The effects of seven days of fasting on body composition, physical capacity and metabolic regulation followed by three days with re-feeding

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Abstrakt

Formål

Langvarig faste har i mange tiår blitt benyttet som et helsefremmende terapeutisk tiltak hos overvektige som vil gå ned i vekt. Langvarig faste reduserer kroppsfett, og dette kan fremme den metabolske helsen. Derimot fører faste til redusert muskelmasse, som øker sjansen for å utvikle sarkopeni. Langvarig faste kan også være en vektregulerende strategi for å forebygge overvekt hos friske, normalvektige mennesker. Bare et fåtalls studier har undersøkt effekten av langvarig faste hos normalvektige, og det er lite kunnskap om hvordan kroppen tilpasser seg spising etter en langvarig faste. Formålene med studien var å undersøke effektene av syv dagers langvarig faste etterfulgt av tre dager med næringsinntak hos normalvektige på 1) kroppssammensetning, 2) fysisk kapasitet og 3) konsentrasjoner av hormoner og metabolitter i blod som regnes som markører for metabolsk helse.

Metode

12 friske, normalvektige forsøkspersoner (5 kvinner: gj.snitt vekt (SD) 64.8 kg (6.42), gj.snitt BMI (SD) 23 kg/m^2 (1.3), og 7 menn: gj.snitt vekt (SD) 86.6 kg (13.83), gj.snitt BMI (SD) 26 kg/m^2 (2.6)) gjennomførte studien som bestod av a) tilvenningstest, b) pre-test (utgangspunkt), c) syv dagers langvarig faste med bare vanninntak, og d) post-test (tre dager etter fasten). Forsøkspersonene hadde et kosthold etter eget ønske i dagene før og etter fasten. Kroppsvekt, kroppssammensetning med DXA (fettfri masse og fettmasse), og markører og energisubstrater i blod (glukose, triasylglyseroler, frie fettsyrer, β-hydroksybutyrat og skjoldbruskhormonmetabolitt T_3) ble analysert på pre-test, på dag 7 av fasten, og på post-test. Nitrogenutskillelse i urin ble målt hver dag under fasten. Fysiske tester (maksimalt oksygenopptak, VO_{2maks} og maksimal fettoksidering, $fettoks_{max}$) ble gjennomført på ergometersykkel på pre-test og post-test. Statistisk signifikansnivå var satt til $p<0.05$. Analyser inkluderte Student T-test og one-way repeated measures ANOVA for gjennomsnitt (gj.snitt) (SD), og Wilcoxon Signed Rank Test og Friedman test for median (IQR).

Resultater

For kroppskomposisjon så var vekt, fettfri masse og fettmasse redusert i løpet av fasten (alle $p<0.05$), og deltagerne hadde i gj.snitt mistet 2,4 kg med muskler. Næringsinntak fra dag 7 til post-test medførte økning i vekt og fettfri masse (begge $p<0.05$), men ikke i fettmasse. Gj.snitt vekt var redusert med 3,2 kg (0.68), median fettfri masse var redusert med 0.8 kg (-1.23, 0.11) og gj.snitt fettmasse var redusert med 1.8 kg (0.54) (alle $p<0.05$) fra pre-test til post-test. Alle parameterne for kroppskomposisjon endret seg mellom de tre tidspunktene (alle $p<0.001$). For fysisk kapasitet så var median absolutt VO_{2maks} redusert med 245 ml·min^{-1} (-383.1, -85.0)) ($p<0.05$) fra pre-test til post-test. Det var reduksjon i både gj.snitt relativ VO_{2max} (-1,8 ml·kg^{-1}·min^{-1} (3.03)) og gj.snitt $fettoks_{maks}$ (-0.07 g·min^{-1} (0.160)) fra pre-test til post-test, men dette var ikke statistisk signifikante endringer (begge $p>0.05$). For blodmarkører så var glukose, frie fettsyrer, β-hydroksybutyrat og T_3 endret i løpet av fasten og mellom de tre tidspunktene (alle $p<0.05$), med unntak av triasylglyserol ($p>0.05$). Alle blodmarkørene var normalisert fra pre-test til post-test som følge av tre dager med næringsinntak etter fasten (alle $p\geq0.05$), med unntak av median T_3 som var redusert med 0.55 pM (-1.200, -0.475) ($p<0.05$).

