FACULTY OF BIOSCIENCE ENGINEERING

THE EFFECT OF OMEGA-3 FATTY ACID INTAKE AT BREAKFAST ON ENERGY EXPENDITURE AND APPETITE IN YOUNG WOMEN

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Preface

This master's dissertation was performed in order to obtain the degree of Master of Science in Bioscience Engineering in Food Science and Nutrition. I had the amazing opportunity to perform my thesis at the University of Arkansas (U.S.). During these months of living in America, I gained a wealth of experience and knowledge on so many levels. Finishing this thesis has been the outcome of a battle striving for no dropouts and good results, and writing and rewriting. I am proud and exhilarated to be able to state that this has indeed become a reality, after several months and 26,130 words later.

Many thanks to professor John Van Camp who was open to guide me as my professor back in Ghent. His infinite motivation, knowledge and valuable support were essential to finishing this thesis with success. I also want to express my sincere gratitude to Dr. Jamie I. Baum for supporting and guiding me at the UofA, teaching me the skills I needed. The door of her office was always open when I needed some advice or had a question about my research or writing.

Throughout the years of studying bioscience engineering, a lot of friends came on my way and are still so precious to me. I want to thank every single one of them giving me these unforgettable years of their friendship, laughter, and inexhaustible valuable advice.

Finally, but foremost I want to thank my family. I am so thankful to my mother for giving me these beautiful opportunities to get me to the point where I am now and I am blessed with a brother like mine, who's the best best example of what I want to become in my life.

Contents

List of Abbreviations

AgPR	Agouti-Related Peptide
ALA	α-Linolenic Acid
AMPK	5' AMP-activated protein Kinase
ANOVA	Analysis of Variance
BMR	Basal Metabolic Rate
CART	Cocaine- and Amphetamine-Regulated Transcript
CCK	Cholecystokinin
COX	Cyclooxygenase
CVD	Cardiovascular Disease
DHA	Docosahexaenoic Acid
DLW	Double Labeled Water
DXA	Dual-Energy X-ray Absorptiometry
EE	Energy Expenditure
EFSA	European Food Standards Authority
EI	Energy Intake
EPA	Eicosapentaenoic Acid
FA	Fatty Acid
F_{ECO2}	Fraction of Expired Carbon Dioxide
F_{EO2}	Fraction of Expired Oxygen
FFM	Fat-Free Mass
FM	Fat Mass
GIP	Glucose-dependent Insulinotropic Polypeptide
iAUC	Incremental Area Under the Curve
IC	Indirect Calorimetry
IL-6	Interleukin-6
IRB	Institutional Review Board
IUD	Intrauterine Device
LCFA	Long-Chain Fatty Acids
LC n-3 PUFA	Long-chain Omega-3 Polyunsaturated Fatty Acids
MCFA	Medium-Chain Fatty Acids
MUFA	Mono-Unsaturated Fatty Acids

NFκB	Nuclear Factor kappa B
NHANES	National Health and Nutrition Examination Survey
niAUC	Net Incremental Area Under the Curve
npRQ	Non-Protein Respiratory Quotient
NPY	Neuropeptide Y
NW	Normal Weight
O3FA	Omega-3 Fatty Acids
OW	Overweight/Obese
PFC	Prospective Food Consumption
POMC	Pro-Opiomelanocortin
PP	Pancreatic Polypeptide
PPAR	Peroxisome Proliferator Activated Receptor
PYY	Peptide YY
REE	Resting Energy Expenditure
RER	Respiratory Exchange Ratio
RMR	Resting Metabolic Rate
SD	Standard Deviation
SFA	Saturated Fatty Acids
SPSS	Statistical Package for the Social Sciences
T2DM	Type 2 Diabetes Mellitus
TDEE	Total Daily Energy Expenditure
TNF$-\alpha$	Tumor Necrosis Factor α
USDA	U.S. Department of Agriculture
VAS	Visual Analogue Scale
V_E	Expired Ventilation
VCO_2	Carbon Dioxide Production Volume
VO_2	Oxygen Consumption Volume
WHO	World Health Organization

Summary

Worldwide, overweight and obesity are a growing health problem. Both in the United States and in Europe, obesity is a public health challenge, considering it is a multifactorial diseases and individuals suffering have difficulties following a healthy lifestyle, especially over the longer term. Young women are considered to be at high risk for weight gain. Trying to identify novel strategies (e.g. food components with potential beneficial health effects) might be of great importance to lead to new ways to treat or prevent weight gain. Previous studies have investigated the potential beneficial health effects of omega-3 fatty acids from marine sources (i.e. eicosapentaenoic acid, EPA; and docosahexaenoic acid, DHA), however they are limited, with conflicting results regarding potential anti-obesity effects. The purpose of this master thesis was to set up a pilot study using a double-blind, randomized crossover design trying to investigate the possible effects of LC n-3 PUFA (from marine sources) supplementation at breakfast in two different weight groups of young women (normal weight and overweight/obese). This study served as a first step to examine the effects on energy expenditure and appetite, by utilizing a high dose of LC n-3 PUFA (2000 mg EPA + 2000 mg DHA). Appetite and craving responses were measured with visual analogue scale, resting energy expenditure and substrate utilization were measured through indirect calorimetry and following energy intake was measured through 24-hour food intake record. This pilot study showed that consumption of breakfast supplemented with LC n-3 PUFA resulted in decreased postprandial hunger sensations and increased sensations of postprandial perceived fullness, compared to consuming the control diet and this effect was prevalent in both normal weight and overweight/obese participants. The breakfast supplemented with LC n-3 PUFA resulted in an increased postprandial resting energy expenditure. There was no difference found in postprandial carbohydrate oxidation, but fat oxidation was significantly higher after consuming the breakfast supplemented with LC n-3 PUFA in the normal weight group but not in the overweight/obese group. The 24-hour food intake record did not show any difference in energy intake (kcal) at lunch, dinner, or snacks between the LC n-3 PUFA and the control breakfast in both the normal weight and overweight/obese weight group.

Samenvatting

Overgewicht en obesitas zijn een groeiend wereldwijd volkgezondheidsprobleem waar zowel mensen in de Verenigde Staten als in Europa aan lijden. Het kan gezien worden als een multifactoriële aandoening waarbij velen vooral moeite hebben om zich te houden aan een gezonde levensstijl, en vooral op lange termijn. Voornamelijk jonge vrouwen hebben een groot risico op gewichtstoename. Het identificeren van nieuwe strategieën (zoals bijvoorbeeld extra voedingscomponenten met mogelijks gunstige gezondheidseffecten) zou kunnen leiden tot nieuwe manieren om gewichtstoename te behandelen of voorkomen. Enkele voorgaande studies onderzochten reeds de potentiële gezondheidseffecten van omega-3 vetzuren afkomstig van mariene bronnen (i.e. eicosapentaeenzuur, EPA en docosahexaeenzuur, DHA). Echter, er zijn slecht beperkte resultaten te vinden over deze studies met betrekking tot mogelijke anti-obesitas effecten. Het doel van deze masterthesis was het ontwikkelen van een proefstudie met een *double-blind, randomized crossover* design waarbij getracht werd om enkele mogelijke effecten van omega-3 vetzuur (i.e. EPA + DHA) supplementatie bij het ontbijt te onderzoeken in twee verschillende gewichtcategorieën (normaal gewicht en overgewicht/obesitas). Deze studie was een eerste stap naar het onderzoeken van de effecten op energieverbruik en eetlust, door een hoge dosis omega-3 vetzuren (2000 mg EPA + 2000 mg DHA) toe te dienen. Eetlust en verlangingsresponsen werden gemeten met visuele analoge schaal, *resting energy expenditure* werd gemeten met indirecte calorimetrie en de energie-inname (kcal) van de rest van de dag werd gemeten via een 24-uurs voedselinname logboek. Deze proefstudie toonde aan dat de consumptie van ontbijt gesupplementeerd met omega-3 vetzuren resulteerde in verminderde postprandiale hongersensaties en een verhoogd gevoel van volheid, in vergelijking met het consumeren van het controle ontbijt (zonder omega-3 vetzuren) en dit effect was aanwezig in zowel participanten met een normaal gewicht als participanten met overgewicht. Het consumeren van het ontbijt aangevuld met omega-3 vetzurenen resulteerde ook in een verhoogde postprandiale *resting energy expenditure*. Er was echter geen verschil merkbaar in postprandiale koolhydraatoxidatie, maar de vetoxidatie was significant hoger na het consumeren van het ontbijt aangevuld met omega-3 vetzuren in participanten met een normaal gewicht, echter dit effect werd niet significant gevonden in participanten met overgewicht. Het 24-uurs voedselinname logboek toonde geen verschil in energie-inname (kcal) tijdens lunch, avondmaal of snacks tussen het omega-3 en het controle ontbijt en dit zowel bij participanten met een normaal gewicht als participanten met overgewicht.

1 Introduction

Overweight and obesity are growing health problems throughout the world in both developed and developing countries. These complicated and multifactorial chronic diseases may result in an increased risk of several noncommunicable diseases (e.g. diabetes), reduced quality of life and life expectancy, as well as an increased health and economic concern for society [21, 61, 117]. According to data from the World Health Organization, more than 1.9 billion adults aged 18 years and older were overweight (approximately 39% of the world's population) in 2014 [154]. Younger women (18-23 years of age) are considered to be at high risk for weight gain [1].
The fundamental cause of weight gain is a chronic energy imbalance between the amount of calories consumed versus the amount of calories expended. Subsequently, most recommendations from known public health organizations for reducing body fat are based on consuming a nutrient dense, healthy and balanced diet, and increasing physical activity. Despite widespread recommendations, many individuals have difficulty sticking to a healthy lifestyle, especially over the long term. Hence, the prevalence of obesity continues to be a mounting health concern. Therefore, identifying novel strategies to treat and prevent weight gain is essential and food components with potential beneficial health effects on weight loss have become attractive. Overall it is necessary to ensure a proposed dietary intervention is applicable for both lean (by preventing the increase of weight gain) and overweight/obese individuals (by reducing weight gain).
The total amount of energy an individual uses in a day can be illustrated as the total daily energy expenditure (TDEE), composed of resting metabolic rate, thermic effect of activity, and the thermic effect of food. The latter one has been shown to be macronutrient dependent. Food products lowering appetite or increasing the satiety during the day, are of interest as well.
Long-chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA) have previously been linked with several health benefits such as cardiovascular health [133]. However, sufficient data is lacking regarding the influence of the consumption of LC n-3 PUFA (in particular eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) and their potential anti-obesity effects.

The aim of this thesis is to establish a pilot study investigating the effect of EPA and DHA (omega-3 fatty acids from marine sources) at breakfast on postprandial energy expenditure (i.e. on thermic effect of feeding), substrate utilization, and the appetite/satiety in young women. This pilot study uses a double-blind, randomized crossover design in which participants act as their own control.
The first section of this thesis illustrates the worldwide problem of obesity with some associated health complications, several causes and potential treatments. Omega-3 fatty acids are discussed with their mechanisms of action and some possible anti-obesity effects that were proposed in the past. In the second section, the problem statement and objective of this research project are further clarified. A total overview of how this pilot study was established, performed, and analyzed is given in the third section of this thesis. The results of this study can be found in the fourth section. Data about the participants characteristics, appetite response, energy expenditure, and a 24-hour food log are presented. Finally, discussion of this study and of the results together with an overall conclusion of this dietary intervention pilot study and some further perspectives are presented in the last section of this thesis.

I LITERATURE STUDY

1 Obesity

According to the World Health Organization (WHO), overweight and obesity are defined as an abnormal or excessive fat accumulation ($>$ 20% of body weight) that may impair health [81]. The term widely used to categorize overweight and obesity is body mass index (BMI) or Quetelet index. The BMI value is derived from an individuals weight and height and is calculated dividing the weight (in kilograms) to the square of height (in meters). The WHO defines obesity by a BMI value of $\geq$ 30.0 kg/m^2. The BMI classification is shown in table 1.1.

Table 1.1: Body Mass Index classification.

BMI (kg/m^2)	**Classification**
$<$ 18.5	Underweight
18.5-24.9	Normal weight
25.0-29.9	Overweight
30.0-34.9	Class I obesity
35.0-39.9	Class II obesity
$\geq$ 40.0	Class III obesity

Although BMI is an easy and quick method, it only gives an impression but no true estimation of the amount of body fat. More accurate methods to determine body fat include skinfold thickness measurements, total body potassium, underwater weighing, and dual-energy x-ray absorptiometry (DXA) [81]. However, measuring body fat using these techniques is generally expensive and not always readily available. For that reason BMI is a more practical approach for a clinical setting.
In this thesis, overweight is defined as a BMI value of 25.0-29.9 kg/m^2 and obesity as a BMI value of $\geq$ 30 kg/m^2.

1.1 Worldwide problem

Overweight and obesity are growing health concerns throughout the world [81]. According to data from the WHO, more than 1.9 billion adults aged 18 years and older were overweight (approximately 39% of the world's population) in 2014 [154]. That 39% consisted of 38% men and 40% women. Over 600 million of those adults were obese (approximately 13% of the world's population) [154]. Comparing the year 2014 to the year 1940, a doubling in the worldwide prevalence of obesity can be observed [155]. Every country that is included in the WHO data repository showed an increase in adult obesity rates from 2010 to 2014. During this time period, none of these country's obesity rates remained the same or decreased [155].

In the United States, the prevalence of obesity among adults aged 20 and over increased from 19.4% in 1997 to 30.4% in 2015 [143]. According to recent data, obesity rates from male and

female adults exceeds 35% in four states in the US (Louisiana, Alabama, Mississippi and West Virginia), 30% in 25 states and are above 20% in all states [139].
Likewise, obesity is a public health challenge in Europe [156]. Since the 1980s, the prevalence of obesity has tripled in many countries of the WHO European Region. In the European Food and Nutrition Action Plan 2015-2020, WHO indicated in that in 46 countries in the WHO European Region (accounting for 87% of the Region) more than 50% of adults are overweight or obese [156]. The prevalence of overweight and obesity among children and adolescents from countries of the Eastern part of the WHO European Region is moving towards the high rates found in the Western part of the WHO European Region.

Young women are considered to be at high risk for weight gain [1]. Studies in Australia and the United States looked into this early adulthood life stage of women and reported an average weight gain of 6.3 to 11.7 kg from the age of 20 to the age of 30 years old [1, 99] which could be due to a lot of changes during this life stage, e.g. education, job rotation, marriage, and pregnancy [24].

1.2 Health complications

Overweight and obesity bring along several health complications. A BMI value higher than 25 is regarded as overweight and is a major risk factor for several noncommunicable diseases [154]. Hypertension, type 2 diabetes mellitus (T2DM), coronary heart disease, dyslipidemia, sleep apnea and respiratory problems, stroke, osteoarthritis, and cancer are some examples of the noncommunicable diseases positively linked with overweight and obesity [33, 60].
As previously mentioned, overweight and obesity are a common problem among young women (at reproductive age). There is also an association between obesity and infertility. Overweight women have a higher risk of anovulatory cycles, menstrual dysfunction (this may cause abnormal ovulation, menstrual abnormalities and excess hair growth in obese women due to hormonal imbalances), difficulties in assisted reproduction, miscarriage, and adverse pregnancy outcomes [61, 44]. In addition, the Nurses' Health Study showed that once the age of 18 was reached, the chance of developing type 2 diabetes in women increased by 1.9 times if they gained 5 to 8 kg during early adulthood and this change increased by even 2.7 times when gaining 8 to 11 kg [37, 149].
Taken together, all of these health complications of overweight and obesity demonstrate the importance of prevention, management and treatment.

1.3 Causes of obesity

As obesity is the second leading preventable cause of disease and death in the United States [62], it is useful to understand the underlying causes of weight gain to help prevent the onset of obesity and the chronic diseases associated with it.
However, overweight and obesity is a complicated and multifactorial chronic disease. It can develop from an interaction of an individuals genotype and the environment [97]. Generally, a complete understanding of the way and reason overweight and obesity develops is incomplete, but it can result from an interaction between various factors including social, behavioral,

cultural, physiological, medical, metabolic and genetics [97] as well as a lack of supportive policies in sectors health, agriculture, transport, urban planning, environment, food processing, distribution, marketing and education [154].

1.3.1 Energy balance

The fundamental cause of overweight, and eventually obesity, is a chronic energy imbalance between the amount of calories consumed (i.e. energy intake, EI) versus the amount of calories spent (i.e. energy expenditure EE). When EI consistently exceeds EE, this will eventually lead to the storage of surplus energy in the white adipose tissue. Harmonizing this energy balance is crucial for treatment and prevention of overweight and obesity [14].
The first modifiable aspect of energy balance is energy intake (EI), which is directly associated with an individual's diet. There is an increased consumption, as well as the overall constant availability, of energy-dense food products, increased consumption of larger portion sizes, the low cost of an unhealthy diet [81], and diet shifts from traditional foods to Western diets [100]. A strong indication exists for a relationship between the increased burden of obesity and energy-dense diets, processed foods, excess consumption of saturated fats, trans fats, free sugars and salt, and low consumption of fruit and vegetables [156].
The second fundamental aspect of energy balance is energy expenditure (EE). Increasing the exercise level (e.g. physical activity) is associated with promoting changes in body composition and weight, even when keeping dietary intake constant [130]. However, the combination of exercise and dietary restriction can promote more favourable changes in body composition than any of those on its own [130]. In addition, physical activity also has other benefits in reducing cardiovascular and diabetes risk [145]. To set a healthy long-term plan, adults should incorporate at least 30 minutes or more of physical activity on most, and preferably all, days of the week [97]. Successful obesity treatments include both increased physical activity and reduced caloric intake.

Energy expenditure

Total daily energy expenditure (TDEE) is composed of three major components, i.e. resting (or basal) metabolic rate (RMR or BMR), the thermic effect of activity (TEA), and the thermic effect of food (TEF).
Resting metabolic rate is the amount of energy expended while being at complete rest, the energy needed to maintain the major body functions at rest, and it constitutes 60 to 75% of the total daily energy expenditure. Significant contributors of RMR are fat mass, sex, age, genetic traits, stress, caffeine, pregnancy, and fat-free mass with the latter one by far the strongest determinant (i.e. accounts for $\sim$70% variance) of RMR [18, 80]. The resting metabolic rate slows down due to weight loss, and this may counteract further weight loss and maintaining lost weight. This phenomenon is called "metabolic adaptation" or "adaptive thermogenesis". A recent study on morbidly obese individuals following an intensive calorie-restricted diet (i.e. 70% of their baseline energy requirements, calculated as 21.6 kcal/kg.d x FFM (fat-free mass, kg) + 370 kcal/d) and exercise interventions (i.e. 90 min/d for 6 d/wk) over a total time of 30 weeks, showed a strong slowing down of RMR at the end of the program (-275 kcal/d) and even an ongoing decline (increased magnitude to -499 kcal/d) at 6 years post-intervention [72, 54].

Thermic effect of activity includes energy expenditure that accounts for energy consumed during energy-related activity thermogenesis and nonexercise activity thermogenesis [57]. This component is a highly variable component, ranging from about 15% of TDEE in very sedentary individuals to 50% or more of TDEE in highly active individuals [86]. Contributors to physical activity level, both spontaneous and voluntary, are genetic traits, age, sex, and the response to environmental stimuli [86].
Thermic effect of food, or dietary induced thermogenesis (DIT), is the energy required during the post-prandial period for digestion, intestinal absorption of nutrients, and assimilation and storage of the nutrients from food, and constitutes approximately 10% of TDEE [147]. However, the extent of TEF has been shown to be macronutrient dependent. The magnitude of thermic response to fat, carbohydrate and protein ingestion ranges respectively from 0 to 3%, 5 to 10%, and 20 to 30% [134]. Besides the diet composition, other contributors of TEF are age, physical activity, obesity, and insulin resistance. For example, a crossover study by Raben et al. [116] investigated the different effects on energy expenditure of breakfast meals with similar energy densities and fiber contents but rich in protein (32% of total energy), fat (65% of total energy), carbohydrate (65% of total energy), or alcohol (23% of total energy). In total 19 young (age: 23.3 ± 0.5 years), normal weight (BMI 22.1 ± 0.4 kg/m^2), healthy men and women were tested. The result showed that the TEF was highest after the alcohol meal (by 27%, $p < 0.01$), followed by the protein meal (by 17%, $p < 0.01$) however this last one was not significantly different compared to the carbohydrate and fat meal [116]. Westerterp [147] reviewed different studies about TEF and concluded that, after energy content, the protein fraction of a meal is the main determinant of TEF.

