$), but there was no difference between treatments ($P = 0.193$). Similarly, niAUC for desire to eat (panel F) over the early and late postprandial period was not different between cricket and beef (Treatment, $P = 0.46$), but was lower over the early compared to late postprandial period (Time, $P < 0.001$). Prospective food consumption (panel G) showed a main effect for time ($P < 0.001$), but there was no difference between treatments ($P = 0.390$). niAUC for prospective food consumption (panel H) over the early and late postprandial period was not different between cricket and beef (Treatment, $P = 0.596$), but was lower over the early compared to late postprandial period (Time, $P < 0.001$).

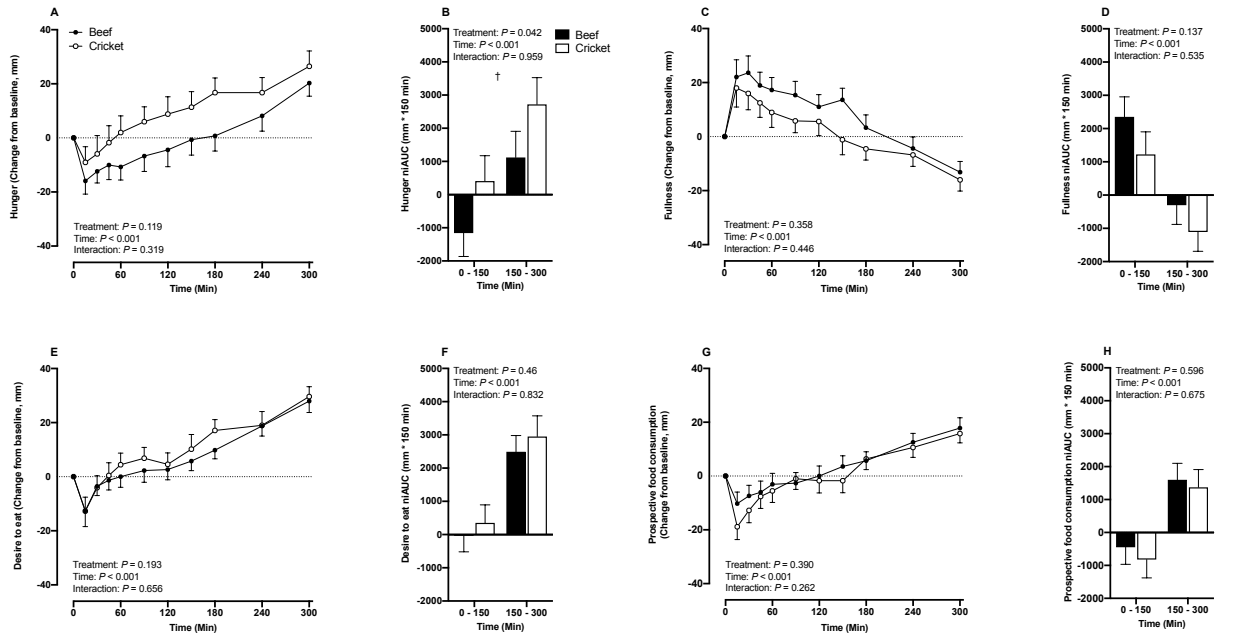

***Figure** 7. Change from postabsorptive conditions (t = 0 min) in visual analogue scale-derived hunger (A) fullness (C) desire to eat (E) and prospective food consumption (G) during postprandial conditions (t = 15-300 min), and corresponding net incremental area-under-the curve for hunger (B) fullness (D) desire to eat (F) and prospective food consumption (H) during the early (0-150 min) and late (150-300) period after protein beverage intake in young males.*

Values represent means ± SEMs, n = 20 study participants. Data for hunger, fullness, desire to eat, and prospective food consumption were analyzed by a 2-factor repeated-measures ANOVA. † indicates a difference between treatments, P < 0.05. Beef, beverage containing 25 g protein derived from beef; Cricket, beverage containing 25 g protein derived from crickets; niAUC, net incremental area-under-the-curve.

3.3.5: Ad libitum energy intake

Ad libitum food energy intake **(Figure 8)** assessed 300 min (5 h) after ingestion of the cricket and beef-derived protein treatments averaged 4466 ± 283 and 4153 ± 264 respectively and was not different between treatments ($P = 0.277$).