Konklusjon

Tross store metabolske endringer i forbindelse med syv dagers langvarig faste, så indikerte denne studien at de fleste metabolske blodmarkørene var normaliserte fra pre-test til post-test målt etter tre dager med næringsinntak. Derimot kan en fastestrategi med denne varigheten føre til en større nedbrytning av muskelmasse sammenlignet med fettmasse, som kan øke risikoen for å utvikle sarkopeni. Man bør derfor kritisk vurdere nødvendigheten av å utføre en langvarig faste hos friske, normalvektige personer.

Abstract

Purpose

Prolonged fasting has for decades been used as a therapeutic approach to promote health in over-weight people who wants to reduce their weight. Prolonged fasting reduces body fat, and this can promote the metabolic health. However, fasting may also reduce muscle mass, which increases the risk for development of sarcopenia. Prolonged fasting can also be a weight regulating strategy to prevent overweight in healthy, normal-weight people. Only a few studies have investigated the effects of prolonged fasting in normal-weight subjects, and there is little knowledge on how the body adapts to eating after a prolonged fast. The aims of the study were to investigate the effects of seven days of prolonged fasting followed by three days with re-feeding in normal-weight participants on 1) body composition, 2) physical capacity, and 3) blood concentrations of hormones and metabolites considered markers of metabolic health.

Method

12 healthy, normal-weight participants (5 females: mean weight (SD) 64.8 kg (6.42), mean BMI (SD) 23 kg/m^2 (1.3) and 7 males: mean weight (SD) 86.6 kg (13.83), mean BMI (SD) 26 kg/m^2 (2.6)) completed the study which consisted of a) familiarization test, b) pre-test (baseline), c) seven days of prolonged fasting with water intake only, and d) post-test (three days after the fast). The participants had a diet according to their desire in the days before and after the fast. Body weight, body composition with DXA (lean and fat mass), and markers and energy substrates in blood (glucose, triacylglycerols, free fatty acids, β-hydroxybutyrate and thyroid hormone metabolite T_3) were analyzed on pre-test, on day 7 of the fast, and on post-test. Urinary nitrogen excretion was measured every day during the fast. Physical tests (maximal oxygen uptake, VO_{2max} and maximal fat oxidation, $fatox_{max}$) were performed on ergometer bike on pre-test and post-test. Statistical significance level was set to $p<0.05$. Analyses included Student T-test and one-way repeated measures ANOVA for mean (SD), and Wilcoxon Signed Rank Test and Friedman test for median (IQR).

Results

Regarding body composition, weight, lean mass and fat mass reduced during the fast (each $p<0.05$), and the participants had lost an average of 2.4 kg of muscles. Re-feeding from day 7 to post-test led to gain in weight and lean mass (each $p<0.05$), but not in fat mass. Mean weight decreased by 3.2 kg (0.68), median lean mass decreased by 0.8 kg (-1.23,0.11) and mean body fat decreased by 1.8 kg (0.54) (each $p<0.05$) from pre-test to post-test. All body composition parameters changed between the three occasions (each $p<0.001$). Regarding physical capacity, median absolute VO_{2max} decreased by 245 ml·min^{-1} (-383.1, -85.0) ($p<0.05$) from pre-test to post-test. There were reductions in mean relative VO_{2max} (-1,8 ml·kg^{-1}·min^{-1} (3.03)) and mean $fatox_{max}$ (-0.07 g·min^{-1} (0.160)) from pre-test to post-test, but these were not statistically significant changes (each $p>0.05$). Regarding blood markers, glucose, free fatty acids, β-hydroxybutyrate and T_3 changed during the fast and between the three occasions (each $p<0.05$), except triacylglycerol ($p>0.05$). All blood markers were normalized from pre-test to post-test as a result of three days with re-feeding after the fast (each $p\geq0.05$), except median T_3 which was decreased by 0.55 pM (-1.200, -0.475) ($p<0.05$).