1.3.2 Breakfast skipping

Breakfast is typically defined as the first meal of the day. However, the term breakfast has no clear and straightforward definition in the literature [104]. Various definitions have been used such as ” An eating occasion that occurred between 5 am and 10 am on weekdays, and 5 am and 11 am on weekends” [3, 141, 10, 2, 4], ”Consumption of food, beverage, or both between 5 am and 10 am” [126], ”First meal of the day” [6, 123] (in the morning) [148], ”The first meal of the day, eaten before or at the start of daily activities (e.g. errands, travel, work), within 2 hours of waking, typically no later than 10 am, and of a calorie level between 20% and 35% of total daily energy needs.” [106, 136], ”The first meal of the day, taken before or at the start of daily activities with an energy content that meets 20% to 25% of total daily energy needs, and which includes dairy products, cereals, fruit, and healthy fats.” [91]. This lack of consistent use of the same interpretation of breakfast makes it difficult comparing study conclusions [104].
The word ”breakfast” is derived from the phrase ”breaking the fast”. As a combination of different interpretations, the following definition of breakfast will be used:

> Breakfast is the first meal of the day that breaks the fast after the longest period of sleep. It is consumed before 10 a.m. and consists of food or beverage from at least one food group. It contains an energy content between 20% and 30% of total daily energy needs.

The prevalence of breakfast skipping in adult Americans both men and women has increased over the years [73, 58]. Based on data from NHANES (National Health and Nutrition Examination Survey), over the last 40 years there was a decline of breakfast consumption in American

adults [74]. Approximately 20% to 30% of both American and European adults do not consume breakfast on a regular basis [128, 76]. Over the past few decades the consumption of breakfast has declined and parallel with that the prevalence of overweight and obesity has increased [128], breakfast skipping has been widely believed to be a possible element influencing weight gain in adults [23]. For example, data from NHANES during 1999-2002 (n=5316, age: 20-39 years) showed individuals consuming ready-to-eat cereal (although these can still contain quite some sugar) were 31% less likely to be classified as overweight/obese and 39% less likely to have abdominal obesity (significantly and independently associated with risk factors of cardiovascular disease and chronic kidney disease in young adults [121]) if compared to individuals who did not consume breakfast [45]. Younger age, smokers, late consumption of dinner, increased alcohol consumption, higher daily energy intake, and not exercising frequently are some of the factors having a positive influence on a breakfast skipping behaviour [128]. A long-term study by Odegaard et al. [102] with young adults (n=3598, 18-30 years) showed an inverse relationship between breakfast consumption and weight gain over a period of 18 years. The weight of participants who reported consumption of breakfast on a daily basis increased 1.9 kg less compared to participants consuming breakfast 0-3 days/week [102]. There is also an association between breakfast consumption and lower BMI values in adults [88, 106, 102, 114, 32]. For example, a 5-year longitudinal study with adolescents (n=2216) reported an inverse association between frequency of breakfast consumption and BMI with adolescents always skipping breakfast experiencing the greatest increase in BMI and adolescents consuming breakfast on a regular basis experiencing the smallest increase [137].

Effect of breakfast skipping on appetite

It is believed that omission of breakfast leads to increased appetite [106]. Visual analogue scales are usually used to assess appetite feelings (e.g. hunger, fullness). Various studies showed an clear pattern of appetite suppression during the morning when subjects consumed breakfast, compared to subjects who skipped breakfast [8, 35, 34]. A randomized crossover study by Astbury et al. [8] with twelve men (age: 23.4 ± 7.3 years, BMI: 23.5 ± 1.7 kg/m^2) demonstrated that consuming breakfast (containing 14%, 14%, and 72% energy from protein, fat, and carbohydrates, respectively) resulted in lower hunger ratings and higher fullness ratings, compared to not consuming breakfast ($p < 0.05$). Another recent randomized study by Clayton et al. [35] with eight male, habitual breakfast eaters demonstrated that omitting breakfast appeared to have a decreasing effect on fullness and an increasing effect on hunger, desire to eat and prospective food consumption before lunchtime ($p < 0.05$) compared to consuming breakfast. However, whether or not the participant consumed breakfast, no differences were shown after lunch ($p > 0.193$). A 7-days randomized crossover study by Leidy et al. [85] with 20 breakfast-skipping adolescent girls (age: 19 ± 1 years, BMI: 28.6 ± 0.7 kg/m^2) investigated the effect of consumption of a 350-kcal normal-protein breakfast (13 grams protein), a 350-kcal high-protein breakfast (35 grams protein), or continuing of skipping breakfast. The study showed an overall reduction of daily hunger and increasing fullness sensations when breakfast is consumed, compared to breakfast omitting. However, the high-protein breakfast had a higher impact on increased fullness feeling. This study also investigated various hormones and demonstrated that the high-protein, but not the normal-protein, breakfast also increased concentrations of peptide YY (a satiety hormone) [85].

However, the effect of breakfast consumption on appetite suppression is possibly only transient

as some studies showed that the appetite response to following meals appears to be not affected by prior breakfast omission [34]. Nonetheless, note that subjective appetite sensations do not always give a right prediction on following energy intake [34].

Effect of breakfast on energy intake

The increased appetite effect when skipping breakfast can cause decreased satiety and eventually overeating (i.e. overcompensation) during the rest of the day [89], with ultimately weight gain over a longer period of time [106]. Although other scenarios could take place as well, i.e. no weight change over time when there is no overeating later in the day when skipping breakfast or even weight loss if there is an under-compensation [89]. The same study by Astbury et al. [8] mentioned before also investigated the energy intake at lunch after consuming or omitting breakfast and demonstrated a 17% lower intake when breakfast was consumed ($p < 0.01$). The 7-days randomized crossover study by Leidy et al. [85] with 20 breakfast-skipping adolescent girls (age: 19 ± 1 years, BMI: 28.6 ± 0.7 kg/m^2) investigated also energy intake after breakfast skipping, normal-protein or high-protein breakfast consumption. Breakfast and lunch were controlled at 350 and 500 kcal, respectively, whereas dinner and snacks were provided ad libitum. This study did not show a significant difference in dinner consumption, but the amount of energy consumed during the ad libitum snacking was higher with breakfast skipping and normal protein breakfast (656 ± 108 and 621 ± 110 kcal, respectively), compared to high protein breakfast (486 ± 84 kcal). Eventually, the total daily energy intake was not much different between breakfast skipping (2002 ± 111 kcal) and high-protein diet (2123 ± 71 kcal). However, the total daily energy intake with normal-protein diet (2292 ± 115 kcal) was significantly different from breakfast skipping and high-protein diet ($p < 0.003$ and $p < 0.05$, respectively).
Altogether, even if suggested that breakfast consumption will lower the energy intake during the rest of the day, various studies do not support this assumption [34].

Effect of breakfast on energy expenditure

Consuming breakfast in the morning will inevitably increase the energy expenditure as a result of the diet induced thermogenesis (or thermic effect of food) [147]. As well, a recent 6-wk randomized controlled trial (the Bath Breakfast Project) by Betts et al. [12] studied all components of energy balance in 33 men and women (age: 21 - 60 years, BMI: 22.4 ± 2.2 kg/m^2) with regular daily breakfast relative to extended morning fasting. This study showed no metabolic adaptation (i.e. increased resting energy expenditure), however breakfast consumption significantly increased the physical activity thermogenesis (1449 ± 666 and 1007 ± 370 kcal/day for breakfast and fasting group, respectively). The increased physical activity thermogenesis especially originated from higher light intensity activity during the morning in the breakfast consuming group.

Effect of breakfast quality

Observational studies focusing on breakfast quality and found that habitual breakfast consumers, compared to breakfast skippers, are more likely to consume a higher-quality diet [84].

Such a higher-quality diet is linked with a higher intake of vitamins, minerals, and dietary fiber but lower amounts of dietary fat and cholesterol [84]. Despite the possibility of the scenario that energy intake remains the same when skipping breakfast, skipping breakfast and the normally concomitant provision of vitamins, minerals, and trace elements during breakfast are not compensated for during the rest of the day [158, 98]. In the Third National Health and Nutrition Examination Survey, the meal and snack patterns of 15,978 US adults (age: 20 years and older) were examined and demonstrated that individuals who skip breakfast had the lowest intakes of all micronutrients, except for sodium [75]. Additionally, a decreased consumption of unhealthy snacks (e.g. soft drinks, sweets, and salty snacks) that are energy-dense, high in saturated fats, and/or have a high sugar level is noticed in breakfast consumers [84]. Dietary factors as consumption of high-fat and/or high-sugar snacks and soft drinks are indicated as having an importing role in the etiology of obesity [111].

Even though some studies showed some effects of breakfast frequency on BMI in adults, not many research studies were actually able to proof this statement and it is only presumed to be true [23]. This cumulative meta-analysis of cross-sectional studies showed that the proposed effect of breakfast on obesity only has ambiguous evidence as well as there are a lot of non probative studies on this proposed effect [23]. These different and sometimes ambiguous results may be caused by the diverse ways of how individuals respond to habitual breakfast skipping [89]. Finally it is important to take in mind the methodological differences across experimental studies when comparing them [89].

1.4 Influence of body composition on energy intake

Energy homeostasis, i.e. the mechanism that makes sure that over the longer term the amount of food intake will provide energy equivalent to the amount of energy expended, will realize body weight regulation [153, 16]. Fat-free mass is the largest contributor to resting metabolic rate (RMR), this latter is on its turn the largest contributor of total energy expenditure [16]. All these, i.e. FFM and/or RMR, may be related with energy intake during the day. A 12-week intervention study by Blundell et al. [16] with obese adults (n=92, age: 40.8 ± 9.2, BMI: 31.6 ± 4.3 kg/m^2) investigated these possible interactions. This study demonstrated a clear and significant positive relationship between meal size and daily energy intake with the amount of FFM, but not with fat mass (FM) nor with BMI. It was suggested that a possible mechanism for this positive relationship between FFM and EI as the amount of energy that is required to maintain lean tissues which regulates the minimum level of energy intake [16], meaning obese individuals (having a higher amount of lean mass in support of their higher amount of FM) and individuals with a great amount of muscle mass (e.g. athletes) probably have a higher penchant for consuming bigger meals [16].
Blundell et al. [17] also investigated a group of young lean adults (n=47, mean age: 20 years, mean BMI: 22 kg/m^2) to find out if the effects are the same in lean individuals. In obese individuals there was also a clear and significant positive relationship between meal size and daily energy intake with the amount of FFM. However, in lean individuals FM had a strong significant inhibitory effect on EI. Blundell et al. suggested this effect as a result of fat being a storage of energy, and adipose tissue generating negative feedback signals when sufficient energy reserves in the body [17]. This feedback system being highly sensitive and both insulin

and leptin playing a role in this mechanism. However, insulin and leptin sensitivity decreases with increasing adiposity which could mean that overweight and/or obese individuals could encounter a lowering effect the inhibitory action of FM on EI [17].

1.5 Treatments of obesity

Overweight and obesity have clear disadvantages to human health, with increased morbidity and mortality [154]. Reducing weight in overweight and obese people is recommended due to several beneficial effects. There is strong evidence that reducing weight in overweight and obese individuals accompanies reducing risk factors for diabetes and cardiovascular disease (CVD) [97]. The basic fundamental principle for successful weight loss is to create a negative energy balance (i.e. EI $<$ EE) [81]. Overweight/obesity treatment requires an achievable and flexible long-term management[81]. Essential for interventions is dietary modification, cognitive behavior therapy, and lifestyle modification, and physical activity [81]. Other approaches for the treatment of obesity include drug therapy and weight loss surgery [81]. Diets exist, including low-fat, low-carbohydrate, meal replacements, intermittent energy restriction diet, etc. [135].
Interestingly to mention, the resting metabolic rate (RMR) slows down due to weight loss, and this may counteract further weight loss and maintaining lost weight. This phenomenon is called ”metabolic adaptation” or ”adaptive thermogenesis”. A recent study on morbidly obese individuals following intensive diet and exercise interventions, showed a strong slowing down of RMR at the end of the program (-275 kcal/d) and even an ongoing decline (increased magnitude to -499 kcal/d) at 6 years post-intervention [54].

2 Omega-3 fatty acids

Omega-3 (ω-3 or n-3) fatty acids are polyunsaturated fatty acids (PUFA) with a double bond at the third carbon atom counting from the methyl (-CH3) end of the acyl chain. The most simple omega-3 fatty acid is α-linolenic acid (ALA) [18:3(n-3)]. The human body is able to metabolize and convert a small amount of ALA to the more biologically active longer chain omega-3 PUFA eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) by a series of linked desaturation and elongation reactions (see Figure 2.1), largely conducted in the liver in the endoplasmic reticulum. This conversion is relatively inefficient in the human body. In particular the conversion to the end product DHA appears to be limited [133, 7]. Subsequently, the conversion of ALA cannot produce sufficient amounts o EPA and DHA, meaning these omega-3 fatty acids should come from the diet [94]. The process of retroconversion to generate DHA and EPA is possible through limited peroxisomal β-oxidation of DHA. Figure 2.2 shows the structures of ALA, EPA, and DHA.
The conversion of linoleic acid to arachidonic acid (both omega-6 fatty acids) uses the same enzymes as the conversion of α-linolenic acid to EPA which leads to a competition. Δ6-desaturase is considered to be rate limiting in this pathway. Nutritional status, hormones, as well as feedback inhibition systems by the end products are considered to regulate the Δ6- and Δ5-desaturases activities. It is important to note that aging, alcohol consumption, nutrient deficiencies, trans fatty acids and elevated cholesterol levels can impair the action of Δ6-enzymes [5].

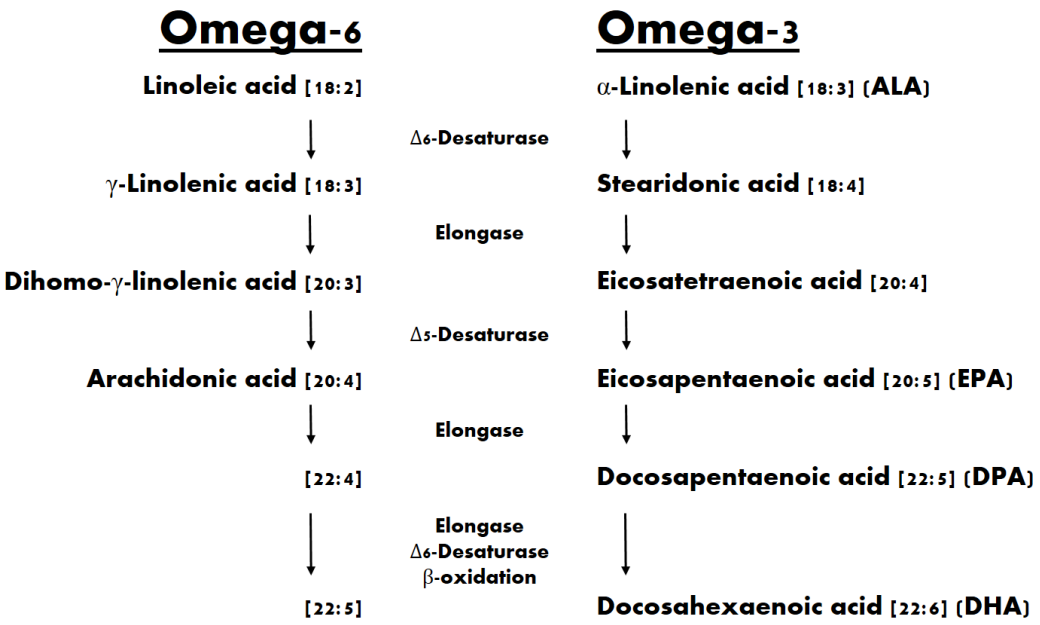

Figure 2.1: The biosynthesis of LC n-3 PUFA EPA and DHA, starting from ALA. [28][7].

α-linolenic acid (n-3) is an essential fatty acid, as well as linoleic acid (n-6). ALA is mostly found in plant oils. EPA and DHA are considered conditionally essential and they are commonly found in significant quantities in fish and other seafood, and so they may be referred to as marine n-3 fatty acids. These fatty acids can be found in fish oils, algae-derived marine oils, egg oil, squid oils, and krill oil. The content of LC n-3 PUFA changes significantly among and within the fish species, as well as in function of the fat content of the fish. The richest sources of

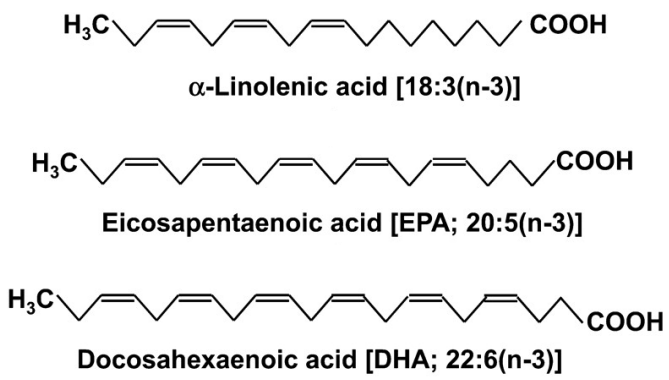

Figure 2.2: Structures of ALA, EPA, and DHA [28].

EPA and DHA are found in the flesh of high-fat (10-15%) cold-water fish like salmon, sardines, mackerel, and sprat [87], and in the livers of some lean fish (e.g. cod).

2.1 Current intake and recommendations

The American diet has shifted over time to contain high amounts of saturated fatty acids, and low amounts of omega-3 PUFA [141]. According to data from NHANES 2003-2008, intakes of ALA, EPA and DHA from food only were 1.4 g/d, 18 mg/d and 50 mg/d respectively, in American adults (age $>$ 19 years) [105]. If including the dietary supplements, these adults reached total intakes for ALA, EPA, and DHA of 1.6 ± 0.04 g/d, 41 ± 4 mg/d, and 72 ± 4 mg/d, respectively [105]. This paper also demonstrated a lower total intake of EPA in women versus men (39 ± 4 versus 44 ± 6 mg/d) and DHA (59 ± 4 versus 90 ± 4 mg/d) [105]. The low intake of dietary EPA and DHA, the two biologically important dietary LC n-3 PUFA, is considered to be associated with increased inflammatory processes, poor fetal development, general cardiovascular health, and risk for the development of Alzheimer's disease [133]. Generally, the recommendation is to eat at least 8 ounces ($\simeq$ 230 grams) of seafood per week, to get the target amount of at least 250 mg/d of EPA and DHA [142].

2.2 Beneficial health effects

Throughout the life cycle, LC n-3 PUFA are important nutrients and the potential beneficial health effects of consuming supplemental omega-3 fatty acids have been examined in a considerable number of clinical trials. However, the health benefits are still somewhat unclear.
LC n-3 PUFA are incorporated into cell membrane phospholipids of different tissues, where they can alter the composition and modify the permeability and viscosity of the cell membrane, and influencing metabolic processes [83, 70]. Another health benefit is the role of LC n-3 PUFA on anti-inflammatory processes. Both EPA and DHA have previously been shown to have protective effects in diseases with inflammation as key component of the pathology (e.g. coronary artery disease, rheumatoid arthritis, inflammatory bowel disease, and asthma) [51, 113].