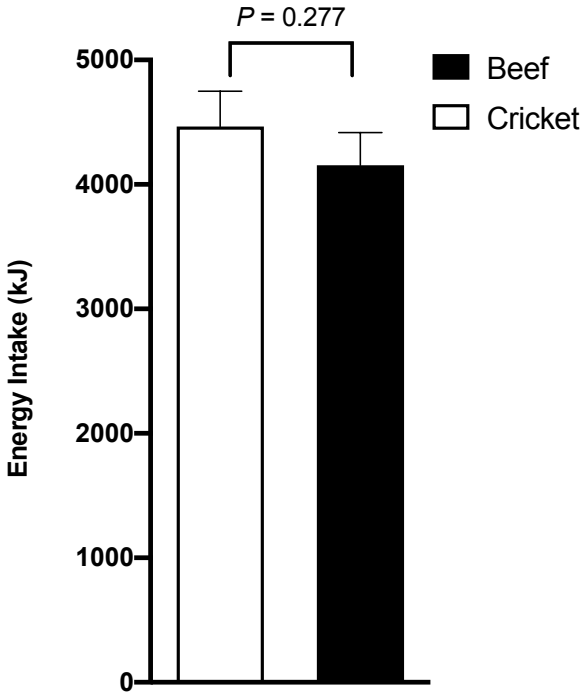

Figure 8. *Ad libitum energy intake (kJ) 300-min after protein beverage intake in young males. Values represent means ± SEMs, n = 20 study participants. Data for energy intake were analyzed using a paired sample t test. Beef, beverage containing 25 g protein derived from beef; Cricket, beverage containing 25 g protein derived from crickets; kJ, kilojoule.*

3.4 Discussion

In the present study we evaluated postprandial changes in plasma glucose, insulin, and amino acid (leucine, BCAA, EAA, NEAA and TAA) concentrations, VAS-derived appetite sensations (hunger, fullness, desire to eat, and prospective food consumption), and *ad libitum* food energy intake in response to the ingestion of 25 g protein derived from crickets or beef in healthy

young men. Protein ingestion increased plasma insulin concentrations, but to a greater extent following beef compared to cricket protein from 30-60 min in the postprandial period. Protein intake resulted in a rise in plasma amino acid concentrations during the postprandial period. Leucine, BCAA and EAA niAUC over the entire 300 min postprandial period was greater for cricket vs. beef-derived protein, while NEAA and TAA niAUC was greater for beef protein. VAS-derived niAUC for sensations of hunger was lower with beef compared to cricket protein; however, there were no differences between protein sources for VAS-derived fullness, desire to eat, or prospective food consumption. No differences in *ad libitum* food energy intake between protein sources were observed when assessed 300-min following the protein intake.

It is well established that on an energy-matched basis, protein is more satiating than carbohydrate or fat (120). However, the satiating influence of protein is variable and may be influenced by the source of ingested protein (5, 66). Sources of dietary protein differ in their respective amino acid content and digestibility, and ingestion of different sources of dietary protein can result in differences in postprandial insulin and/or amino acid concentrations (5, 10, 121, 122), which may alter appetite, satiety, and/or subsequent food intake (4, 5). The present study is the first to our knowledge to compare the acute effects of ingesting cricket- vs. beef-derived protein on postprandial plasma insulin (Figure 4B) and amino acid concentrations in healthy young men (Figure 5, panel A-D and Figure 6, panel A-F). Plasma insulin concentrations increased following protein intake but were greater with beef vs. cricket-derived protein from 30-60 into the postprandial period. Data from animal studies have demonstrated that insulin administration decreases food intake and leads to a decline in bodyweight (123, 124). Alternatively, inhibition of normal insulin action increases energy intake and results in increased bodyweight (125). These results suggest that insulin contributes to satiety. Insulin (along with other hormones) can cross

the blood-brain barrier and act on appetite centres in the brain to induce satiety (126). Specifically, insulin can inhibit orexigenic (appetite-stimulating) pathways in the arcuate nucleus of the hypothalamus in the brain (50). Therefore, it is possible that the greater postprandial increase in plasma insulin observed following beef compared to cricket-derived protein contributed to the lower niAUC for sensations of hunger with beef-derived protein (Figure 7, panel B).

A number of studies have evaluated changes in postprandial amino acid concentrations following the ingestion of different sources of dietary protein (5, 66, 68, 111); however, there is little information available on changes postprandial amino acid concentrations following the ingestion of insect-derived proteins in humans. Vangsoe and colleagues (115) recently compared postprandial blood insulin and amino acid concentrations over 120-min following the ingestion of 25 g of whey, soy, and insect (lesser mealworm) protein in healthy young men; no measures of satiety or food intake were performed. Postprandial insulin concentrations were greater at 20 and 40 min with whey and soy protein compared to insect protein. Postprandial AUC for leucine, BCAA, and EAA was greater for whey compared to soy and insect-derived protein, but equivalent between soy and insect protein. However, a limitation of this study was that the postprandial period was only evaluated for 120 min and amino acid concentrations remained elevated compared to baseline. Furthermore, the proteins were matched on crude protein which resulted in a substantially reduced TAA intake with insect protein (115). Protein content is often calculated from total nitrogen using a nitrogen-to-protein conversion factor of 6.25. However, this factor may overestimate the protein content on insect-derived proteins, due to the presence of non-protein nitrogen in insects (127). Therefore, in the present study comparing cricket- and beef-derived protein, subjects ingested 25 g protein based on total amino acids with blood samples collected over a 300-min postprandial period to assess changes in plasma amino acid concentrations.