Conclusion

Despite major physiological changes occurring during seven days of a prolonged fast, this study indicated that most of the metabolic blood markers were normalized from pre-test to post-test measured after three days with re-feeding. However, a fasting strategy of this duration contributes to a greater breakdown of muscle mass compared to fat mass, which may increase the risk to develop sarcopenia. Thus, critical evaluation of the necessity to perform a prolonged fast in healthy, normal-weight subjects should be thoroughly considered.

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Sarah Victoria Frivold

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List of abbreviations and units of measurements

Abbreviation	Explanation
ATP	adenosine triphosphate
β	beta
BMI	Body Mass Index
bpm	beats per minute
CI	confidence interval
CO_2	carbon dioxide
df	degrees of freedom
DXA	Dual Energy X-Ray Absorptiometry
g	gram
$g \cdot min^{-1}$	gram per minute
$g \cdot kg^{-1}$	gram per kilo
h	hour
H_2O	water
Hb	haemoglobin
HR	heart rate
IQR	interquartile range
IV	intravenous (catheter)
kcal	kilocalorie
$kcal \cdot day^{-1}$	kilocalorie per day
$kcal \cdot g^{-1}$	kilocalorie per gram
$kcal \cdot L^{-1}$	kilocalorie per liter
kg	kilogram

kg/m^2	kilogram meter squared
$kJ \cdot L^{-1}$	kilojoule per liter
m.	muscle
min	minute
$mL \cdot kg^{-1} \cdot min^{-1}$	milliliter per kilogram per minute
$mL \cdot min^{-1}$	milliliter per minute
mM	millimolar
mm	millimeter
mmol/L	millimol per liter
NIH	Norwegian School of Sport Sciences
O_2	oxygen
pM	picomol per liter
RER	respiratory exchange ratio
RMR	resting metabolic rate
rpm	revolution per minute
SD	standard deviation
µg	microgram
VO_{2max}	maximal oxygen uptake
$Watt_{max}$	maximal Watt
WHO	World Health Organization
°C	degree Celsius
>	greater than
≥	greater than or equal to
<	less than
≤	less than or equal to

1. Introduction

1.1 Energy balance

The chemical energy in carbohydrate, fat and protein is the foundation for life, used by all cells in the body (Cahill, 1970). This energy is converted to muscle energy when we perform physical work (Frayn, 2010). The first principle of thermodynamics states that «energy can be neither destroyed nor created» (Goran & Astrup, 2002, pp. 30-31). Energy balance is the sum of dietary energy intake minus the total sum of the energy expenditure (Goran & Astrup, 2002; Jørgensen & Holmquist, 2011). A homeostatic regulation makes sure of a stable body weight when the organism is in energy balance (Goran & Astrup, 2002). In most people, resting metabolic rate accounts for 60-75 % of the total energy expenditure, physical activity for about 15–50 %, and thermic effect from food for about 10 % (Drevon, 2019a). A negative energy balance will lead to weight loss, whether it results from reduced caloric intake and / or increased activity level (Calbet, Ponce-Gonzalez, Perez-Suarez, de la Calle Herrero, & Holmberg, 2015). Excess energy will lead to a positive energy balance and weight gain (Goran & Astrup, 2002).