2.3 Mechanisms of action of n-3 PUFA

Omega-3 polyunsaturated fatty acids can act on and interact with the human body in multiple ways. Overall a few mechanisms can be suggested by which they could affect cell and tissue behavior to elicit their physiological actions.

As indicated previously, LC n-3 PUFA play a protective role in anti-inflammatory processes, such as a decreased production of eicosanoids from arachidonic acid (ARA) due to a lowered membrane content of arachidonic acid and inhibition of ARA metabolism, an increased production of eicosanoids derived from EPA due to an increased content of EPA in the cell membrane, an increased production of pro-resolution resolvins due to and increased membrane content of EPA and DHA, and a decreased production of inflammatory cytokines due to the down-regulated gene expression of inflammatory cytokines (via e.g. NFκB, nuclear factor kappa B, and PPAR-γB, peroxisome proliferator activated receptor gamma) [29, 27, 30, 124]. The main anti-inflammatory processes are illustrated in Figure 2.3.

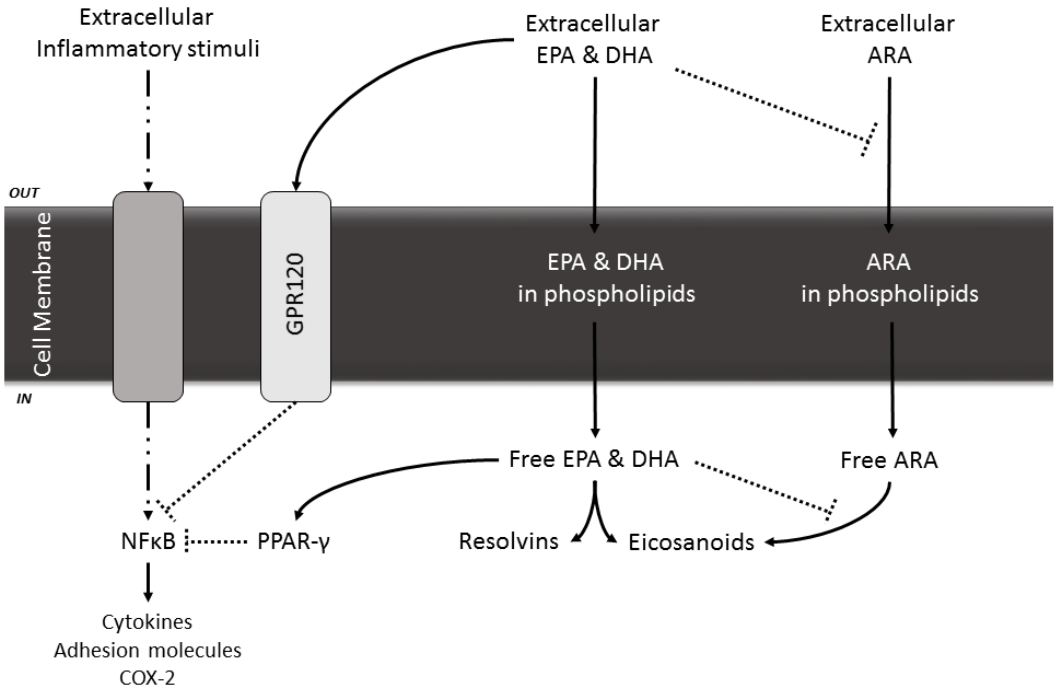

Figure 2.3: Illustration of the anti-inflammatory role of LC n-3 PUFA. Modified from Calder et al. [29]. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; ARA, arachidonic acid; COX, cyclooxigenase; NFκB, nuclear factor kappa B; PPAR-γB, peroxisome proliferator activated receptor gamma.

2.3.1 Direct effects on cell behavior via surface or intracellular fatty acid receptors or sensors

EPA and DHA may act via cell surface (GPR120) and intracellular (PPAR-γ) receptors to control inflammatory cell signalling and gene expression patterns [30].

Interaction with NFκB

Nuclear factor kappa B (NFκB) is one of the key transcription factors involved in activating the genes encoding proteins involved in inflammation, including many cytokines, adhesion molecules, and cyclooxygenase (COX)-2. The inactive state of NFκB exists as a trimer in the cytosol with one of the subunits being inhibitory (IκB). Activation of NFκB occurs through various extracellular inflammatory stimuli, including bacterial endotoxin, inflammatory cytokines, and oxidative stress. IκB has to be phosphorylated which leads to dissociation of this inhibitory subunit from the remaining dimer NFκB and is degraded. The remaining dimer NFκB is able to translocate to the nucleus where it binds to response elements and upregulates gene expression. LC n-3 PUFA EPA and DHA seem to inhibit the production of several inflammatory cytokines and COX-2 metabolites (the actions on COX might be the best characterized anti-inflammatory functions of n-3 PUFA) [77]. This inhibitory effect can be associated with decreased IκB phosphorylation and decreased activation of NFκB [28, 30].

Influence on intracellular PPAR

Peroxisome proliferator activated receptors (PPAR) are transcription factors that regulate gene expression, having a role in cell and tissue responses to the environment. PPAR-α and PPAR-γ are two well known PPAR isoforms [46]. PPAR-α is, for most part, expressed in the liver where it is involved in regulating hepatic responses to the presence and availability of certain fatty acids, and fatty acid metabolites. PPAR-α may be essential in partitioning fatty acids toward hepatic oxidation. PPAR-γ is expressed in the adipose tissue and in inflammatory cells. In the adipose tissue it acts as a regulator of adipocyte differentiation and regulation of the metabolic responses of adipocytes, including promoting insulin sensitivity. In inflammatory cells it has an anti-inflammatory effect. Marine n-3 fatty acids are able to activate PPAR-γ [30]. DHA induced PPAR-γ in dendritic cells was linked with the inhibition of NFκB activation and lower production of the inflammatory cytokines tumor necrosis factor (TNF)-α and interleukin (IL)-6 which are produced following endotoxin stimulation. A randomized 18-week study from Mehra et al. [90] with fourteen patients (with Class III to IV heart failure) demonstrated a significant reduction in the inflammatory cytokes TNF$-\alpha$ and IL-1, when receiving 5.1 g/day of EPA and DHA, compared to the control (placebo of corn oil). PPAR-γ can physically interfere with the translocation of NFκB to the nucleus [30]. Various types of fatty acids are able to activate PPAR however, PUFA have been shown to be the preferred activators for both PPAR-α and PPAR-γ [77]. All together, LC n-3 PUFA activate PPAR and due to this they are able to regulate metabolism and other cell and tissue responses which might explain a few of the physiological actions of LC n-3 PUFA (e.g. lowering fasting plasma triglyceride concentrations, increasing insulin sensitivity, and reducing inflammation) [28, 30, 77].

Involvement of G-protein coupled cell membrane receptor

Long chain fatty acids can be bound by both GPR40 and GPR120. Both these G-proteins coupled receptors are able to activate intracellular signalling pathways. GPR120 is highly expressed on mature adipocytes and proinflammatory macrophages [28, 103] and it is suggested that it is involved in anti-inflammatory signalling [30]. EPA and DHA have shown similar effects compared to a synthetic agonist of GPR120, which is capable of inhibiting the macrophage

response to endotoxin, an effect that involves the maintenance of cytosolic IκB and a decrease in production of TNF and IL-6 [30]. The latter suggests that the G-proteins coupled receptor 120 is related to anti-inflammatory signalling. A study from Oh et al. [103] fed obese WT mice and GPR120 knockout mice a high-fat diet with or without omega-3 fatty acids supplementation. Treatment with omega-3 fatty acids inhibited inflammation and increased systemic insulin sensitivity in WT mice, but there was no significant effect in GPR120 knockout mice. This suggests that GPR120 is a functional ω-3 FA receptor and able to mediate potent insulin sensitizing effects (inflammation is a key mechanism for insulin resistance in obesity) in vivo by suppressing macrophage-induced tissue inflammation [103].

As mentioned before, there are three possible mechanisms by which EPA and DHA might act to suppress inflammatory signalling via NFκB. However, the way or extent these three mechanisms are coupled is not clear yet [103].

2.3.2 Actions mediated via changes in the composition of cell membrane phospholipids

Role in cell membrane phospholipids

Cell membrane phospholipids contain fatty acids, which have several important roles, e.g. creating a suitable environment for membrane protein function, maintaining proper membrane order (i.e. fluidity), and affecting lipid raft formation [28].

Exposure to n-3 PUFA influence cell membrane fatty acid composition

Intake of marine LC n-3 PUFA supplementation modifies the fatty acid profiles of cells and tissues. Increased intake results in an increase of EPA and DHA content in plasma lipids, platelets, erythrocytes, leukocytes, colonic tissue, and liver tissue in a dose-dependent way. Additionally, the dietary supplementation of EPA and DHA is able to replace the n-6 PUFA (e.g. arachidonic acid) present there [28].

Eicosanoids and eicosanoid-like mediators

Eicosanoids are part of mediating and regulating inflammation and are mainly produced by oxidation of PUFA with a length of 20 carbon units, mainly the n-6 PUFA arachidonic acid (ARA) [27]. Besides being involved in modulating the intensity and duration of inflammatory processes, eicosanoids also play a role in the regulation of immunity, platelet aggregation, and smooth muscle contraction [27]. LC n-3 PUFA are able to act directly by decreasing the availability of extracellular ARA and by inhibiting the ARA metabolism, eventually affecting the eicosanoids synthesis (from ARA) negatively. EPA and DHA are also able to synthesize resolvins (see Figure 2.3), which are alternative eicosanoids acting as anti-inflammatory mediators [27].

2.4 Possible anti-obesity effects

It has been suggested that PUFA may have a beneficial influence on fat metabolism. Several studies in the past were conducted on rodents investigating the effects of LC n-3 PUFA supplementation. However, these studies gave conflicting results so no straightforward conclusions about possible effects reducing adiposity or preventing weight gain could be made [25]. An animal study with rats and mice from Ruzickova et al. [120] investigated possible anti-obesity effects of supplementation with EPA and DHA. They demonstrated a reduced weight gain when supplementation with EPA and DHA increased from 1 to 12% (wt/wt) of dietary lipids as well as a reduction in adipose tissue accumulation. This study demonstrated that the adiposity lowering effect LC n-3 PUFA from marine sources (EPA and DHA) tends to be higher than the n-3 PUFA from plant sources (ALA). Some human trials also support the idea of LC n-3 PUFA having this inverse relationship between supplementation with LC n-3 PUFA and obesity, while other human studies show conflicting results and causality should be questioned [67, 64, 69]. Possible effects include suppression of appetite, improvements in circulation which might facilitate nutrient delivery to skeletal muscle and changes in gene expression which shift metabolism toward increased accretion of lean tissue, enhanced fat oxidation and energy expenditure and reduced fat deposition [25].
Two large epidemiological studies, the Health Professional Follow-Up Study and the Nurses' Health Study, provided opposing evidence on the association between LC n-3 PUFA intake and obesity prevalence. The Health Professional Follow-Up Study data in men (n=43,671, age: 40 - 75 years) demonstrated that men with high fish consumption were less likely to be overweight compared to those with low fish consumption (54.2% for individuals who consumed fish $<$ once a month versus 49.4% for individuals consuming fish $\geq$ 5 times a week) [64]. However, data from the Nurses' Health Study (a study in 79,839 women) reported a higher prevalence of having a BMI $\geq$ 29 kg/m^2 with higher fish and LC n-3 PUFA intake (14.6% for individuals who consumed fish $<$ once a month versus 24.0% for individuals consuming fish $\geq$ 5 times a week) [69]. Taking in consideration that both studies estimated dietary intakes from food frequency questionnaires, which can be seen as a limitation to accurately assess intakes of different types of fat, as this is a semi-quantitative approach.
Measuring fatty acid levels in plasma phospholipids gives an indication of dietary fatty acid consumption over a time period of weeks, whereas measuring erythrocyte levels gives information about dietary intake over a time period of months. A cross-sectional analysis of erythrocyte LC n-3 PUFA content in 476 adults (291 women, 185 men) [68] found an inverse relationship between erythrocyte LC n-3 PUFA levels and BMI, waist circumference, and percentage body fat assessed by DXA. The results of this study showed important gender differences in the relationship of specific LC n-3 PUFA in erythrocytes to markers of adiposity. This study indicated that higher erythrocyte levels of LC n-3 PUFA, particularly DHA in females, are associated with less adiposity.
Increasing the LC n-3 PUFA intake by 0.3-3.0 g/day may benefit body weight and fat reduction by various mechanisms, described below.

2.4.1 Effect on the expression of genes regulating metabolic pathways

Improving body composition after increasing the intake of LC n-3 PUFA is most likely due to an altered gene expression that shift the metabolism towards increased fat oxidation in adipose, liver, cardiac, intestinal, and skeletal muscle tissue: a reduced fat deposition in adipose tissue; and increased energy expenditure. The intake of LC n-3 PUFA is suggested to increase the expression of genes coding for key enzymes playing a role in fatty acid (FA) transport and β-oxidation, e.g. lipoprotein lipase, acetyl-CoA carboxylase-2, FA translocase, and mitochondrial uncoupling protein 3 [65].

5' AMP-activated protein kinase (AMPK) is a heterotrimeric protein, formed by three subunits (i.e. α, β, and γ subunits) with each of them having a specific role in both the stability and activity of AMPK. AMPK plays a role in cellular energy homeostasis, and is expressed in liver, brain, and skeletal muscle tissue. AMPK activation results in stimulation of hepatic fatty acid oxidation, ketogenesis, stimulation of skeletal muscle fatty acid oxidation and glucose uptake, inhibition of cholesterol synthesis, lipogenesis, triglyceride synthesis, inhibition of adipocyte lipolysis and lipogenesis, and modulation of insulin secretion by pancreatic beta-cells [150]. A comparative study investigated the effect of n-3 PUFA on the possible activation of AMPK-α1 in high-fat fed rats [93]. Consumption of a high-fat diet altered the AMPK-α1 gene expression in liver and skeletal muscle of rats, with an outcome of body weight gain and hyperglycemia. The study showed that n-3 PUFA could efficiently activate AMPK-α1 mRNA expression in the skeletal muscle, counteracting the effects of the high-fat diet.
An animal study from Todoric et al. [138] investigated the effect of including LC n-3 PUFA in the diet of obese diabetic db/db mice and lean non-diabetic mice after feeding them with a low-fat standard diet or a high-fat diet. This study demonstrated that inclusion of LC n-3 PUFA in a high-fat diet modified inflammatory gene expression and completely prevented macrophage infiltration in adipose tissue, with an increase in circulating adiponectin levels, in the obese diabetic db/db mice. Down-regulation of genes involved in lipid metabolism (including genes for fatty acid synthase and hormone-sensitive lipase) in adipose tissue of the obese diabetic db/db mice, as well as lowered levels of circulating triglyceride, was observed. Another animal study from Mori et al. [92] demonstrated that obesity-prone mice fed with a diet containing 8% fish oil had a lower body weight gain compared with the mice fed with a diet containing 0% fish oil. This lower rate of weight gain was associated with a higher expression of oxidative enzymes in intestinal tissue. Due to these results the authors concluded that the fish oil might increase intestinal lipid oxidation, leading to the observed anti-obesity effect.

2.4.2 Effect on obesity-related inflammation

Obesity can be characterized by low levels of chronic inflammation [77]. Overweight and obese individuals show infiltration of inflammatory cells in adipose tissue and show moderately raised concentrations of inflammatory mediators in the systemic circulation [29]. Inflammation of adipose cells is a result of the chronic nutrient overload, leading to the release of pro-inflammatory cytokines (TNF-α and IL-6) which ultimately results in cell death [53].
As previously mentioned, LC n-3 PUFA has some possible anti-inflammatory processes (see section 2.3 Mechanisms of action of n-3 PUFA). An intake concentration of $>$2 g/day EPA +

DHA was previously proposed as the required amount to evoke these actions in adults. However, only a few dose-response studies with significant results have been completed [29]. Inconsistent, not significant results, and lack of research are reasons that there are no actual recommendations for effective doses and treatment duration [118].

2.4.3 Appetite

Appetite is the desire to eat food and the control of appetite is an important factor in the success of dietary treatments for overweight and obesity, due to its role in the control of energy intake. The essence of appetite regulation lies in the gut-brain axis [31]. Satiety is the sensation of postprandial feeling of fullness and subsequently results in ending the meal, therefore limiting the meal size [15].

2.4.3.1 Hunger

The key element that acts as control center for hunger is the hypothalamus. A part of the mediobasal hypothalamus, i.e. the arcuate of infudibular nucleus, lets peripheral peptides and other proteins that may interact with its neurons enter through a blood-brain barrier. Those neurons are the ones that are expressed together with peptides stimulating food intake (i.e. neuropeptide Y, NPY) and weight gain (i.e. agouti-related peptide, AgRP). The neurons also co-express with those expressing pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) which are known for inhibiting consumption of food and promote weight loss [9]. Neuropeptide Y and AgRP are known as orexigenic (i.e. induce appetite), while POMC and CART are knows as anorexigenic (i.e. induce satiety) [31].

Ghrelin is a circulating orexigenic, growth hormone-releasing peptide which plays and important role in appetite behavior. Besides the role in appetite behavior, it also plays an important role in regulating the distribution and rate of energy use. This ”hunger hormone” is mainly produced by endocrine cells of the gastric mucosa of the fundus and it is secreted when the stomach is empty [9]. Ingestion of nutrients results in the quick postprandial fall in circulating ghrelin levels [42]. Ghrelin plays a role in secretion of growth hormone, food intake, stimulating gastric motility and secretion of gastric acid [157]. Increasing food intake and body weight because of the stimulating effect on the production of NPY and AgRP in the arcuate nucleus in the hypothalamus [9]. Overall, circulating ghrelin levels are a good indication of the nutritional status and body fat stores being inversely correlated with adiposity. Obese individuals tend to have low ghrelin levels, while lean individuals tend to have higher levels [9].

2.4.3.2 Satiety

The control center for satiety is the same one as for hunger, i.e. the hypothalamus. Production of POMC and CART are the peptides responsible for this capability. POMC undergoes tissue-specific post-translational cleavage. Both POMC and CART neurons are directly stimulated by leptin, a peptide produced by the adipose tissue. CART neurons are able to target various areas throughout the hypothalamus and these neurons have a role in the neural circuits controlling reward and reinforcement [78], sensory processing, and stress and endocrine regulation [9].

Many peripheral peptides are known to be associated with satiety. The gastrointestinal tract, pancreas, and adipose tissue are examples of regions where these hormones are secreted. Satiety hormones include cholecystokinin (CCK), peptide YY (PYY), pancreatic polypeptide (PP), incretins, glucose-dependent insulinotropic polypeptide (GIP), adiponectin, insulin, and leptin [9].
Insulin is produced by the β-cells of the pancreatic islets when glucose levels in the blood are high and so insulin levels increase rapidly after consumption of a meal. This hormonal regulator of appetite vary directly with changes in adiposity [9]. Insulin diminishes the appetite, when entered the brain.
Leptin (also called the "satiety hormone") is also able to regulate appetite. Various places produce leptin, e.g. white and brown adipose tissue, stomach, placenta, mammary gland, ovarian follicles, and certain fetal organs such as heart, bone or cartilage, and perhaps the brain [9]. In obese individuals, there is tend to occur a decreased sensitivity to leptin which eventually results in failing to detect satiety despite high energy stores. Leptin binding on the receptors results in the stimulation of a specific signaling cascade that eventually results in the inhibition of NPY and AgRP and stimulation of POMC and CART [9]. Overall, this gives an outcome of decreased appetite and increased energy expenditure.