Ingestion of 25 g cricket-derived protein resulted in greater increases in postprandial niAUC for leucine, BCAA, and EAA (Figure 5, panel B, D; Figure 6, Panel B) than an equal dose of beef-derived protein. Alternatively, ingestion of beef-derived protein resulted in greater increases in postprandial niAUC for NEAA and TAA (Figure 6, panel D and F) than cricket protein. Based on analysis of the amino acid content of the two nutritional treatments (Table 1), leucine, BCAA and EAA content was higher in the cricket-derived protein compared to the beef-derived protein source which likely explains the greater postprandial plasma aminoacidemia for these amino acids. Overall, the ingestion of insect-derived protein from crickets increases circulating concentrations of key amino acids such as leucine and EAA that are important in the regulation of appetite and food intake, as well as whole-body and muscle protein synthesis.

As early as the 1950's, Mellinkoff and colleagues (4) demonstrated a reciprocal relationship between circulating amino acid concentrations and appetite. Specifically, a rise in postprandial serum amino acids concentrations was accompanied by a reduction in appetite, while an increase in appetite was accompanied by a decline in amino acid concentrations. Mellinkoff's amino static theory (4) states that an increased concentration of circulating amino acids which are not utilized for protein synthesis may serve as a satiety signal for a food intake regulating mechanism in the brain. Once the concentration of amino acids reaches a certain threshold, this serves as a signal that is detected in the brain and results in a suppression of appetite and decreased food intake. In support of this theory, Hall and colleagues (5) reported that the ingestion of a whey protein beverage resulted in a 28% increase in postprandial plasma amino acid concentrations over 180 min compared with a casein protein beverage and was accompanied by a 19% reduction in energy intake at a subsequent meal. Therefore, the source of ingested protein and the resulting hyperaminoacidemia may be an important regulator of the satiety response after meal intake. In

the present study, the niAUC for postprandial plasma NEAA and TAA concentrations was greater for beef compared to cricket-derived protein, while the niAUC for VAS-derived hunger was lower for beef compared to cricket-derived protein. However, despite lower ratings of subjective hunger with beef- vs. cricket-derived protein, there was no difference between the protein sources for *ad libitum* energy intake at the subsequent meal.

Although amino acids appear to be important in the regulation of appetite, satiety, and food intake, specific amino acids may be particularly important. Evidence from animal (128) and human studies (111) suggests that leucine may be a key amino acid in appetite control via acting as a signaling molecule in the brain for satiety (113). Bolster and colleagues (111) recently reported that addition of leucine (2 g) to a low-dose of ingested protein (9 g) resulted in greater increases in plasma leucine and VAS-derived fullness, and greater decreases in VAS-derived hunger, prospective food consumption, and desire to eat when compared to a low-dose of ingested protein without added leucine. In the present study, cricket-derived protein resulted in a greater leucinemia than beef protein; however, all VAS-derived appetite sensations except hunger were similar between protein sources and there were no differences in *ad libitum* energy intake at the subsequent meal. Alternatively, it has been suggested that incomplete proteins that are deficient in one or more EAAs and higher in NEAAs may be more satiating than complete proteins in the acute setting (129) based on the observation that consumption of diets low in EAAs will induce a decrease in plasma concentration of these amino acids, which in rodents is found to be detected in the brain and lead to a behavioral response rejecting consumption of imbalanced diets and consequently a suppression of hunger (130, 131). The overall EAA content of the beef-derived protein was substantially lower compared to the cricket-derived protein and may have been deficient in one or more EAA's leading to a greater suppression of hunger. Nonetheless, the two protein sources were

not different when examining the other VAS-derived appetite sensations or *ad libitum* energy intake.