1.2 Overweight as a risk to health

Humans have the ability to store energy primarily as body fat (Frayn, 2010). Overweight is a huge health challenge of today, and this epidemic is highly complex (Centers for Disease Control and Prevention, 2020). Overweight is the cause of many diseases such as diabetes type 2 (World Health Organization, 2016, n.d.), and it is most likely the direct cause of various forms of cancer (World Cancer Research Fund International, n.d.). Overweight is defined with a body mass index (BMI) ≥ 25 kg/m^2, and obesity is defined with a BMI ≥ 30 kg/m^2 (World Health Organization, 2016). 1 in 5 Norwegians have BMI ≥ 30 kg/m^2 (Helsedirektoratet, 2010a). Worldwide 1 in 3 adults are overweight, and 1 in 10 adults are obese (World Health Organization, 2016, n.d.). Many tools and resources are available to help reduce the risks associated with overweight (Centers for Disease Control and Prevention, 2020). The World Health Organization (2002) address the importance of considering a more holistic approach involving recommendations of health, diet and physical activity. With the growing interest in interdisciplinary health approaches and the need for more

evidence-based knowledge, finding new solutions on how to prevent overweight in the healthy population *before* the person becomes overweight, should be considered. Prevention of diabetes type 2 is a shared responsibility across many sectors, and the knowledge and competence which can prevent and reduce the epidemic should be approached more proactively rather than reactively (Bergman et al., 2012). Tools for prevention of overweight is highly needed already in the primary health care (Helsedirektoratet, 2010a). Tools helping to assess the risk for e.g. diabetes type 2 is important as it can « (...) identify high-risk subjects when they are still in a normoglycemic state and to treat them by interventions that prevent their transition from normoglycemia to impaired glucose tolerance and to overt diabetes.» (Lindström & Tuomilehto, 2003, p. 725). The Norwegian Directorate of Health underlines the importance of gaining knowledge from various methodological studies to explore the causality between body weight, diet and diseases (Helsedirektoratet, 2010a, 2010b, 2011). National recommendations in prevention of overweight are based upon evidence-based data from many studies (Helsedirektoratet, 2010a, 2010b). New knowledge and more data derived from studies may contribute to updated guidelines.

1.3 Prolonged fasting as a therapeutic approach

Prolonged fasting may be *one* of many solutions to prevent and overcome the over-weight epidemic in normal-weight people, if used occasionally as a weight-regulating strategy before one become overweight. It is already well established that prolonged fasting in overweight and obese patients is a highly effective tool to reduce weight and fat mass, besides preventing and treating many chronic diseases, lowering the blood pressure and improving the insulin sensitivity (Drevon, 2019d; Johnson & Drenick, 1977; Longo & Mattson, 2014; Longo & Panda, 2016; Runcie & Thomson, 1970; Thomson, Runcie, & Miller, 1966). Fasting will reverse the insulin resistance in overweight people (Furmli, Elmasry, Ramos, & Fung, 2018). Weight loss for overweight subjects is highly recommended: for people with a BMI 25-29.5 kg/m^2 a 3-5 % weight reduction is advised, whereas a loss of 5-10 % will contribute to increased health benefits (Nasjonalt råd for ernæring, 2019). For patients with diabetes type 2 reducing energy intake is necessary to reduce weight and improve hyperglycemia (Drevon & Blomhoff, 2019). People with obesity and diabetes type 2 have decreased fat oxidation which is related to low physical fitness and increased skeletal muscle insulin resistance (Kelley et al., 2001). «Interventions aimed at

increasing fat metabolism could potentially reduce the symptoms of the disease in these groups of patients and might have tremendous clinical relevance» (Achten & Jeukendrup, 2004, p. 716). Prolonged fasting increases fat oxidation (Brufladt, 2018). Fat oxidation will in turn contribute to a reduced fat mass. Fatty acids reaches a peak after five days of a prolonged fast (Balasse & Fery, 1989). Safety and feasibility in overweight subjects performing a prolonged fast have been thoroughly evaluated in the last decades, and much data exists on fasting adaptations in this population. During a prolonged fast weight loss in this population is most striking in the first week, and the heavier subject, the greater weight loss (Thomson et al., 1966). Fasting durations from 12 to 382 days have been reported (Drenick, Swendseid, Blahd, & Tuttle, 1964; Runcie & Thomson, 1970; Stewart & Fleming, 1973; Thomson et al., 1966). Despite the favorable reduction in weight, prolonged fasting more than three days should be clinically supervised (Longo & Mattson, 2014; Runcie & Thomson, 1970; Thomson et al., 1966).