2.4.3.3 Effects of LC n-3 PUFA

The effect of PUFA on appetite has been studied, with contradictory results. DHA has the capacity to transport appetite-regulating molecules such as dopamine [125]. PUFA can interact with various neuroendocrine factors that play a role in brain−gut loop signals related to energy metabolism, for instance insulin [63, 95] and leptin [151, 110]. Some studies show an increased feeling of satiety following a meal containing a high LC n-3 PUFA content, using a visual analogue scale (VAS) to measure hunger sensations. A nutritional weight loss intervention study from 2008 [107] with 278 overweight and obese participants, showed that LC n-3 fatty acids modulate hunger signals and postprandial satiety. Subjects who ate a dinner rich in LC n-3 fatty acids felt less hungry and more full immediately following and two hours after consuming the meal. However, in this study pre-meal appetite sensations were not measured which could have influenced the post-meal appetite sensation. Another randomized, crossover study showed that consuming a walnut-based breakfast, which contained a substantial amount of the n-3 fatty acids α-linolenic acid (22.82 grams of PUFA), increased satiety in subjects with the metabolic syndrome. However, the satiation effects could come from the difference in fiber (walnut shake 9.76 g vs. placebo 7.21 g) [22]. Furthermore, a randomized, crossover study in healthy adults from 2013 found that taking fish oil for 3 weeks (3.5 grams of LC n-3 PUFA, of which 1.9 g EPA and 1.1 g DHA) resulted in a significant increase in appetite. The desire to eat more was modified by gender, as women reported an increased desire to eat after the fish-oil period [43].

2.4.3.4 Effects of different fat sources

In previous studies, other fat sources (i.e. medium-change fatty acids, MCFA; long-chain fatty acids, LCFA; saturated and unsaturated fatty acids) and their influence on appetite, food intake, and body weight were investigated. A 3-day randomized crossover study from Poppitt et al.

[112] in lean men (n=18) investigated the effect of high-fat diets with different sources of fat (short-chain, medium-chain, and long-chain breakfast). They demonstrated that there was no significant effect of chain length on feelings of hunger, fullness, satisfaction or current thoughts of food. In addition, energy and macro-nutrient intake during the next meal did not differ between diets. Lawton et al. [82] investigated the effect of different high-fat lunch meals with the different fat sources having a different degree of saturation of fatty acids. Overall, including PUFA in the diet enhanced satiety relatively stronger compared to MUFA (mono-unsaturated fatty acids) and SFA (saturated fatty acids).

2.4.4 Effects on muscle blood flow

Endothelium-dependent vasodilation [129], an early step in the development of atherosclerosis [59] and skeletal muscle blood flow [66], are impaired in obese individuals. Impaired functioning of these may be associated with reduced nutrient delivery and disposal in humans. The microvascular action of insulin also appears to be essential in enhancing the delivery of insulin and glucose to the skeletal muscle. Eventually, impaired responses to insulin in obese individuals may contribute to the impaired metabolic response to insulin which results in impaired to glucose disposal [36].
A 12-week intervention study by Hill et al. [65] with overweight individuals (n=75, age: 25-65 years) with high blood pressure, cholesterol, or triacylglycerols demonstrated an improved endothelium-dependent arterial vasodilation ($p < 0.05$) when supplemented with LC n-3 PUFA (260 mg DHA + 60 mg EPA). Another 6-week study by Walser et al. [144] with thirteen healthy subjects showed that supplementation with LC n-3 PUFA (2 g/day DHA + 3 g/day EPA) enhanced brachial artery dilation and blood flow during rhythmic contractions of the forearm as well as increased skeletal muscle blood flow during exercise. Along with these findings, some of the conclusions drawn were that supplementation with LC n-3 PUFA may reduce body fat due to the improved vasodilator function increasing blood flow and nutrient delivery to skeletal muscle resulting in improved nutrient utilization for energy production and less conversion to fat available for storage [25, 67].

2.4.5 Effects on resting metabolic rate and substrate oxidation

Another proposed mechanism of LC n-3 PUFA is the influence on metabolic processes. The results from studies of LC n-3 PUFA supplementation on RMR and substrate oxidation are limited and open to question. A study from Couet et al. [39] showed a stimulated fat oxidation by 22% (as measured by respiratory exchange ratio) and an increase in RMR by 4% (however this effect was no longer noticeable when adjusted for lean body mass) in healthy adults (n=6, age: 23 ± 2, BMI: 21.9 ± 1.6) when 6.0 g/d of visible fat was replaced by 6.0 g/d of fish oil for three weeks. While Gerling et al. [55] found a significant increase in RMR (5.3%, $p = 0.040$) but no effect on fat and carbohydrate oxidation in a 12 week study with active males (n=21) supplemented with 3.0 g/day of EPA and DHA. However, the increased RMR effect found in this study was no longer statistically significant when normalizing for lean body mass, which was similar to the study by Couet et al. [39]. Another study from Bortolotti et al. [20] found no significant effect on energy metabolism and energy efficiency during exercise in healthy males

(n=8) when supplemented with fish oil (containing 1.1 g/day EPA and 0.7 g/day DHA) for two weeks.

2.5 Potential adverse effects of LC n-3 PUFA

Although there are several beneficial effects of consuming LC n-3 PUFA, considering and being aware of both the benefits and risk of the potential adverse effects of LC n-3 PUFA consumption is necessary. Two examples of adverse effects of n-3 fish oil supplements, which are dose-dependent, are a fishy aftertaste and gastrointestinal disturbances [40]. High doses (> 3 g/day) of fish oil can have drug interactions, although not necessarily clinically significant, which could potentially be a concern. Interaction with drugs such as aspirin and warfarin can cause enhanced bleeding [51].
An animal study from Feillet-Coudray et al. [49] demonstrated that a diet high in PUFA through fish oil supplementation elevated the liver fat content in rats with a resulting increased oxidative stress in liver and muscle tissue.
As mentioned previously, methylmercury exposure is a health risk related to fish consumption. Children and pregnant and lactating women may have a higher risk for intoxication with methylmercury from fish consumption [109], so avoidance of potentially contaminated fish is should be a priority in this stage of life group. Fish species that contain high levels of mercury are shark, swordfish, king mackerel, and tilefish [40].
Oxidative degradation of LC n-3 PUFA supplements could cause problems as well. Different factors can influence the degree and rate of oxidation such as fatty acid composition, exposure to oxygen and light, and temperature. Both primary and secondary oxidation can be measured by the peroxide value and the anisidine value, respectively. However, the European Food Standards Authority (EFSA) Panel on Biological Hazards suggested in 2010 that information is lacking about the level of oxidation of fish oil and the associated toxicological effects in humans.

II PROBLEM STATEMENT AND OBJECTIVE

Worldwide, overweight and obesity are a growing health problem. The prevalence of obesity among American adults increased from 19.4% in 1997 to 30.4% in 2015 [143]. Young women are considered to be at high risk for weight gain [1]. Overweight and obesity are complicated and multifactorial diseases and individuals suffering have difficulties following a healthy lifestyle, especially over the longer term. Identifying novel strategies (e.g. food components with potential beneficial health effects) to treat and prevent weight gain is of great importance.
The trend of breakfast skipping has been increasing over the past few decades and parallel with that, the prevalence of overweight and obesity has increased. Some believe that omission of breakfast could be a possible element influencing weight gain in adults [23, 128]. With increasing data suggesting that breakfast may be the most important meal of the day, it becomes an interesting meal time to investigate the impact of dietary interventions.
Previous studies have investigated the potential beneficial health effects of omega-3 fatty acids from marine sources (i.e. eicosapentaenoic acid, EPA; and docosahexaenoic acid, DHA), however they are limited, with conflicting results regarding potential anti-obesity effects. Variable results in previous studies may come from low doses of LC n-3 PUFA, small numbers of participants [39, 20], and no control group [39, 20].
Therefore, this pilot study tried to investigate the effect of LC n-3 PUFA (from marine sources) supplementation at breakfast in young women on energy expenditure and appetite, by utilizing a high dose of LC n-3 PUFA (2 g EPA, 2 g DHA), and the use of a control group (with high oleic sunflower oil).

This thesis presents a pilot study investigating the possible effects of EPA and DHA at breakfast on postprandial energy expenditure (i.e. thermic effect of feeding), substrate utilization, and appetite in young women. This pilot study used a double-blind, randomized crossover design in which participants act as their own control and included two groups, normal weight versus overweight/obese individuals. Including both normal weight and overweight/obese individuals in the study gives insight into possible differences between BMI groups. The hypothesis is that an increased consumption of EPA and DHA at breakfast will increase postprandial energy expenditure and decrease hunger compared to a breakfast without EPA and DHA to a greater extent in overweight/obese young women compared to normal weight young women.

III MATERIALS AND METHODS

1 Participants

Females, 18-30 years of age, were recruited to participate in the study via advertisements in the University of Arkansas daily newsletter, social media, flyers, and word of mouth. Women who had food allergies, had dietary restrictions, were habitual breakfast skippers (defined as skipping breakfast $\geq$ 2 days/week), were actively trying to lose weight, had lost weight in the last three months, were competitive athletes, were pregnant or had any chance of being pregnant, were taking medication (excluding hormonal birth control), had any pre-existing metabolic conditions (e.g., type 1 or 2 diabetes mellitus), or had any other diet-related conditions that would prevent them from eating the breakfasts were excluded from the study.
A total of forty three women were screened of which thirty five (17 normal weight, BMI = 18.5-24.9 kg/m^2, and 18 overweight/obese, BMI $\geq$ 25.0 kg/m^2) were selected to participate in the study. Eight women did not meet the criteria to participate in the study (not in a specific BMI category, taking anxiety medication, having irritable bowel syndrome, taking dietary supplements, and taking prescription medication). Twenty seven women (15 normal weight and 14 overweight/obese) completed the study and their results were used in the data analysis (see Figure 1.1). Five women dropped out of the study due to scheduling conflicts, and one women mentioned not being able to participate due to personal reasons. Three of the subjects that dropped out already had their DXA scan, so their data was not further used.
Before the start of the study, ethical approval for the study was obtained from the Institutional Review Board (IRB) at the University of Arkansas on January 20th 2017. The ethical approval number for this study is #16-09-124 (see Appendix A). It was also checked with the University of Ghent and no additional request for approval from the Ethical Committee UZGent was necessary for this thesis. Written consent was obtained from all participants prior to starting the study and following a detailed explanation of the study protocol and any associated risks.

1.1 Screening

Potential participants underwent a telephone screening to determine if they met the minimum study qualifications (the participant screening questionnaire can be found in Appendix B). During this screening, information about the potential participant was asked (e.g. age, ethnicity, estimated height and weight, contact information, current health status, possibility on pregnancy, being a competitive athlete, being a regular breakfast consumer, medication, menstrual cycle, and availability).

After the phone screening, it was determined if the person qualified for the study and if so, an email was sent with suggested dates. Women were only scheduled during the follicular phase of their menstrual cycle as this is the time period when hormones may be least likely to influence appetite and food intake [56]. During the luteal phase of the menstrual cycle water retention, increased body weight and energy demand, changes in lipid profile and metabolism

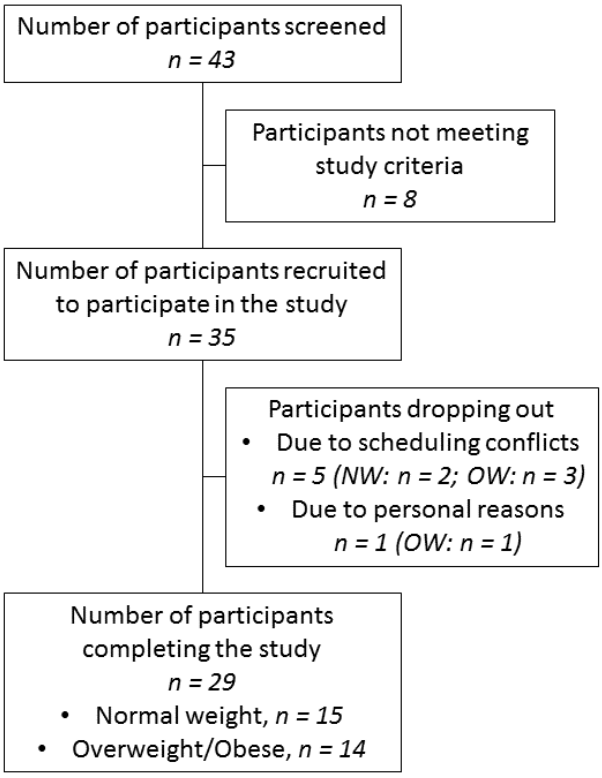

Figure 1.1: Flow diagram of participant screening and selection process.

of some nutrients, emotional hypersensitivity, and aches can occur and for this reason as well participants were scheduled during the follicular phase [119].

2 Study design

This pilot study used a double-blind, randomized crossover design in which participants acted as their own control. Figure 2.1 shows an schema of the study design with starting from the screening, followed by a grouping according to BMI, followed by a randomized crossover comparison including two study days and a washout period. Participants were divided into two groups based on their BMI, i.e. normal weight (BMI = 18.5-24.9 kg/m^2) and overweight/obese (BMI $\geq$ 25.0 kg/m^2). Subsequently, each participant was randomly assigned to one the dietary interventions: study day one with the control breakfast (beverage without supplementary omega-3 fatty acids) or study day one with the treatment breakfast (beverage with supplementary omega-3 fatty acids). The two study days had to be completed with one to two weeks between visits.
Participants were instructed to fast overnight (10-12 hours) and refrain from strenuous physical activity the day before testing. Figure 2.2 illustrates the time-line of how a study day looked like. On the study day, participants arrived at the Food Science Department at the University of Arkansas between 07:30 and 8:00 am. Upon arrival, body weight and height were measured. Also resting energy expenditure (REE), and baseline appetite assessments were measured. Subsequently, participants were provided with the breakfast (with or without supplementary omega-3 fatty acids) and had to consume the test breakfast within 10 minutes. Immediately after consumption of the breakfast drinks, participants were asked to evaluate the taste using a visual

analogue scale (VAS). Appetite assessments were collected at 15, 30, 60, 90, 120, 180 and 240 minutes postprandial. Resting energy expenditure and substrate utilization was measured 30, 60, 90, 120, 180, and 240 minutes following consumption of the breakfast. At the end of the study day, participants were provided with a 24-hour food intake log that they were required to complete for the rest of that day. Participants were also required to maintain their typical physical activity level throughout the day. The protocol of study day one and study day two was the same, with the only difference being the breakfast provided (with or without supplementary omega-3 fatty acids).

At the end of study day two and upon completion of the whole study, participants were compensated with a Walmart gift card for $50.

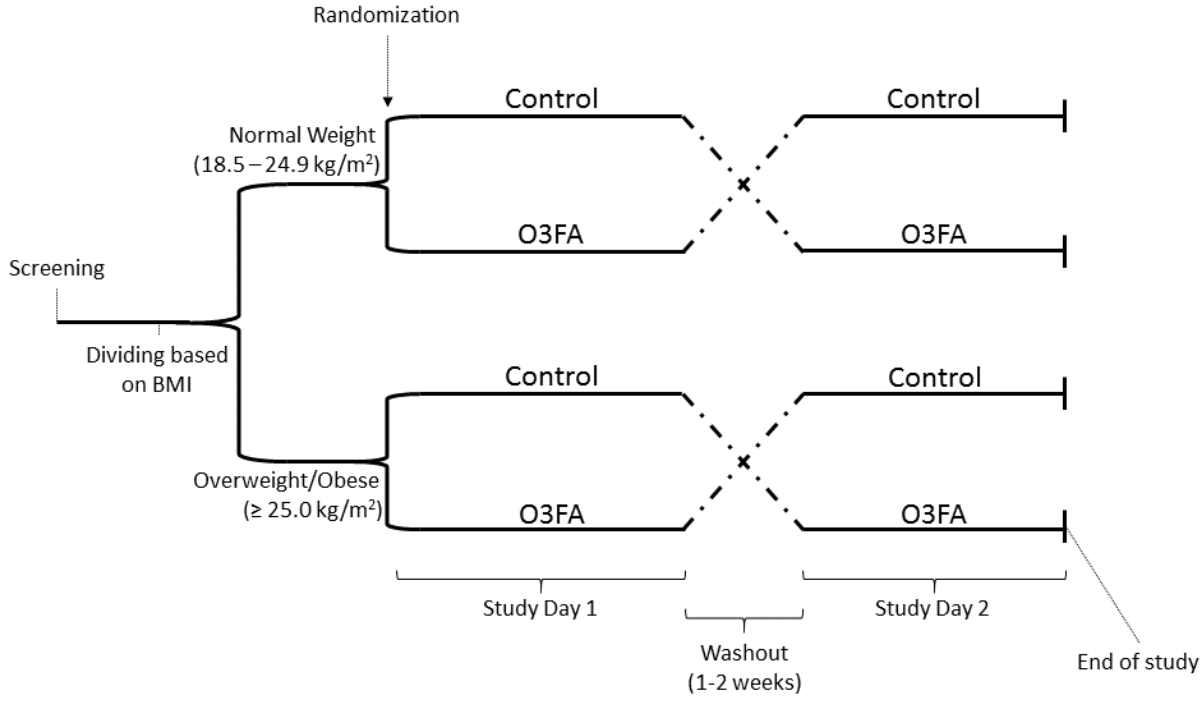

Figure 2.1: Study with randomized crossover design. O3FA: Omega-3 Fatty Acids.

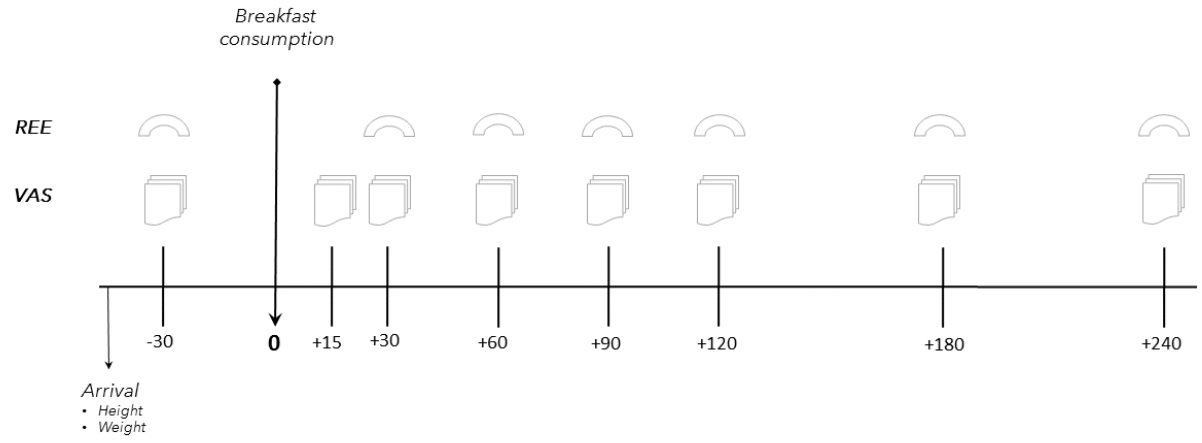

Figure 2.2: Time-line of a study day. REE: Resting Energy Expenditure; VAS: Visual Analogue Scale.

3 Anthropometric measurements

Body composition was determined using dual energy X-ray absorptiometry (DXA) analysis (Lunar Prodigy, GE Healthcare, Madison, WI, USA). This measurement was executed by a trained person and took place in the Human Performance Laboratory at the University of Arkansas. With DXA it is possible to determine fat mass, fat-free mass, bone mineral density and content, and bone area.
Body weight was measured using a standard calibrated balanced scale, and was re-measured every time participants came to the research center. Height was measured using a standing stadiometer with participants barefoot, in the free-standing position. Height and weight was measured by the same person on both study days. BMI was calculated dividing the weight (in kilograms) by height (in meters) squared.