Several studies have been conducted to date examining the effects of different sources of dietary protein on appetite control, satiety, and food energy intake; however, this is the first to our knowledge to evaluate insect-derived protein. Uhe and colleagues (66) compared the satiating effects of animal-derived beef, chicken, and fish protein over 180 min. The authors reported that VAS-derived measures of satiety were lower with beef and chicken compared to fish protein; however, energy intake was not assessed. Lang and colleagues (67) compared egg, casein, gelatin, soy, pea, and wheat protein on satiety and food intake and reported no difference between the protein sources. A more recent study by Douglas et al (11) compared macronutrient and fiber-matched doses of beef and soy protein (24 g). The authors reported no differences between protein sources in VAS-derived hunger or fullness, circulating concentrations of PYY or GLP-1, and energy intake during an *ad libitum* buffet meal. Therefore, while some studies have reported protein source-dependent differences, there is inconclusive evidence that one source of protein is most effective at decreasing appetite and subsequent food intake. Differences between studies comparing the effect of protein source on appetite regulation and subsequent energy intake may relate to the dose or amount of protein consumed.

In the present study, a 25 g dose of protein was chosen as 25-30 g proteins has been suggested to represent the meal-specific protein quantity to support improvements in appetite control and bodyweight management (2). There is also evidence that at relatively higher doses of ingested protein (25% vs. 10% energy from ingested protein), it may not be possible to detect differences between protein sources because the amino acid concentrations are above a threshold (i.e., a ceiling effect) for all protein sources. For example, Veldhorst et al. (9) compared the

appetite-regulating effects of whey, casein, and soy and reported that at 10% energy from protein, whey decreased hunger compared with casein and soy. However, there were no differences between protein sources at the high protein dose (25% energy from protein). Therefore, at high protein concentrations, it may not be possible to discriminate between protein sources because the amino acid concentrations are above the threshold for all protein sources (9).

In conclusion, cricket- and beef-derived proteins elicited very similar effects on appetite control, satiety, and subsequent food intake in healthy young men, despite differences in postprandial plasma hyperinsulinemia and hyperaminoacidemia. The two protein sources appear similar and may represent equivalents when developing higher protein meal plans.

Chapter 4. Conclusion and Summary

The present investigation represents an acute, tightly controlled, laboratory-based feeding study assessing protein source-related differences over the course of a single day. This type of study design is the "most influential" experimental approach to evaluate appetite regulation as it provides the highest level of control over the study intervention and outcome measures (55). However, whether these acute meal findings obtained within a single test day are qualitatively predictive of what would happen over the long-term (i.e., with consumption of cricket- and beef-derived protein over several days or weeks) is unknown. Furthermore, other factors present in the 'real-world' under free-living conditions (i.e., outside the laboratory) can also influence appetite and satiety responses. For example, external factors such as the presence of other people during a meal, food palatability, food variety, portion size, and distractions such as television viewing are all factors that can influence satiation and satiety (50). Therefore, this study serves as a first step in examining the influence of cricket-derived protein on appetite control and satiety. Future longer-term feeding trials, both laboratory-based and free-living, examining practical outcomes such as weight management and daily food intake are required.

In the current study, only a single type of edible insect (the cricket species *Acheta domesticus*) was evaluated and compared to beef-derived protein. This insect species was chosen as it is currently reared on an industrial scale for use in human food products. Cricket-based protein powders, protein bars, and pasta are among the foods currently available on the market for human consumption. Given that there are over 2000 species of insects currently consumed around the globe by humans as food, it is unclear whether other insect species would elicit similar responses in terms of postprandial insulin and amino acid concentrations, VAS-derived appetite sensations, and *ad libitum* meal intake as that observed in the present study. Future research is warranted to

evaluate the effects of other species of edible insects on factors such as appetite and satiety, muscle metabolic responses, and bodyweight regulation in humans. Furthermore, it should be noted that the form in which protein is consumed can influence subsequent appetite sensations and satiety. It is well understood that food presented in a solid form is more satiating than its equivalent liquid counterpart (132). With regards to beverages, the same trend can be observed with a greater satiating effect seen with beverages demonstrating a more viscous consistency (133). The current study has only evaluated the protein in liquid form whereas future studies could explore the role of food form on satiety with respect to the consumption of insect-derived protein and their subsequent impact on satiety.

In conclusion, the ingestion of insect-derived protein from crickets resulted in a postprandial increase in plasma amino acid concentrations. The temporal and net (niAUC) increase in leucine, BCAA, and EAA was greater for cricket compared to beef-derived protein. Alternatively, the ingestion of beef-derived protein led to a greater temporal and net increase in NEAA and TAA. The niAUC for VAS-derived hunger was lower following beef- compared to cricket-derived protein; however, there were no other differences in VAS-derived appetite sensations or subsequent *ad libitum* energy intake. Cricket- and beef-derived proteins appear similar with regards to their capacity to regulate appetite and food intake and may represent equivalents when developing higher protein meal plans.

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