1.4 Fasting in other contexts

At any given time about 1/5 of men and 2/5 of women are dieting (Johnstone, 2015). We usually associate fasting as an option or a desire to reduce weight and to promote health. Prolonged fasting is in this thesis defined as a *voluntary absence* from energy intake in food and drinks for a consecutively amount of days (Maughan, 2010). *Starvation* on the other hand, is a chronic nutritional insufficiency (Longo & Mattson, 2014), and normally unintended. However, the metabolic changes that occur along with a prolonged fast have occurred throughout human history when our ancestors could not find sufficient food. There are other clinical situations today when the person cannot ingest energy for an extended period of time, such as trauma, cancer, burns, and surgery (Ferrier, 2014). Fasting is also performed in some cultures, religions, and even sports (Maughan, 2010). Besides, there is evidence supporting that short-term fasting can promote the efficacy of chemotherapy in oncology, and in the future fasting may be included in these clinical trials (de Groot, Pijl, van der Hoeven, & Kroep, 2019). No matter the purpose to perform a fast, a reduction in lean mass is usually not wanted: if used as a weight-loss strategy, the optimal weight loss will improve the body composition by reducing body fat without reducing the lean mass and / or the physical capacity. With aging we usually lose muscle mass (sarcopenia) (Wackerhage, 2017), while the fat mass usually increases and the

combination of these two elements are unfavourable to metabolic health (Lee, Shook, Drenowatz, & Blair, 2016). A body composition of low muscle mass and high fat mass (sarcopenic obesity) is associated with increased risk of mobility impairment, cardiometabolic disease and earlier mortality, whereas a body composition with high muscle mass and low fat mass is considered healthy (Lee et al., 2016). Physical capacity, especially handgrip strength, is a strong predictor for metabolic health and early mortality, as it indicates low muscle mass which is directly linked to sarcopenia (Lee et al., 2016). Therefore, losing muscle mass should be avoided. On the other hand, losing fat mass can be beneficial in sports as it is regarded an undesirable ballast only adding to weight and not contributing to strength (Venkata Ramana, Surya Kumari, Sudhakar Rao, & Balakrishna, 2004). That is why some athletes wants to reduce weight and fat mass to improve body composition when partaking in weight-sensitive sports such as long distance running and road cycling (Ackland et al., 2012). Body composition and weight influence physical capacity (Venkata Ramana et al., 2004). Optimizing body composition could be preferred by some. Thus, both positive and negative consequences on fitness are seen in healthy-weight subjects during a prolonged fast (Brufladt, 2018; Nilsen, 2019).

1.5 Metabolic adaptations to prolonged fasting

A prolonged fast leads to increased breakdown of energy stores in the body. The fast puts the body in a stress situation leading to various adaptations in hormones, cells, and energy substrates that serves vital organs with energy (Beer et al., 1989; Bergendahl, Vance, Iranmanesh, Thorner, & Veldhuis, 1996; Frayn, 2010; Komaki et al., 1990; Longo & Mattson, 2014; Palmblad et al., 1977; Vance & Thorner, 1989). In a normal (non-fasting) state the brain utilizes primarily glucose as an energy substrate (Frayn, 2010). In times of low blood glucose the limited carbohydrate stores in the body will serve the brain with glucose (Frayn, 2010). A fasted-state will empty these stores in order to maintain energy utilization to the brain (Frayn, 2010). To continue to serve the brain with sufficient energy other energy substrates will break down and be converted to carbohydrates (Frayn, 2010). This will lead to a decreased muscle mass (Felig, Owen, Wahren, & Cahill, 1969). Eventually the increased breakdown of skeletal muscles slows down (Felig et al., 1969). This is because fasting adaptations will contribute to a slow switch in energy substrate utilization in the brain through ketogenesis (Frayn, 2010). After some days primarily fat tissue will be oxidized and