4 Test breakfast

The test breakfasts were provided by Smartfish, a Norwegian nutrition research-based company. The treatment breakfast contained supplementary omega-3 fatty acids (O3FA), while the control breakfast did not. Both drinks were taste-matched and the nutritional values were the same for both drinks. The supplementary omega-3 fatty acids were derived from fish oil sourced from Norwegian catch. The fatty acids in the Smartfish emulsion are well protected against oxidation, making them odorless, tasteless and with prolonged durability. Buckley et al. [25] reviewed several human intervention trials indicating potential anti-obesity effects of LC n-3 PUFA supplementation. They mentioned increasing the LC n-3 PUFA intake by 0.3-3.0 g/day could improve body composition through certain mechanisms (see Literature Study), while a supplementation concentration of 1.9-5.0 g/day of LC n-3 PUFA could reduce the availability of nutrients for lipogenesis and storage in adipose tissue. The total fat fraction of each breakfast drink includes 19.8 grams of fat. In the O3FA breakfast, 4928 mg of the fat consists out of omega-3 and of this 4928 mg, 2000 mg is EPA and 2000 mg is DHA. An amount of 4000 mg omega-3 fatty acids (EPA + DHA) seemed an appropriate concentration comparing with the ranges of LC n-3 PUFA supplementation mentioned in the review of Buckley et al. [25].
The fat source in the control drink was high oleic sunflower (HO-sunflower) oil. The fatty acid composition (in %) of this HO-sunflower oil is 85, 10, 5, and 5 of monounsaturated (oleic acid), saturated, polyunsaturated, and omega-6 fatty acids, respectively.
The drink was juice based with juices of aronia, pomegranate, apple and pear, and did not contain any added sugars, sweeteners or preservatives [127]. The palatability (”How much do you like or dislike the taste of the drink?”) of both drinks (control and O3FA) were measured using a visual analogue scale (VAS) right after consumption of the breakfast (with opposing extreme responses ”dislike extremely” or ”like extremely”). The result of this VAS-question is shown in Table 4.1. A paired samples t-test was used to determine if there was a significant difference between the palatability of both drinks. No significant difference was found between the palatability of the two drinks ($t_{28} = 1.602$, $p > 0.05$).
Each breakfast provided contained a total of two beverages (two cartons of 200 mL, 8 oz), Table 4.1 shows the nutrient values and composition for the total breakfast (i.e. sum of two drinks).

Table 4.1: Nutrients of the two test breakfast beverages.

	Control breakfast (code NFAC)	O3FA breakfast (code NF2)
Energy (kJ (kcal))	1556 (372)	1556 (372)
Fat (g)	19.8	19.8
Omega-3 (mg)	-	4928
EPA (mg)	-	2000
DHA (mg)	-	2000
Carbohydrates (g)	34	34
Protein (g)	10.4	10.4
Palatability (mm) [a]	63.6 ± 19.3	68.6 ± 19.8

DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.
[a] Mean ± standard deviation.

Figure 4.1: Beverages; left (NFAC) control beverage, right (NF2) O3FA beverage.

Smartfish delivered the beverages in white coded juice boxes (see Figure 4.1). After delivery, the codes NFAC and NF2 were labeled with a yellow and blue sticker, respectively. On May 1st 2017, the company unblinded the codes: NFAC = control, NF2 = O3FA.

5 Appetite and craving response

Appetite, cravings and palatability were assessed using VAS. This questionnaire is the most common method for measuring subjective ratings of appetite [80]. Each question hold a 100 mm straight line below with opposing extreme responses at the ends and participants were asked to place an ”X” on the 100 mm VAS to indicate their sensations, using pen and paper. Appetite VAS was conducted at time points 0, 15, 30, 60, 90, 120, 180 and 240 minutes. The questions asked about appetite were ”How hungry do you feel at this moment?”, ”How full do you feel at this moment?”, ”How strong is your desire to eat at this moment?”, and ”How much food do you think you could eat at this moment?”. Cravings VAS was also conducted at the same time points as appetite (i.e. 0, 15, 30, 60, 90, 120, 180 and 240 minutes) asking the questions ”How strong is your desire for something salty at this moment?”, ”How strong is your desire for something sweet at this moment?”, and ”How strong is your desire for a snack at this moment?”.

5.1 Restrictions

While the use of VAS is an easy and efficient method for assessing appetite, a major drawback using VAS is the validity and reproducibility of subjective appetite ratings. Appetite is, be-

sides having a physiological basis, strongly influenced by cognitive and environmental factors (e.g. surroundings, habitual meal times, sensory stimulation, and familiarity) [80, 132]. At the start of each study day, the VAS questions were explained to attempt to overcome differences in individuals' interpretation of the scale. However, it is still the participant's own interpretations of their feelings, sensations and motivations towards eating with underlying physiological processes, which is a limitation of the methodology [132].

5.2 Processing VAS data

The distance from every VAS question was measured from the point indicated by the participant to the left using a ruler to the nearest of mm. Data from each participant's VAS was recorded and transposed from the paper into a Microsoft Excel document, subdividing the different questions.

6 Energy expenditure and substrate oxidation

Resting energy expenditure (REE; kcal/min) was measured via indirect calorimetry (IC) with a TrueOne 2400 metabolic cart and ventilation hood (Parvo Medics, Sandy, Utah, USA). The hood captures the exhaled gas and is connected to analyzers on the mobile cart. This metabolic measurement system uses a Rudolph heated pneumotachometer to measure the flow and volume with a range of 0-800 L/min. The accuracy is $\pm$ 2% with Precision Yeh Algorithm to correct for the linearity at low flow rates as it is non-linear at flow rates less than 80 L/min [108][41]. The TrueOne 2400 is a mixing chamber system using a paramagnetic O_2 analyzer (with a range of 0-25%) and an infrared, single beam, single wave-length CO_2 analyzer (with a range of 0-10%) [108] to measure fractions of expired oxygen (F_{EO2}) and carbon dioxide (F_{ECO2}). At the beginning of each study day, the metabolic cart had to warm up for at least 30 minutes. Once warmed up, calibrations were performed according to the manufacturer's instructions, to account for changes in environmental conditions. The TrueOne 2400 metabolic cart system is provided with a weather station and ambient temperature, barometric pressure, and relative humidity were entered into the computer. First, the gas analyzer was calibrated via a room air auto-calibration routine and a two-point gas calibration with a single certified gas tank (15.99% O_2, 1.002% CO_2). After that, the flowmeter was calibrated using a 3 L Hans Rudolf calibration syringe (series 5530) by using a five-stroke calibration. Once these two calibrations were completed correctly and saved, measurements could begin. Gas and flow rate and volume were calibrated to $\leq$ 3% error.
Bassett et al. [11] demonstrated the TrueOne metabolic measurement system from Parvo Medics to be an accurate gas exchange measurement device with minimal errors in gas volumes. Crouter et al. [41] also demonstrated this system to be have a good reliability for measuring expired ventilation (V_E), oxygen consumption (VO_2), and carbon dioxide production (VCO_2). Cooper et al. [38] demonstrated that TrueOne 2400 is a valid system to measure both RMR and respiratory exchange ratio (RER). Research demonstrated that using metabolic carts to measure energy expenditure has advantages as having a quick response time, easy to operate and feasible in clinical setting, while limitations include restricted respondent mobility [80].

Using indirect calorimetry under standard conditions for measuring REE gives information at

rest in the form of the amount of oxygen consumption (VO_2; mL/min), and the amount of carbon dioxide production (VCO_2; mL/min). This amount of O_2 consumption for oxidation and CO_2 production depends on the type of substrate that is being oxidized at that moment. From the VO_2 and VCO_2 data, the non-protein respiratory quotient(npRQ) (see equation 6.1), and the resting energy expenditure (based on the Weir equation [146]) was calculated by the TrueOne 2400 metabolic cart software.

$$\mathbf{RQ} = \frac{\mathrm{VCO_2\ (mL/min)}}{\mathrm{VO_2\ (mL/min)}} \tag{6.1}$$

The value of RQ varies with the nutrients that are being oxidized and can give a percentage contribution of carbohydrates and fats. Carbohydrate oxidation and fat oxidation can be calculated from the non-protein RQ [115].

Resting energy expenditure was measured before consuming the breakfast (time point 0), and at 30, 60, 90, 120, 180, and 240 minutes after consuming the breakfast beverages. During measurements, participants were required to be awake and lying down in the supine position. The first measurement was a total of 30 minute rest period (measuring the BMR, at time point 0) while the rest of the REE measurements were only 20 minutes. REE was measured in 30 second increments during the rest periods.

During one study day, the power went out so no more metabolic cart data could be collected for the time points 180 and 240 minutes. All metabolic cart data from this participant (both study days) were excluded for analysis.

6.1 Processing metabolic cart data

The data output of the metabolic measurement system came in the form of an excel file, with the output of time (min), VO_2 (mL/min), VCO_2 (mL/min), npRQ, carbohydrate oxidation KCHO (kcal/min), fat oxidation KFAT (kcal/min), and REE (kcal/day and kcal/min). The metabolic cart system generated an output every 1 minute. The first five minutes of every measurement were discarded from each data set [19]. Eventually, all data per subject per study day was put together in one file.

7 Self-reported dietary intake record

At the end of each study day participants were given a food intake log to record their food intake for the remainder of that test day (the document that was given to the participants can be found in Appendix C). The record included detailed instructions and an example for correctly completing this form. The record was divided into lunch, dinner, and snacks. This document was previously composed together with a registered dietitian.

8 Statistical analysis

Power analysis.
To identify an appropriate sample size for this study, a power analysis can be performed. However, due to lack of data about this topic on LC n-3 PUFA influence on energy expenditure and appetite response, power analysis were based on earlier studies using VAS-data. According to Flint et al. [52] recruiting of ≥ 8 subjects was necessary to detect an effect of 10 mm on mean 4.5 hour appetite VAS-scores (hunger, fullness, prospective food consumption) and ≥ 15 for mean 4.5 hour craving VAS-scores (desire for something salty or sweet) in a paired design at a significance level of 0.05 and a study power of 0.8. It was decided to recruit 15 subjects for both groups (normal weight and overweight/obese). This number was reached in the normal weight group however, due to dropouts, only fourteen participants completed the study in the overweight/obese group.s
Summary Statistics.
Statistical analyses were conducted using the statistical software of SPSS Statistics (Version 24, IBM Corp., Armonk, NY, USA). All results in this thesis are reported as mean $\pm$ standard deviation (SD). For all statistics, a significance level of 95% was applied.
Participants Characteristics.
Two-sample independent t-tests were used to determine initial differences between the participants characteristics means (age, height, weight, BMI, and DXA results) of the normal weight and overweight/obese group. To conduct a t-test, some conditions should be met. First of all, the two groups being compared should follow a normal distribution. Normality was tested using the Shapiro-Wilk test. Subsequently, the homoscedastic condition was tested using the Levene's test (which is already implemented in the t-test in SPSS). If the Levene's test shows a significant difference between variances, the Welch-test was performed instead of the t-test.
VAS Data.
Changes from baseline (Δ) were calculated by subtracting the baseline VAS response (point 0 min) from the score at a certain time point (at 15, 30, 60, 90, 120, 180, and 240 min postprandial). With this data, further calculations were made. The incremental area under the curve (iAUC) of changes from baseline over time was calculated for each subject for appetite responses (perceived hunger, perceived fullness, desire to eat, and prospective food consumption) and craving responses (salty, sweet, and snack craving) using the conventional trapezoidal rule. Summation of the different iAUC values gave the net incremental area under the curve (niAUC) and was used in the analysis [96, 152]. Changes from baseline of appetite and craving responses across time per group and for the two study days (control and O3FA) and niAUC over the 240 minute period are represented in figures. A paired samples t-test was conducted on the ΔniAUC data per group as well as comparing control and O3FA (putting normal weight and overweight/obese group data together). A Shapiro-Wilk test was conducted on the difference between the paired values to check for normal distribution.
Energy Expenditure.
Thermic effect of food (TEF, kcal/min) was determined for each time point by calculating the difference between REE (kcal/min) at time point 0 and time points 30, 60, 90, 120, 180, and 240 minutes after breakfast consumption (e.g., TEF at time point 90 = REE_{90} - REE_0).
The incremental area under the curve (iAUC) was calculated for for each subject and each time point for REE, TEF, carbohydrate oxidation (KCHO), and fat oxidation (KFAT) using the trape-

zoidal rule. Summation of the different iAUC values gave the net incremental area under the curve (niAUC) and was used in further analysis.

Dietary Intake Records.

All data from the food intake records was analyzed using the Genesis R&D diet analysis software package (Salem, OR, USA), which was available in the lab. The records were entered manually and subdivided per subject per study day. Eventually, total energy and macronutrient composition was collected in one excel file and reported in a summarizing table.

IV RESULTS

1 Participants characteristics

Table 1.1 represents a summary of the demographic and anthropometric characteristics of the participants who completed the study. This table shows the variables age, height, body mass, BMI, DXA results (i.e. body fat mass, percentage body fat, fat free mass, and lean tissue mass), and ethnicity of the participant. Data is represented as mean ± standard deviation. The last column of this table shows the result of the independent samples t-test.

Table 1.1: Demographic and anthropometric characteristics

	Normal weight (n=15)	**Overweight/Obese** (n=14)	t[a]
Age (y)	24.1 ±3.0	24.7 ±3.8	-0.522 [ns]
Anthropometric measurements			
Height (m)	1.66 ±0.05	1.64 ±0.07	0.848 [ns]
Body mass (kg)	58.0 ±5.5	77.4 ±11.1	MWU [***]
BMI (kg/m^2)	21.2 ±1.6	28.9 ±3.9	-6.924 [***]
DXA			
Body fat mass (kg)	15.5 ±4.2	32.1 ±7.6	-7.214 [***]
Percentage body fat (of total)	27.9 ±6.0	43.1 ±5.5	-7.077 [***]
Fat free mass (kg)	42.3 ±4.1	44.6 ±5.6	-1.261 [ns]
Lean tissue mass (kg)	39.8 ±3.9	41.8 ±5.3	-1.133 [ns]
Ethnicity			
Caucasian (n)	13	7	
Hispanic (n)	2	1	
African American (n)	-	2	
Asian (n)	-	2	
Indian (n)	-	2	

Data is represented as mean ± standard deviation. BMI, body mass index; DXA, dual-energy X-ray absorptiometry; MWU, Mann-Whitney U test.
[a] independent samples t-test; *** $p < 0.001$; [ns] not significant

The represented body mass in Table 1.1 is the average of the participants weight on study day one and study day two. The represented BMI is the value calculated from this average weight. A total of twenty-nine participants completed the entire study, fifteen in the normal weight group (NW) and fourteen in the overweight/obese group (OW). All the subjects were young women. Table 1.1 shows that the mean age between the two groups was not found to be statistically significant (t = -0.522; ns). The height between both groups was also found to be not significant (t = 0.848; ns) , while body mass (t = -5.916; $p < 0.001$) was significantly different between the groups. BMI was found not to be normally distributed in the overweight/obese group ($p < 0.05$ in Shapiro-Wilk test). Subsequently, the Mann-Whitney U test was performed for BMI and results showed a significant difference between both groups ($p < 0.001$). From the DXA results were body fat mass (t = -7.214; $p < 0.001$) and percentage (t = -7.077; $p < 0.001$) different between the normal weight and the overweight/obese group, while fat free (t = -1.261; ns) and

lean tissue mass (t = -1.133; ns) were not found to be different.
The only ethnic backgrounds in the normal weight group were Caucasian and Hispanic, while the overweight/obese group had a greater variability with Caucasian, Hispanic, African American, Asian, and Indian. This was a clear difference between both groups. Results from a univariate analysis of variance (tests of between-subjects factors) on ethnicity showed that both weight groups (p = 0.904) and type of ethnicity (p = 0.070) did not have a significant influence on the amount of participants present. The adult (men and women) obesity facts from Arkansas shows a current obesity rate of 34.5%. The obesity distribution rate by gender in Arkansas (in 2012) was 34.1% of men and 35.1% of women. The numbers of obesity rate by race in Arkansas (2015) illustrate that largest amount are black individuals (with 43.9%), followed by Latino individuals (with 36.9%) and then white individuals (with 33.2%) [140].
As mentioned before, all participants were scheduled during the follicular phase of their menstrual cycle. Fifteen of the twenty-nine participants took birth control pill, and two women had IUD (intrauterine device) birth control.

2 Appetite

Changes in perceived hunger (A), perceived fullness (B), desire to eat (C), and prospective food consumption (D) (all expressed as Δ mmVAS compared to baseline) across time per group (normal weight and overweight/obese) after consuming the control and O3FA breakfast are shown in Figure 2.1, left. The ΔniAUC over the 240 minute period is shown in Figure 2.1, right.

Before evaluating the difference between the changes from baseline in niAUC of control and O3FA breakfast on perceived hunger, perceived fullness, desire to eat, and prospective food consumption, results from the Shapiro-Wilk test of normality on ΔniAUC were checked (see Table 2.1 with both the combined (normal weight and overweight/obese) and separate per group). If normally distributed, a t-test could be performed. With $p > 0.05$ for combined, normal weight and overweight/obese, it could be concluded with 95% certainty that the difference between the paired values of all the ΔniAUC (of perceived hunger, perceived fullness, desire to eat, and prospective food consumption) was normally distributed so a paired t-test could be conducted on all these data sets. Results from this latter test are shown in Table 2.2.

2.1 Perceived hunger

Question: "How hungry do you feel at this moment?".

Visual evaluation of the postprandial perceived hunger feeling across time showed a slight distinction between the O3FA breakfast and the control breakfast with the O3FA breakfast having a greater decrease in perceived hunger throughout the 240 minutes postprandial.

The paired samples t-test showed that NW control and NW O3FA ΔniAUC of perceived hunger responses were significantly positively correlated (r = 0.701). Consumption of the O3FA breakfast (ΔniAUC perceived hunger -3010.7 $\pm$ 4359.9 mm*240 min in NW; -5317.2 $\pm$ 8728.5 mm*240 min in OW) resulted for both weight groups in a significant lower hunger sensation

compared to consuming the control breakfast (ΔniAUC perceived hunger 1201.0 ± 5169.6 mm*240 min in NW; -516.6 ± 6211.6 mm*240 min in OW), with t_{14} = 4.337 and one-tailed $p < 0.001$ in NW and t_{13} = 1.897 and one-tailed $p < 0.05$). With 95% certainty it could be concluded that both normal weight and overweight/obese participants had a decreased perceived hunger sensation after consuming the O3FA breakfast compared to consuming the control breakfast, based on the results from the changes from baseline in niAUC. To compare NW and OW, a Welch-test was performed (as the Levene's test for equality of variances showed a significant difference, $p < 0.05$). Results from this Welch-test showed that NW and OW ΔniAUC of perceived hunger responses were not significantly different ($t_{46.289}$ = 1.147 and one-tailed p = 0.1285).

2.2 Perceived fullness

Question: "How full do you feel at this moment?".

Visual evaluation of the postprandial perceived feeling of fullness across time showed a slight distinction between the O3FA breakfast and the control breakfast starting from the 30 minute mark point with the O3FA breakfast having a greater increase in perceived fullness throughout the 240 minutes postprandial.

The paired samples t-test showed that NW control and NW O3FA, and OW control and OW O3FA ΔniAUC of perceived fullness responses were significantly positively correlated (r = 0.643 for NW, r = 0.623 for OW). Consumption of the O3FA breakfast (ΔniAUC perceived fullness 4287.2 ± 4211.6 mm*240 min in NW; 5785.7 ± 5224.4 mm*240 min in OW) resulted for both weight groups in a significant larger sensation of fullness compared to consuming the control breakfast (ΔniAUC perceived fullness 1994.388 ± 5760.0 mm*240 min in NW; 1388.5 ± 5912.3 mm*240 min in OW), with t_{14} = -1.999 and one-tailed $p < 0.05$ in NW and t_{13} = -3.376 and one-tailed $p < 0.05$, based on the results from the changes from baseline in niAUC.

2.3 Desire to eat

Question: "How strong is your desire to eat at this moment?".

Visual evaluation of the postprandial desire to eat across time shows a slight distinction between the O3FA breakfast and the control breakfast. There is not a real difference noticeable between the NW and OW groups. When consuming the control breakfast, the desire to eat was the same as the fasting state (baseline) after approximately 110 minutes postprandial. When consuming the O3FA breakfast, the return to baseline desire to eat was reached after approximately 210 minutes postprandial. An independent t-test was performed to look at the real difference between the two weight groups (NW versus OW group). Results from this t-test showed that NW and OW ΔniAUC of desire to eat responses (changes from baseline) were indeed not significantly different (t_{56} = 0.159 and one-tailed p = 0.437).

Consumption of the O3FA breakfast (ΔniAUC desire to eat -3884.7 ± 3507.2 mm*240 min

in NW; -4177.5 $\pm$ 7364.0 mm*240 min in OW) resulted in a significantly lower increase of niAUC of the desire to eat compared to consuming the control breakfast (ΔniAUC desire to eat 806.6 $\pm$ 6421.4 mm*240 min in NW; 562.0 $\pm$ 6464.0 mm*240 min in OW), with $t_{14} = 3.226$ and one-tailed $p < 0.05$ in NW and $t_{13} = 2.873$ and one-tailed $p < 0.05$ in OW. Based on these results from evaluating the changes from baseline in niAUC on the desire to eat, it can be concluded that both NW and OW participants had a lower desire to eat when consuming the O3FA breakfast compared to when consuming the control breakfast.

2.4 Prospective food consumption

Question: "How much food do you think you could eat at this moment?".

Visual evaluation of the postprandial PFC across time shows a slight distinction between the O3FA breakfast and the control breakfast. There is not a noticeable difference between the NW and OW groups and an independent samples t-test confirmed this ($t_{56} = 0.811$, $p = 0.2105$). Approximately 90 minutes after consumption of the control breakfast, participants indicated they could eat more compared to the fasting state (baseline). When consuming the O3FA breakfast, this baseline value was reached much later.

The paired samples t-test showed that NW control and NW O3FA, and OW control and OW O3FA ΔniAUC of prospective food consumption responses were significantly positively correlated ($r = 0.602$ for NW, $r = 0.714$ for OW). Consumption of the O3FA breakfast (ΔniAUC PFC -1950.0 $\pm$ 4036.0 mm*240 min in NW; -3207.5 $\pm$ 5151.0 mm*240 min in OW) had a significantly different effect on changes from baseline of niAUC of the prospective food consumption compared to consuming the control breakfast (ΔniAUC PFC 1771.4 $\pm$ 4188.2 mm*240 min in NW; 808.9 $\pm$ 6091.8 mm*240 min in OW), with $t_{14} = 3.925$ and one-tailed $p < 0.05$ in NW and $t_{13} = 3.462$ and one-tailed $p < 0.05$ in OW. Both NW and OW participants indicated they could eat less food when consuming the O3FA breakfast compared to consuming the control breakfast, based on the results from the changes from baseline in niAUC.

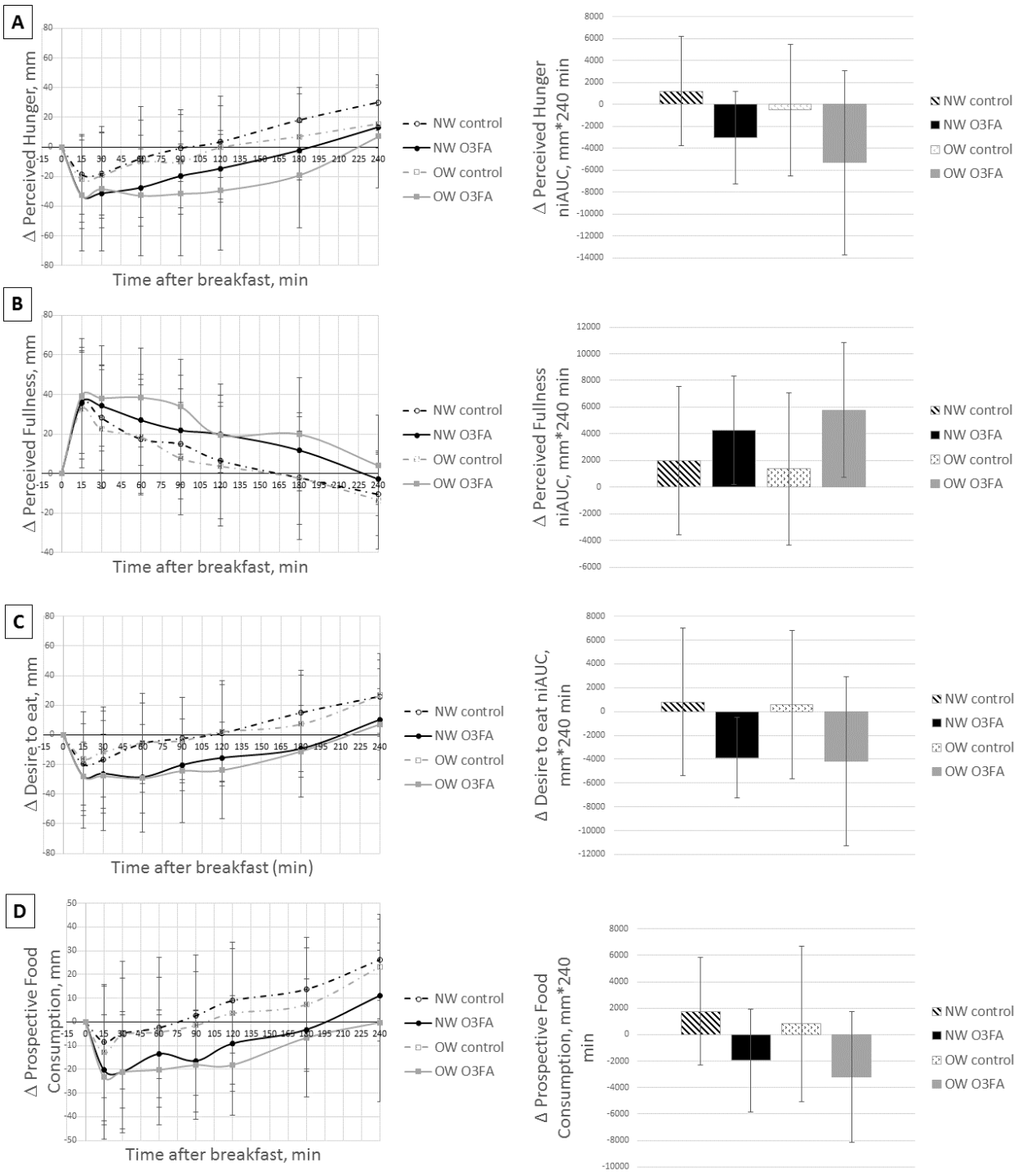

Figure 2.1: Changes in perceived hunger (A), perceived fullness (B), desire to eat (C), and prospective food consumption (D) across time (left) and ΔniAUC over the 240 minute period (right). Data is represented as mean ± standard deviation. NW, normal weight; OW, overweight/obese; O3FA, omega-3 fatty acids.

Table 2.1: Test of normality. Shapiro-Wilk test results of difference in ΔniAUC over the 240 minute period for perceived hunger, perceived fullness, desire to eat, and prospective food consumption.

	Shapiro-Wilk	
	Statistic	**p**
Perceived hunger (combined)	0.954	0.230
Normal weight	0.970	0.853
Overweight/Obese	0.923	0.241
Perceived fullness (combined)	0.971	0.577
Normal weight	0.912	0.147
Overweight/Obese	0.961	0.734
Desire to eat (combined)	0.963	0.388
Normal weight	0.969	0.836
Overweight/Obese	0.899	0.107
PFC (combined)	0.956	0.256
Normal weight	0.961	0.707
Overweight/Obese	0.925	0.260

Table 2.2: Paired samples statistics and results of t-test of ΔniAUC over the 240 minute period for perceived hunger, perceived fullness, desire to eat, and prospective food consumption.

	Paired Samples Statistics		**Paired Samples Test**		
	Correlation	**Sig. correlation**	**t**	**df**	**Sig. (2-tailed)**
Perceived hunger (combined)	0.383	0.040	3.466	28	0.002 *
Normal weight	0.701	0.004	4.337	14	0.001 *
Overweight/Obese	0.232	0.426	1.897	13	0.080 ns
Perceived fullness (combined)	0.611	0.000	-3.796	28	0.001 *
Normal weight	0.643	0.010	-1.999	14	0.065 ns
Overweight/Obese	0.623	0.017	-3.376	13	0.005 *
Desire to eat (combined)	0.534	0.003	4.383	28	0.000 ***
Normal weight	0.484	0.067	3.226	14	0.006 *
Overweight/Obese	0.608	0.021	2.873	13	0.013 *
PFC (combined)	0.675	0.000	5.283	28	0.000 ***
Normal weight	0.602	0.018	3.925	14	0.002 *
Overweight/Obese	0.714	0.004	3.462	13	0.004 *

Sig., significance; t, paired T test statistic; df, degrees of freedom. ns not significant; * $p < 0.05$; *** $p < 0.001$.

3 Craving response

Changes in the desire for something salty (A), desire for something sweet (B), and desire for a snack (C) (all expressed as Δ mmVAS compared to baseline) across time per group (normal weight and overweight/obese) after consuming the control and O3FA breakfast are shown in Figure 3.1, left. The ΔniAUC over the 240 minute period is shown in Figure 3.1, right.

Before evaluating the difference between the changes from baseline in niAUC of control and O3FA breakfast on desire for something salty, desire for something sweet, and desire for a snack results from the Shapiro-Wilk test of normality on ΔniAUC were checked (see Table 3.1 with both the combined (normal weight and overweight/obese) and separate per group). If normally distributed ($p > 0.05$ on Shapiro-Wilk test), a t-test could be performed. If not normally distributed ($p < 0.05$ on Shapiro-Wilk test), a non parametric sign test for paired observations was performed. Results from these tests are shown in Table 3.2.

3.1 Salty cravings

Question: "How strong is your desire for something salty at this moment?".

Visual evaluation of the postprandial desire for something salty across time showed no real distinction between the O3FA breakfast and the control breakfast. Only the OW group consuming the O3FA breakfast showed a slight postprandial average decrease in desire. Visual evaluation of ΔniAUC changes from baseline of desire for something salty showed that only the OW group consuming the O3FA breakfast underwent a slight decrease in average desire. All the other ΔniAUC average results showed a positive value.

The significance p-value of the Shapiro-Wilk test of normality is > 0.05, so it could be concluded with a 95% certainty that the difference between the paired values of ΔniAUC of desire for something salty was normally distributed (for NW and OW combined, as well as for the groups separately) so a paired t-test could be conducted. Results from this latter test are shown in Table 3.2. Overweight/obese participants were less craving something salty after consuming the O3FA breakfast (-1354.0 $\pm$ 4951.7 mm*240 min) compared to when they consumed the control breakfast (2317.2 $\pm$ 4565.4 mm*240 min), $t_{13} = 1.874$ and one-tailed $p < 0.05$. This cannot be concluded with 95% certainty for the normal weight group (ΔniAUC for O3FA breakfast: 1752.0 $\pm$ 5155.7 mm*240 min, ΔniAUC for control breakfast: 3080.1 $\pm$ 4174.4 mm*240 min).
To compare the NW and OW group, an independent samples t-test was performed on the data from ΔniAUC of desire for something salty. The Levene's test showed that equal variances could be assumed ($p = 0.728$). The independent samples t-test showed no significant difference between the ΔniAUC of desire for something salty between the NW and OW group ($t_{56} = 1.520$, $p = 0.067$).

3.2 Sweet cravings

Question: "How strong is your desire for something sweet at this moment?".

Visual evaluation of the postprandial desire for something sweet across time showed no clear distinction between the O3FA breakfast and the control breakfast. However, the NW group consuming the control breakfast could be distinguished from the rest, since the average effect of this group was the only time course showing a difference of desire for something sweet from baseline (at approximately 150 minutes postprandial).

With $p < 0.05$ on the Shapiro-Wilk test, it could be concluded with 95% certainty that the difference between the paired values of ΔniAUC of desire for something sweet in the combined group is not normally distributed. The NW and OW group separately were normally distributed, so on these data sets a paired t-test could be performed. Results from the sign test showed no significant difference between the control and O3FA breakfast on ΔniAUC desire for something sweet (one-tailed p = 0.1325). When evaluating the two weight groups separately, a significant difference ($t_{14} = 1.872$, one-tailed $p < 0.05$) was found in the normal weight group with the change in desire for something sweet showing a greater decrease from baseline with the O3FA breakfast (-3259.2 $\pm$ 4260.8 mm*240 min) compared with the control breakfast effect (-786.7 $\pm$ 3254.7 mm*240 min).
According to the Mann-Whitney U test, ΔniAUC of desire for something sweet between the NW and OW group differed ($p < 0.05$).

3.3 Desire for a snack

Question: "How strong is your desire for a snack at this moment?".

Visual evaluation of the postprandial desire for a snack across time showed a slight distinction between the O3FA breakfast and the control breakfast, however both followed the same trend. The O3FA breakfast caused a higher decrease in postprandial desire and this desire stayed lower during the following 240 minutes after breakfast compared to consuming the control breakfast. Visual evaluation of the ΔniAUC change from baseline of desire for a snack showed that the average trend of the control breakfast gave a positive value while the average trend of the O3FA breakfast showed a negative value, both in the NW and OW group.

With $p > 0.05$ on the Shapiro-Wilk test, it could be concluded with 95% certainty that the difference between the paired values of ΔniAUC of desire for a snack was normally distributed so a paired t-test could be conducted. Results from this latter test are shown in Table 3.2. For the combined group, the NW, and the OW group was a significant difference found. In all the cases it could be concluded with 95% certainty that consumption of the O3FA breakfast resulted in a greater decrease of the change in desire for a snack from baseline, compared to when the participants consumed the control breakfast.
From the Mann-Whitney U test, insufficient evidence existed in the data set to say that ΔniAUC of desire for a snack was different between the NW and OW group (p = 0.312).

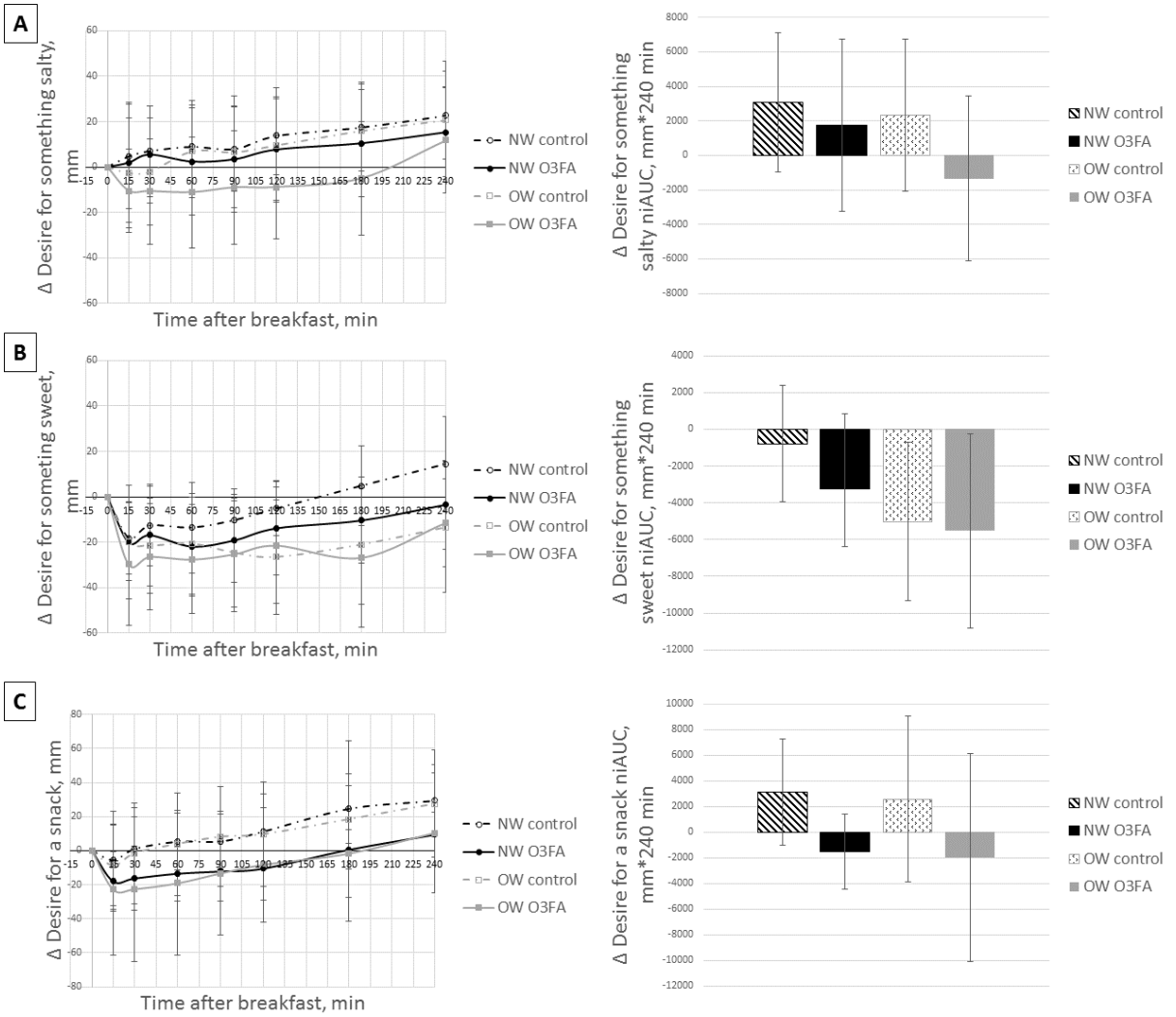

Figure 3.1: The desire for something salty (A), something sweet (B), and a snack (C) across time (left) and ΔniAUC over the 240 minute period (right). Data is represented as mean ± standard deviation. NW, normal weight; OW, overweight/obese; O3FA, omega-3 fatty acids.

Table 3.1: Test of normality. Shapiro-Wilk test results of difference in ΔniAUC over the 240 minute period for desire for something salty, something sweet, and a snack.

	Shapiro-Wilk	
	Statistic	**p**
Desire for something salty (combined)	0.973	0.646
Normal weight	0.964	0.764
Overweight/Obese	0.925	0.256
Desire for something sweet (combined)	0.908	0.015 *
Normal weight	0.893	0.075
Overweight/Obese	0.926	0.269
Desire for a snack (combined)	0.983	0.901
Normal weight	0.946	0.466
Overweight/Obese	0.940	0.419

* $p < 0.05$.

Table 3.2: Paired samples statistics and results of t-test of ΔniAUC over the 240 minute period for desire for something salty, and desire for a snack.

	Paired Samples Statistics		Paired Samples Test		
	Correlation	**Sig. correlation**	**t**	**df**	**Sig. (2-tailed)**
Desire for something salty (combined)	-0.109	0.572	1.861	28	0.073 ^{ns}
Normal weight	-0.105	0.710	0.738	14	0.472 ^{ns}
Overweight/Obese	-0.185	0.527	1.874	13	0.084 ^{ns}
Desire for something sweet (combined)	No paired samples t-test				
Normal weight	0.093	0.742	1.872	14	0.082 ^{ns}
Overweight/Obese	0.565	0.035	0.409	13	0.690 ^{ns}
Desire for a snack (combined)	0.493	0.007	4.215	28	0.000 ***
Normal weight	0.667	0.007	5.614	14	0.000 ***
Overweight/Obese	0.466	0.093	2.133	13	0.053 ^{ns}

Sig., significance; t, paired T test statistic; df, degrees of freedom. ***; * $p < 0.05$; *** $p < 0.001$.

4 Energy expenditure and substrate utilization

Results for resting energy expenditure (REE) and thermic effect of food (TEF) are represented in Figure 4.1 A and B, respectively. Results for carbohydrate and fat oxidation are shown in Figure 4.2 A and B, respectively. Both figures contain the over time (top) and niAUC graphs (bottom). Data is represented as mean ± SD and ime 0 represents the fasting state before consuming the breakfast. The line graphs show the data for the individual time points per group (normal weight and overweight/obese) after consuming the control and O3FA breakfast. The bar graphs represent the niAUC data. To evaluate if using a t-test on these data was eligible, a Shapiro-Wilk test for normality was performed and results are shown in 4.1.