provide energy to organs (Frayn, 2010). Ketone bodies produced from the breakdown of free fatty acids in the liver will supply energy to the brain for the remaining fast (Evans, Cogan, & Egan, 2017). The synthesis of ketone bodies contributes to increased breakdown of body fat, which in turn slows down the breakdown of muscle mass (Felig et al., 1969). In addition, the fasting adaptations will change the availability and utilization of energy stores and energy substrates in blood used both at rest and during exercise (Frayn, 2010). In a normal (non-fasting) state the ability to oxidize fat is important in sports, and it is substantial on low to medium intensities (Romijn et al., 1993b). The carbohydrate stores in the body are important in aerobic activity, especially during high intensities (Bergstrom, Hermansen, Hultman, & Saltin, 1967; Christensen & Hansen, 1939; Jensen, Rustad, Kolnes, & Lai, 2011; Romijn et al., 1993b). «As glycogen stores are limited, a higher reliance on fat oxidation during long duration endurance events could spare muscle glycogen and be beneficial for performance» (Andersson-Hall et al., 2018, p. 37). One study found a reduced rate of carbohydrate and muscle glycogen oxidization during 45 % of VO_{2max} during fasted-state (Knapik et al., 1988). Only a few studies investigate how the body will respond to energy intake *after* a prolonged fast, and a few days with a high-carbohydrate diet will replenish blood glucose and glycogen stores (Nilsson & Hultman, 1973). It is also unknown if the increased ability to oxidize fat during a prolonged fast is maintained or normalizes along with re-feeding.

1.6 The purpose of the study

Periods of short-term fast with only drinking water may be an efficient preventative approach to reduce the risk of metabolic disease in healthy, normal-weight subjects (Horne et al., 2013). Reducing weight and fat mass without reducing lean mass or physical capacity can be beneficial for health. A prolonged fast more than five days will contribute to a maximal breakdown of fatty acids, and the benefits of this fast on overweight subjects, are many. However, there is a knowledge gap on the effects of fasting in normal-weight subjects (Wilhelmi de Toledo, Grundler, Bergouignan, Drinda, & Michalsen, 2019), especially on prolonged fasting (Brufladt, 2018; Consolazio, Matoush, Johnson, Nelson, & Krzywicki, 1967; Nilsen, 2019; Palmblad et al., 1977). Not much is known on how the body responds to eating after a prolonged fast. The aims of the thesis were to investigate the effects of seven days of

prolonged fasting followed by three days with re-feeding. Healthy, normal-weight subjects were recruited to partake in this experimental intervention study.

1.7 Hypotheses

The following alternative hypotheses (Ha) were investigated and tested:

The first hypothesis: Weight, lean mass and fat mass changes from pre-test, to day 7, to post-test.

The second hypothesis: VO_{2max} and $fatox_{max}$ changes from pre-test to post-test.

The third hypothesis: Blood concentrations of hormones and metabolites considered markers of metabolic health changes from pre-test, to day 7, to post-test.

2. THEORY

2.1 Fasting

2.1.1 Fasting and overweight

Obesity is a huge problem today, and many strategies are used to prevent development of overweight and reduce body weight. From the 1960-1970s physicians started to gain interest on the physiological adaptations occurring during a *prolonged fast* as there were more frequent clinical cases of overweight. Some studies reported of obese subjects fasting for periods of 12-249 days (Drenick et al., 1964; Runcie & Thomson, 1970; Thomson et al., 1966). Another successful prolonged fast reported of one man reducing his weight from 200 kg to 85 kg during 382 days, with no prominent side effects (Stewart & Fleming, 1973). Many weight-loss interventions were made where participants only would drink water, caffeinated drinks, juices and vitamin- and mineral supplements (Consolazio et al., 1967; MacCuish, Munro, & Duncan, 1968; Palmblad et al., 1977; Stewart & Fleming, 1973). As caffeine is a diuretic substance contributing to dehydration, it should not be combined with a prolonged fast (Campbell, Wickert, Magner, & Shumak, 1994).