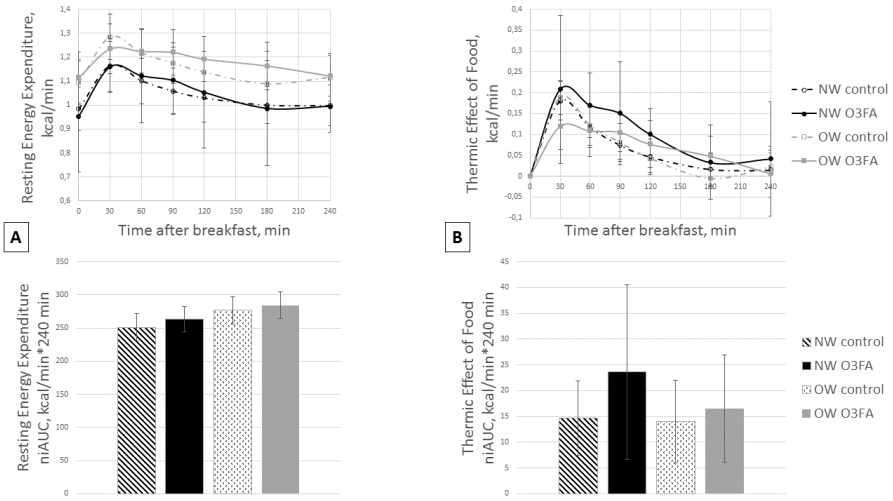

Figure 4.1: Postprandial REE (left) and TEF (right) after consumption of either the control or O3FA breakfast in the normal weight group (n=14) and overweight/obese group (n=14). Data is represented as mean ± standard deviation. NW, normal weight; OW, overweight/obese; O3FA, omega-3 fatty acids; niAUC, net incremental area under the curve.

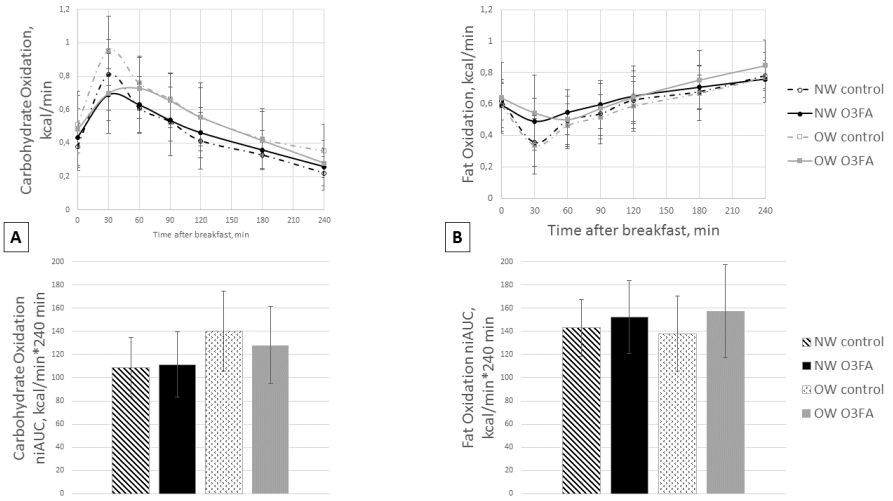

Figure 4.2: Postprandial substrate oxidation. Carbohydrate (left) and fat oxidation (right) after consumption of either the control or O3FA breakfast in the normal weight group (n=14) and overweight/obese group (n=14). Data is represented as mean ± standard deviation. NW, normal weight; OW, overweight/obese; O3FA, omega-3 fatty acids; niAUC, net incremental area under the curve.

Table 4.1: Test of normality. Shapiro-Wilk test results of niAUC over the 240 minute period for REE, TEF, carbohydrate oxidation, and fat oxidation.

	Shapiro-Wilk	
	Statistic	**p**
REE (combined)	0.971	0.597
Normal weight	0.990	1.000
Overweight/Obese	0.915	0.188
TEF (combined)	0.919	0.033
Normal weight	0.934	0.346
Overweight/Obese	0.983	0.989
Carbohydrate oxidation (combined)	0.877	0.004
Normal weight	0.820	0.012
Overweight/Obese	0.932	0.362
Fat oxidation (combined)	0.834	0.001
Normal weight	0.779	0.004
Overweight/Obese	0.833	0.017

The test for normality showed that data from TEF (combined), carbohydrate oxidation (combined), carbohydrate oxidation (normal weight), fat oxidation (combined), fat oxidation (normal weight), and fat oxidation (overweight/obese) were not eligible for a t-test. This was because it could not be concluded with 95% certainty that the difference between the paired values of these data sets was normally distributed. Subsequently, for the data sets mentioned before a non parametric sign test for paired observations was performed and results for this test are represented in Table 4.2. For data sets that were normally distributed, a paired t-test was performed and results can also be found in Table 4.2.

Visual evaluation of the resting energy expenditure across time showed the expected perception of the basal metabolic rate (i.e. post-absorptive state, time point 0) being initially higher in OW participants. The BMR value was not different between the control and O3FA study day in both NW and OW participants ($p > 0.05$), but the BMR value was significantly different between the NW and the OW group ($p < 0.001$). The following course of the REE had approximately the same development, with the OW group staying higher compared to the NW group. Both NW (250.8 ± 21.4 kcal/min*240 min for control, and 263.3 ± 20.1 kcal/min*240 min for O3FA) and OW (276.8 ± 21.4 kcal/min*240 min for control, and 284.3 ± 20.7 kcal/min*240 min for O3FA) participants consuming the O3FA breakfast had a significantly higher postprandial REE ($p < 0.05$ for both NW and OW) compared to consuming the control breakfast, based on the niAUC data.

Remarkably, when visually evaluating the TEF time course data, the OW group consuming the O3FA breakfast had a notable smaller increase at 30 minutes postprandial, while it seemed that in the NW group the TEF increased more when consuming the O3FA breakfast. Results from the paired t-test illustrated that for both NW (14.7 ± 7.5 kcal/min*240 min for control, and 23.6 ± 17.6 kcal/min*240 min for O3FA) and OW (14.0 ± 8.3 kcal/min*240 min for control, and 16.6 ± 10.8 kcal/min*240 min for O3FA) participants the TEF niAUC data did not differ significantly ($p = 0.0545$ for NW, and $p = 0.1405$ for OW) between the control and O3FA breakfast.

The niAUC for carbohydrate oxidation was for both NW and OW not different between the control and O3FA breakfast. However, when examining the first postprandial time point (30

minutes after consuming the breakfast), the difference between the carbohydrate oxidation (kcal/min) was significantly different between the control and O3FA breakfast for both weight groups ($p < 0.05$). At the next time point (60 minutes after consuming the breakfast) this significant difference already disappeared again in both weight groups (p = 0.3425 for NW, and p = 0.341 for OW).
The niAUC for fat oxidation was different between the control and O3FA breakfast for the NW group ($p < 0.05$) but not for the OW group (p = 0.3955). Examining the first postprandial fat oxidation (kcal/min) time point (30 minutes after consuming the breakfast) showed that a significant difference between the control and O3FA breakfast for both weight groups.

The metabolic variables VO_2 (mL/min), VCO_2 (mL/min), and npRQ are represented in Table 4.3. Time 0 represents the fasting state before consuming the breakfast.

To evaluate the breakfast effect, control and O3FA data were put in separate groups (normal weight and overweight/obese together) so a paired samples t-test could be conducted. Testing for normality with the Shapiro-Wilk test showed that only data from VO_2 and VCO_2 were eligible a t-test on (Shapiro-Wilk: VO_2 p = 0.498, VCO_2 = 0.295, npRQ $p < 0.05$). Subsequently, npRQ was tested with a sign test (non parametric test).
Results from the sign test showed no significant difference between the control and O3FA breakfast on npRQ (p = 0.2855). Results from the paired samples t-test on VO_2 showed a significant average difference between consuming the control (228.8 $\pm$ 21.2 mL/min) and the O3FA breakfast (236.8 $\pm$ 20.3 mL/min), t_{27} = -3.345 and $p < 0.05$. The paired samples t-test on VCO_2 showed no significant difference (t_{27} = -1.618, p = 0.0585). With 95% certainty it can be concluded that consuming a breakfast supplemented with EPA and DHA increased postprandial VO_2.

To evaluate the group effect (normal weight versus overweight/obese), control and O3FA data were put together. Testing for normality with the Shapiro-Wilk test showed that only data from VO_2 and npRQ were eligible a t-test on. Subsequently, VCO_2 was tested with a Mann-Whitney U test (non parametric test).
Results from the Mann-Whitney U test showed that VCO_2 was significantly different ($p < 0.001$) between both groups, with Z = -3.818. Results from the independent samples t-test on VO_2 showed that the consumed oxygen in the normal weight group (223.1 $\pm$ 18.9 mL/min) was significantly lower compared the amount consumed in the overweight/obese group (242.5 $\pm$ 18.5 mL/min) with t_{54} = -3.878 and two-tailed $p < 0.001$. Performing an independent samples t-test on npRQ data showed that the npRQ of the normal weight group (0.83 $\pm$ 0.04) was significantly lower compared to the npRQ of the overweight/obese group (0.84 $\pm$ 0.04) with t_{54} = -1.898 and one-tailed $p < 0.05$.

Table 4.2: Paired samples statistics and results of t-test of niAUC over the 240 minute period for REE (combined, NW, and OW), TEF (NW, and OW), carbohydrate oxidation (OW). Results of sign test for paired observations results of TEF (combined), carbohydrate oxidation (combined, and NW), fat oxidation (combined, NW, and OW).

	Paired Samples Statistics		Paired Samples Test			Sign Test
	Correlation	Sig. correlation	t	df	Sig. (2-tailed)	Sig. (2-tailed)
REE (combined)	0.827	0.000	-3.721	27	0.001 *	
Normal weight	0.800	0.001	-3.538	13	0.004 *	
Overweight/Obese	0.743	0.002	-1.841	13	0.089 ns	
TEF (combined)						0.089 ns, a
Normal weight	-0.027	0.927	-1.720	13	0.109 ns	
Overweight/Obese	0.624	0.017	-1.124	13	0.281 ns	
Carb. oxidation (combined)						0.700 ns, a
Normal weight						0.581 ns, b
Overweight/Obese	-0.215	0.460	0.835	13	0.419 ns	
Fat oxidation (combined)						0.054 ns, a
Normal weight						0.022 *, b
Overweight/Obese						0.791 ns, b

NW, normal weight; OW, overweight/obese; Sig., significance; t, paired T test statistic; df, degrees of freedom. ns not significant; * $p < 0.05$. a Asymptotic Significance (2-Tailed Sign.); b Exact Significance (2*(1-Tailed Sig.)).

Table 4.3: Postprandial metabolic variables following consumption of the breakfast[1].

Time following	Normal Weight *(n=14)*		Overweight/Obese *(n=14)*	
breakfast (min)	Control	O3FA	Control	O3FA
VO_2 (mL/min)				
0	205.4 ± 18.6	210.5 ± 19.1	227.2 ± 18.6	231.9 ± 22.1
30	238.1 ± 22.3	241.9 ± 20.7	261.4 ± 19.3	254.5 ± 21.9
60	227.2 ± 19.9	241.8 ± 24.8	249.9 ± 20.4	251.8 ± 19.0
90	219.3 ± 19.6	236.0 ± 21.4	242.3 ± 17.4	252.0 ± 20.1
120	214.9 ± 20.9	231.6 ± 19.4	235.7 ± 17.5	247.4 ± 19.3
180	209.8 ± 16.7	220.3 ± 14.5	227.2 ± 19.8	243.2 ± 21.2
240	210.7 ± 17.2	215.7 ± 16.1	234.0 ± 20.7	236.0 ± 18.5
VCO_2 (mL/min)				
0	166.3 ± 18.2	171.8 ± 19.6	190.3 ± 20.4	191.9 ± 22.1
30	214.3 ± 24.7	209.9 ± 19.6	240.2 ± 21.3	220.5 ± 21.6
60	195.6 ± 18.0	206.3 ± 23.9	220.8 ± 20.9	220.3 ± 17.6
90	184.7 ± 20.8	196.6 ± 15.7	209.9 ± 17.3	216.2 ± 17.7
120	174.8 ± 21.3	188.4 ± 18.1	199.0 ± 18.3	207.1 ± 19.3
180	165.8 ± 14.1	174.1 ± 11.9	185.3 ± 19.1	196.0 ± 18.4
240	160.7 ± 16.4	166.0 ± 12.7	185.9 ± 18.9	183.1 ± 14.7
Non-protein RQ				
0	0.81 ± 0.04	0.82 ± 0.06	0.84 ± 0.04	0.83 ± 0.06
30	0.86 ± 0.14	0.87 ± 0.04	0.92 ± 0.045	0.87 ± 0.06
60	0.86 ± 0.05	0.85 ± 0.04	0.88 ± 0.04	0.88 ± 0.04
90	0.85 ± 0.05	0.87 ± 0.12	0.87 ± 0.04	0.86 ± 0.04
120	0.83 ± 0.07	0.81 ± 0.05	0.84 ± 0.04	0.84 ± 0.05
180	0.80 ± 0.04	0.79 ± 0.05	0.82 ± 0.05	0.81 ± 0.05
240	0.77 ± 0.04	0.77 ± 0.04	0.79 ± 0.04	0.78 ± 0.04

[1]Data is represented as mean ± standard deviation. REE, resting energy expenditure; RQ, respiratory quotient; O3FA, omega-3 fatty acids.

5 24-hour food intake

Subsequent food intake after the test days assessed through the 24-hour food intake record is shown in Table 5.1.

Table 5.1: Energy and macronutrient composition of food intake during the remainder of the study days.

	Normal Weight *(n=15)*		**Overweight/Obese** *(n=14)*	
	Control	**O3FA**	**Control**	**O3FA**
LUNCH				
Total energy intake (kcal)	579 ± 341	688 ± 576	672 ± 276	648 ± 344
Protein (g)	28 ± 12	34 ± 21	27 ± 15	29 ± 14
Carbohydrate (g)	56 ± 38	66 ± 60	87 ± 37	79 ± 58
Fat (g)	27 ± 21	32 ± 35	24 ± 15	24 ± 22
DINNER				
Total energy intake (kcal)	598 ± 502	496 ± 310	698 ± 359	586 ± 320
Protein (g)	39 ± 37	22 ± 16	29 ± 19	34 ± 18
Carbohydrate (g)	52 ± 36	57 ± 28	96 ± 55	54 ± 35
Fat (g)	26 ± 32	20 ± 16	22 ± 16	26 ± 18
SNACKS				
Total energy intake (kcal)	335 ± 333	402 ± 370	315 ± 329	322 ± 243
Protein (g)	10 ± 13	11 ± 11	11 ± 15	10 ± 12
Carbohydrate (g)	40 ± 30	49 ± 43	43 ± 41	48 ± 33
Fat (g)	15 ± 23	18 ± 22	11 ± 15	10 ± 12
TOTAL				
Total energy intake (kcal)	1512 ± 656	1586 ± 904	1685 ± 484	1556 ± 524
Protein (g)	77 ± 39	67 ± 35	67 ± 27	73 ± 31
Carbohydrate (g)	148 ± 70	172 ± 93	226 ± 81	181 ± 72
Fat (g)	68 ± 43	70 ± 49	57 ± 25	60 ± 32

All data is represented as mean ± standard deviation.

All data sets were normally distributed according to the Shapiro-Wilk test, with the exception of data regarding the difference between control and O3FA breakfast in total energy intake at dinner for the NW group. A paired t-test was performed on the normally distributed data sets, while a sign test for paired observations was performed for the data set that was not normally distributed.

Results from these tests showed that the breakfast type (control versus O3FA) had no significant effect on the energy intake during lunch, dinner, or snacks in both the NW and OW group ($p > 0.05$). Also no significant difference was found in energy intake between weight groups.

V DISCUSSION AND CONCLUSIONS

1 Discussion

The aim of this thesis was to examine if a breakfast supplemented with LC n-3 PUFA (EPA and DHA) could affect postprandial appetite and craving responses, and energy expenditure in young women. To evaluate this, a pilot study using a double-blind, randomized crossover design was set up where appetite and craving responses were measured through VAS, resting energy expenditure were measured through indirect calorimetry and subsequent energy intake was measured through 24-hour food intake record. To our knowledge, this is the first pilot study to examine the effect of omega-3 fatty acids intake at breakfast on the postprandial energy expenditure, substrate utilization, and appetite in normal weight and overweight/obese young women.

Appetite sensations are suggested by Drapeau et al. [48] to be an effective way to predict spontaneous energy intake and body weight, and Stubbs et al. [131] proposed measuring appetite to be a long-term indicator of energy intake. Controlling appetite sensations could be a practical approach to decrease the energy intake consistently exceeding energy expenditure, eventually leading to overweight and obesity. Therefore, the effects of LC n-3 PUFA on appetite and satiety sensations were measured in this study. Satiety is the process that causes inhibition of further eating, lowering hunger sensations, increasing the sensation of fullness after a meal has ended and satiety is also known as the post-ingestive satiety or inter-meal satiety, while satiation is the process leading to finishing eating, also known as intra-meal satiety [15]. What has been examined in this thesis is the post-ingestive satiety, by measuring perceived hunger and perceived fullness after consuming the breakfast. The results from this thesis show that a supplementation of LC n-3 PUFA at breakfast leads to greater postprandial reductions of niAUC in perceived hunger, desire to eat, and prospective food consumption and increase of perceived fullness in both NW and OW young women when corrected for baseline measurements. The data suggest that consuming LC n-3 PUFA at breakfast could improve appetite control and satiety. Previous studies on the effect of LC n-3 PUFA on appetite and satiety have showed contradictory results [107, 22, 43]. A study from Lawton et al. [82] showed that PUFA in the diet enhanced satiety compared to monounsaturated fatty acids. Results from this latter study were in accordance with the findings from the current study as the fat source in the control breakfast was high oleic sunflower oil, which is high in monounsaturated fats. Even though sensations of hunger were lower and sensations of fullness were higher when consuming a LC n-3 PUFA rich breakfast, it is important to understand that appetite and satiety can also be affected by other factors such as environmental influences, macronutrient and energy content, and palatability [26]. Palatability was not found to be significantly different between the breakfast drinks, therefore this should not have affected the appetite sensations. The observed appetite lowering and fullness increasing effect of LC n-3 PUFA could be explained by the effect on the transport of appetite-regulating molecules such as dopamine [125] and the interaction they have with various neuroendocrine factors that play a role in the brain-gut loop signals related to energy metabolism (e.g. insulin) [63, 95] and leptin [151, 110]. However, this was not measured in this current study.
Supplementation of LC n-3 PUFA could be helpful and could increase compliance during energy restricted and/or regular exercise weight loss programs. A study from Parra et al. [107]

showed that LC n-3 PUFA had a modulating effect on hunger signals and postprandial satiety, especially when consuming a dinner rich in LC n-3 PUFA. However, Parra et al. mentioned that it is not sure if the observed satiety response would continue to show while in energy equilibrium state and not in energy restriction phase because this latter could have modified participants appetite sensations. Another study, from Kunešová et al. [79], investigated if LC n-3 PUFA had an additional effect with a weight loss program including dietary energy restriction and regular exercise for 3 weeks. Severely obese women (BMI $\geq$ 40 kg/m^2) experienced greater weight, BMI, and hip circumference reductions when consuming LC n-3 PUFA (in combination with the assigned weight loss program with a very low-calorie diet and regular exercise).

Results from this thesis showed that consumption of LC n-3 PUFA at breakfast resulted in a greater decrease in the change in desire for a snack from baseline, both in normal weight and overweight/obese participants. However, the 24-hour food intake for the rest of that day did not show a higher subsequent (energy) intake in snacks on days when the control breakfast was consumed. Consuming a breakfast in the morning is important, as it might help to decrease the consumption of unhealthy, energy-dense snacks [84]. The consumption of high-fat and/or high-sugar snacks and soft drinks were previously indicated as having an important role in the etiology of obesity [111]. A study from O'Connor et al. [101] reported that overweight participants (BMI $\geq$ 25 kg/m^2) consumed more snacks like crisps, sweets, chocolates and ice-creams while they consumed less snack products like yogurt and nuts compared to normal weight participants (BMI 18 - 24.9 kg/m^2). In this thesis study, normal weight participants mainly reported fruits, nuts, cereal bars, and yogurt as snacks while overweight/obese participants mainly chose for cookies, fruits, juice, and candy. Developing snack decisions towards choosing for more healthier snacks might be a possibility to improve anti-obesity public health.

Women from the overweight/obese weight group had higher basal metabolic rate, which could be expected since BMR increases with e.g. body weight. Even though the breakfast drinks had the same macronutrient composition, calorie content and volume, resting energy expenditure turned out to be increased in both weight groups when consuming a breakfast supplemented with LC n-3 PUFA, compared to a control breakfast (without supplemented LC n-3 PUFA). Some authors have previously reported contradictory results about the effect on RMR. Gerling et al. [55] reported an increasing of RMR with consumption of LC n-3 PUFA however, this was a longer-term study so an effect of adaptation might have been present.
The indirect calorimetry metabolic measurement system calculated the carbohydrate and fat oxidation from the non-protein RQ. The value of RQ varies with the nutrients that are being oxidized and can give a percentage contribution of carbohydrates and fats (some values are shown in Appendix D). A RQ value typically lies between 0.7 and 1.0, when closer to 0.7 it typically means that fat is the main fuel being burned while a value closer to 1.0 typically means more carbohydrates are being burned as fuel. In this study, npRQ of the normal weight group was significantly lower compared to the npRQ of the overweight/obese group.
Both breakfast drinks contained 36.6% of carbohydrates (percentage of total kcal). However, compared to the control breakfast (same amount of fat content), the O3FA breakfast did not cause an initial peak 30 minutes postprandial in the carbohydrate oxidation time course.
Results from Couet et al. [39] showed a stimulated basal fat oxidation in healthy adults (BMI: 21.9 $\pm$ 1.6 kg/m^2) when supplemented with LC n-3 PUFA for 3 weeks. These results from Couet et al. could be in agreement with the observation in this study where only the normal

weight group showed a significant higher fat oxidation however, Couet et al. only measured basal substrate oxidation while this pilot study measured the postprandial effect of an acute trial. Zurlo et al. [159] suggested that a reduced fat oxidation might be a factor in the development of obesity. A study from Mori et al. [92] demonstrated that obesity-prone mice fed with a diet containing 8% fish oil had a lower body weight gain and higher expression of oxidative enzymes in intestinal tissue compared with the mice fed with a diet containing no fish oil. Mori et al. concluded that the LC n-3 PUFA oil might increase fat oxidation, leading to the observed anti-obesity effect. Improving body composition after increasing the intake of LC n-3 PUFA is most likely due to an altered gene expression that shift the metabolism towards increased accretion of lean tissue, enhanced fat oxidation in adipose, liver, cardiac, intestinal, and skeletal muscle tissue, a reduced fat deposition in adipose tissue, and increased energy expenditure [25, 65]. The previously mentioned study from Kunešová et al. [79] demonstrated this as well. There was a greater increase reported in serum levels of β-hydroxybutyrate in the severely obese women supplemented with LC n-3 PUFA, associated with an increased fat oxidation. In this thesis, fat oxidation was found to be significantly higher after consuming the breakfast supplemented with LC n-3 PUFA in the normal weight group but not in the overweight/obese group. The results of the higher fat oxidation in normal weight group together with the suggestion of Zurlo et al. suggests that consuming LC n-3 PUFA could prevent the development of obesity in normal weight young women. Binnert et al. [13] examined oxidation rates in obese and non-obese subjects consuming long-chain triacylglycerol and found a lower oxidation in the obese subjects compared to the control subjects. They also found that the amount oxidized was negatively correlated with the body fatness. The conclusion from Binnert et al. was that obesity could be associated with a defect in dietary fat oxidation, probably due to an excessive long-chain fatty acid uptake in the adipose tissue, which may play a role in human obesity.

While consumption of the O3FA breakfast led to a decreased sensation of perceived hunger and an increased sensation of perceived fullness, consuming omega-3 fatty acids at breakfast did not have any influence on the energy intake during following lunch, dinner, or snacks in both the normal weight and overweight/obese weight group in this pilot study. These findings are in agreement with the observations in the long-term study from Couet et al. [39], where energy intakes were unchanged when healthy volunteers were supplemented with fish oil for 3 weeks. This thesis study made use of 24-hour food intake records however, it has to be mentioned that the use of these records have well known limitations (see more about this in the section Strengths and additional limitations of this study). Average values of perceived hunger were exceeding baseline fasted levels after 240 minutes postprandial in all groups, which could have led to overeating at lunch. However, this last statement is not sure as no 24-hour food intake record was registered on a regular day so normal food intake patterns were not known. Interestingly, there were no significant differences found in energy intake between the normal weight and overweight/obese weight group, which would have been expected as overweight/obese participants have a larger body weight compared to the normal weight participants.

The American diet is low in LC n-3 PUFA intake and women tend to have a lower intake of EPA and DHA [105]. Incorporating more fish meals might be a possibility to consume more LC n-3 PUFA, as fish are the major food source of EPA and DHA (the PUFA of interest). However, the content changes significantly among and within the fish species. An estimation of the EPA and DHA content in different fish species can be made, but can vary remarkably (up to 300%) with

season, packaging and preparing of the fish [109]. Appendix D gives an overview of an estimation of EPA + DHA content in several fish species, as well as the amount of fish consumption required to provide an intake of approximately 1 gram of EPA + DHA per day. Keeping in mind that a health risk related to fish consumption is greater exposure to methylmercury. Potentially contaminated fish should be avoided by children and pregnant and lactating women as they may have a higher risk for intoxication. For these population groups, functional foods enriched with LC n-3 PUFA could be an alternative to include more LC n-3 PUFA in their diet. However, fish or fish products contain additional nutrients next to these LC n-3 PUFA, such as high quality protein, vitamins and minerals. Fish species with a high fat content (e.g. salmon, sardines, and mackerel) contain also a range of fat-soluble vitamins [50]. However, when consuming function foods enriched with LC n-3 PUFA, these additional beneficial nutrients are not consumed.

Strengths and additional limitations of this study

One strength of this pilot study is the strong double-blind, randomized crossover design. Both normal weight and overweight/obese individuals were included in the study, which gave extra perception on possible different ways these two weight groups would react on the supplementation of LC n-3 PUFA. However, this research was as a pilot study so the results of this study were based on only a limited amount of participants in both groups (normal weight group n = 15, overweight/obese group n = 14). During the progress of the study, a total of six scheduled participants dropped out for several reasons, which delayed finishing the study. Including more participants could have made the outcome of this study better and the power could have been greater. However, because of delayed start of the study and unexpected setbacks (e.g. several last-minute dropouts), there was limited time to collect replacements for participant dropouts or to include more participants. Another benefit is the amount of time points where measurements were taken during the study days, which gave a good representation of how appetite, cravings, energy expenditure, and substrate oxidation progressed during the 240 minutes postprandial and for instance when they turned back to baseline. Also, this study measured preprandial appetite sensations so those influences could be considered as well as differences from baseline could be calculated.

A limitation in this study is the use of 24-hour food logs. Results from this technique to assess food intake in humans should be severely questioned. Dhurandhar et al. [47] mentioned the significant limitations of self-reports and point them out as so poor that they are not at all acceptable for scientific research on energy intake. They call the errors being not random. Schoeller et al. [122] tested the accuracy of these self-reported energy intake records, compared with measures from the doubled labeled water (DLW) technique and showed that there was a systematic underestimation of energy intake by hundreds of kcal/day. They also mentioned that the bias was highest in obese subjects. Participants can fill in the records carelessly, vague, and can decide not to report side food products (such as condiments, cooking oils, and salad dressing) as these are much more difficult to measure precisely. Another major challenge is the analysis of dietary intake records [80]. Food databases might have some limitations as many specific food products are not included in the software. However, to keep an overall alignment and if possible, food products noted with the U.S. Departement of Agriculture (USDA) as supplier were selected. Even with all these well known limitations on the use of self-reported food

records, this method is still used in scientific research as there is not yet an acceptable alternative available.
During the screening procedure, there was no question on the current fish intake of the participant, nor if they were currently taking any omega-3 supplements.
Another possible limitation of this study might be the caloric content of the test breakfasts. Both weight groups (normal weight and overweight/obese) were served the same breakfast with the exact same caloric content. Overweight/obese participants could be used to consuming typically breakfasts higher in calories compared to normal weight participants. However, it can be debated if test meals in studies should be admitted as a function of body size/weight, rather than a general meal (so without taking the absolute energy requirements of the individual into account) [15].
Appetite-related hormones were not measured in this study however, this additional data could have been useful. In a previous study from Parra et al. [107] leptin (according to Joannic et al. [71] leptin is a long-term energy controller) was found to be highly significantly related with perceived fullness and the desire to eat after consuming the test meal.
Another crucial portion of the study is to ensure that participants adequately followed the 10 to 12 hour fasting period before data collection. Because of the study design it was unable to ensure that participants followed this procedure correctly.
Comparing with other human studies investigating possible anti-obesity effects of LC n-3 PUFA supplementation can be hard because a lot of discrepancies arise due to lack of comparable experimental designs, concentration administered, the way of consuming LC n-3 PUFA (pill supplementation, added to a drink which can maybe cause an interaction effect with the food matrix, or as consuming real fish), the type and concentrations of LC n-3 PUFA, study length (with or without adaptation period), and different subject size. However, if a good control is used, results from different studies may be compared to each other.
Lastly, because this study was designed to be an acute trial, the longevity of the results were unable to be verified. Future studies on this topic should increase the analyzing time frame, which could give different results. Acute trials are widely used, however acute changes (or no changes) do not mean immediately that these would continue and observed over the longer term.

2 Conclusions and further perspectives

This pilot study served as a first step in examining possible anti-obesity effects in young women when consuming LC n-3 PUFA at breakfast. Possible effects that were investigated were appetite and craving control, as well as postprandial energy expenditure and substrate utilization.

Results from this pilot study suggest that consumption of breakfast supplemented with LC n-3 PUFA could improve appetite control and satiety in both normal weight and overweight/obese young women. Resting energy expenditure turned out to be increased in both weight groups when consuming a breakfast supplemented with LC n-3 PUFA, compared to a control breakfast (without supplemented LC n-3 PUFA). There was no difference found in postprandial carbohydrate oxidation, but fat oxidation was significantly higher after consuming the breakfast supplemented with LC n-3 PUFA in the normal weight group but not in the overweight/obese group. Even though participants indicated to be more hungry and less full after consuming the control breakfast compared to the omega-3 breakfast, the 24-hour food intake record did not show any difference in energy intake (kcal) at lunch, dinner, or snacks in both the normal weight and overweight/obese weight group.

While some results are promising in this pilot study investigating the possible anti-obesity effects of LC n-3 PUFA consumption, there are still several limitations that must be considered. To provide more proof and a stronger study, more well-controlled research should be done including more participants and with a longer duration. Investigating possible outcomes such as weight management over longer-term and measuring appetite-related hormones may provide a greater insight into the possible anti-obesity effects of consuming LC n-3 PUFA.

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Appendices

Appendix A: IRB Approval, Protocol 16-09-124

UNIVERSITY OF ARKANSAS

Office of Research Compliance
Institutional Review Board

January 20, 2017

MEMORANDUM

TO:	Jamie Baum	Eva Dehaene
	Hexirui Wu	Charlayne Mitchell
	Sarah Russell	Aubree Worden
	Regan Burgess	Katie Cloud
	Lauren Landwehr	
FROM:	Ro Windwalker IRB Coordinator	
RE:	PROJECT MODIFICATION	
IRB Protocol #:	16-09-124	
Protocol Title:	*The Effect of Breakfast Intake on Energy Expenditure and Hunger in Young Women*	
Review Type:	☐ EXEMPT ☒ EXPEDITED ☐ FULL IRB	
Approved Project Period:	Start Date: 01/20/2017 Expiration Date: 10/13/2017	

Your request to modify the referenced protocol has been approved by the IRB. **This protocol is currently approved for 300 total participants.** If you wish to make any further modifications in the approved protocol, including enrolling more than this number, you must seek approval *prior to* implementing those changes. All modifications should be requested in writing (email is acceptable) and must provide sufficient detail to assess the impact of the change.

Please note that this approval does not extend the Approved Project Period. Should you wish to extend your project beyond the current expiration date, you must submit a request for continuation using the UAF IRB form "Continuing Review for IRB Approved Projects." The request should be sent to the IRB Coordinator, 109 MLKG Building.

For protocols requiring FULL IRB review, please submit your request at least one month prior to the current expiration date. (High-risk protocols may require even more time for approval.) For protocols requiring an EXPEDITED or EXEMPT review, submit your request at least two weeks prior to the current expiration date. Failure to obtain approval for a continuation *on or prior to* the currently approved expiration date will result in termination of the protocol and you will be required to submit a new protocol to the IRB before continuing the project. Data collected past the protocol expiration date may need to be eliminated from the dataset should you wish to publish. Only data collected under a currently approved protocol can be certified by the IRB for any purpose.

If you have questions or need any assistance from the IRB, please contact me at 109 MLKG Building, 5-2208, or irb@uark.edu.

109 MLKG • 1 University of Arkansas • Fayetteville, AR 72701-1201 • (479) 575-2208 • Fax (479) 575-6527 • Email irb@uark.edu

Appendix B: Participant Screening Questionnaire

Participant information:
Name:
Age (must be 18-30 years of age to participate):
Date of birth:

Ethnicity:
Caucasian African American Asian or Asian American
Latino/Hispanic Indian Other:

What is your estimated height (indicate inches and feet):
What is your estimated weight (indicate lbs):

(Calculate BMI to determine qualification. If not qualified, STOP HERE).

Contact Information:
Phone Number:
Email Address:
Preferred Method of Contact: phone email text message

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1. Do you currently have (check all that apply):
__ Lactose Intolerance
__ Food Allergies (if yes, please specify):
__ Gluten Intolerance
__ Celiac Disease
__ Other Intestinal Diseases (if yes, please specify):
__ Asthma/Allergies (if yes, please specify):
__ Eye Injury/Surgery
__ Color Blindness
__ High Blood Pressure
__ Kidney Disease
__ Diabetes
__ Cardiac Disease __ Other Heart Problems (if yes, please specify):
__ Inherited/Genetic Disease (if yes, please specify):
__ Cancer
__ Other (if yes, please specify):

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2. Are you pregnant or is there any change you are pregnant?
Yes **No**
(If yes, STOP SCREENING process)

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3. Are you a competitive?
Yes **No**
(If yes, STOP SCREENING process)

4. Do you consume breakfast on a regular basis (five or more times per week)?
Yes **No**
(If no, STOP SCREENING process)

5. Are you currently taking any medication (over the counter and/or prescribed)?
Yes **No**
If yes, please list medication and reason(s) prescribed:

(STOP SCREENING process if on any prescribed medication. Birth control is ok).
(Ask Dr. Baum about over the counter medication).

6. Are you currently dieting or did you lost a lot of weight the last three months?
Yes **No**
(If yes, STOP SCREENING process)

7. Are you a vegetarian or a vegan?
Yes **No**

8. Do you exercise?
Yes **No**
a. If yes, what type of exercise do you participate in?:
b. How long do you exercise per session?:
c. How often do you exercise each week?:

9. Are there any food products that you will not eat?
Yes **No**
If yes, please specify:

10. Would you be willing to rest quietly for 30 minutes intervals, with a metabolic hood place over your head and upper body?
Yes **No**
Would you feel claustrophobic in this situation?
Yes **No**

11. Will you be able to drink 1.5 cup of a beverage within 10 minutes?
Yes **No**

IF THE SUBJECT QUALIFIES, ASK FOLLOWING ADDITIONAL QUESTIONS:

A. Are you available to come to the research center on study days?
This will involve coming to the research center between 7:30 and 8:00 a.m. and leaving between 12:30 and 1:00 p.m.
Yes **No**

B. Are you available on weekdays?
Yes **No**
If yes, please specify:

C. Are you available on weekends?
Yes **No**
If yes, please specify:

D. When was the first day of your last period?
Do you take birth control pill?
Yes **No**

Appendix C: Self-reported dietary intake document

UofA Department of Food Science
Nutrition and Energy Metabolism Lab

24-Hour Food Intake Record

- Provide **all** details regarding food intake
- Be as accurate as possible
- If the food is prepared at home or in a restaurant, please include a description of the preparation techniques (ex. Grilled vs. fried)

For example:

LUNCH												
			Unit of Measurement									
Time	Brand	Product Description	cups	tbsp.	grams	lbs.	tsp.	oz.	fl.oz.	mL	Item	All
12:00	Sara Lee	2 slices of 100% Whole Wheat Bread										X
12:00	Hellman's	Light Mayonnaise					2					
12:00	Hormel Nat. Select	2 slices of oven roasted turkey										X
12:00	Great Value	1 slice of American cheese										X
12:00	Lays	BBQ potato chips					2					X
12:00	Diet Coke	1 can 12 ounce can						12				

- Please keep track of your activities throughout the day
- However, please maintain your typically weekly activity level

Study day ____ **Subject #:**
Date:

UofA Department of Food Science
Nutrition and Energy Metabolism Lab

LUNCH												
			Unit of Measurement									
Time	Brand	Product Description	cups	tbsp.	grams	lbs.	tsp.	oz.	fl.oz.	mL	Item	All

DINNER												
			Unit of Measurement									
Time	Brand	Product Description	cups	tbsp.	grams	lbs.	tsp.	oz.	fl.oz.	mL	Item	All

Study day ____ **Subject #:**
Date:

UofA Department of Food Science
Nutrition and Energy Metabolism Lab

SNACKS												
			Unit of Measurement									
Time	Brand	Product Description	cups	tbsp.	grams	lbs.	tsp.	oz.	fl.oz.	mL	Item	All

ACTIVITIES		
Name of activity	Duration	Intensity (please cirle)
		Low *Medium* *High*
		Low *Medium* *High*
		Low *Medium* *High*
		Low *Medium* *High*

Appendix D: EPA + DHA content in fish

Modified from Penny et al. [109].

	EPA + DHA content g/85-g serving fish (edible portion)	**Amount required to provide ± 1 gram of EPA + DHA per day** grams
Herring (Atlantic)	1.71	57
Herring (Pacific)	1.81	43
Mackerel	0.34 - 1.57	57 - 241
Oyster (Pacific)	1.17	71
Oyster (farmed)	0.37	227
Sardines	0.98 - 1.70	57 - 85
Salmon (pink)	1.09	71
Salmon (Atlantic, wild)	0.9 - 1.56	57 - 99
Shrimp (mixed species)	0.27	312
Trout (rainbow, farmed)	0.98	85
Trout (rainbow, wild)	0.84	99
Tuna (fresh)	0.24 - 1.28	71 - 340
Tuna (light, canned in water)	0.26	340

Appendix D: Non-protein RQ values with corresponding percentage contribution of carbohydrate and fat being oxidized

Modified from Robert Robergs R. [115].

npRQ	% CHO	% FAT
1.00	100.0	0.0
0.95	84.0	16.0
0.90	67.5	32.5
0.85	50.7	49.3
0.80	33.4	66.6
0.75	15.6	84.4
0.71	1.1	98.9
0.707	0.0	100.0

npRQ, non-protein respiratory quotient; CHO, carbohydrate oxidation; FAT, fat oxidation.