2.1.2 Fasting strategies

There are many variations of fasting. Caloric restriction can occur via daily reduction in energy intake, sometimes limited to 20 - 40 % of the usual consumed energy (Longo & Mattson, 2014). Intermittent fasting is another strategy involving alternate periods of eating and fasting (Kim et al., 2017; Longo & Mattson, 2014). Intermittent fasting can include some days with a normal diet, and a deficit in calories the other days, e.g. the 5:2 diet (Patterson et al., 2015). Ramadan fast is another example of intermittent fasting, but usually the same amount of energy is eaten during that month as compared to the rest of the year (Maughan, 2010; Shephard, 2012). An ongoing hunger sensation is reported by people performing caloric restriction diets (Johnstone, 2015), while hunger have been reported to decline after a few days by people performing prolonged fasting (Thomson et al., 1966). Prolonged fasting in normal-weight people are less investigated, but it was of importance when physicians wanted

to investigate physiological adaptations to limited nutrition when soldiers and officers took part in military operations (Consolazio et al., 1967; Palmblad et al., 1977).

2.2 Energy stores in man

Water makes up approximately 60 % of the total body weight to a man of 70 kg; fat mass contains about 10 % of water; and muscles contains about 75 % of water (Sawka, 1992). An athlete have relatively more body water than a sedentary person due to higher muscle mass and more muscle glycogen (Sawka, 1992). Triacyl-glycerols in adipose tissue accounts for the biggest energy store in the body. The average 70 kg man has approximately 15 kg of body fat stored as triacylglycerols, which can potentially give between 135 000 - 141 000 kcal, equivalent to 80 days of energy during starvation (Cahill, 1970; Ferrier, 2014; Frayn, 2010). 10 - 15 kg (20 %) of the human weight are proteins - if skeletal muscles were the only energy source it could last about three weeks when broken down (Frayn, 2010). As the body can only "tolerate" a 50 % loss of muscle proteins (Frayn, 2010), survival during prolonged fasting is linked directly to the protein sparing mechanism (Felig et al., 1969). Based on calculations by Cahill (1970) the following tissues can be broken down to release stored energy in the fasting person: 5 kg of proteins (mostly muscles) can give 20 000 kcal, 150 g of stored m. glycogen can give 600 kcal, 75 g of liver glycogen can give 300 kcal, which is a total of 161 900 kcal, plus 113 kcal from circulating energy substrates in blood.

2.3 Anabolism

2.3.1 Macronutrients

The definition of 1 kcal is the energy needed to rise the temperature of 1000 g H_2O by 1 °C (Drevon, 2019a). Macronutrients are energy-rich molecules from food supplying humans with energy sustaining normal body functions (Ferrier, 2014). We utilize these food constituents in the forms of carbohydrates, protein and fat (Cahill, 1970). Fat contains the most energy of about 9 $kcal \cdot g^{-1}$, and carbohydrate and protein contain about 4 $kcal \cdot g^{-1}$ when completely oxidised (Cahill, 1970; Ferrier, 2014). In

the diet Norwegians get almost 50 % of their total energy intake from carbohydrates (Kolset, 2019). A high-carbohydrate diet is usually consisting of 60 % carbohydrates (Magnusson, Rothman, Katz, Shulman, & Shulman, 1992). Energy and ATP from fatty acids from fats and glucose from carbohydrates provide most of the dietary energy to humans, whereas dietary protein is responsible for only a limited energy supply and its levels are adjusted with amino acid oxidation versus -intake for a constant level of proteins in the body (Flatt, 1995). Excess dietary energy (caloric intake exceeding energy balance) from fat, carbohydrates and protein can all be converted to triacylglycerols and stored as adipose tissue (Ferrier, 2014). Excess energy is also stored as glycogen in liver and skeletal muscles (Drevon, 2019d). Tracking and measuring biomarkers in blood can give information and status of absorption, distribution, excretion and status of metabolic activity and energy substrates (Drevon, 2019b).

2.3.2 Carbohydrate metabolism

The brain needs about 120 grams of glucose every day in a non-fasting state (Frayn, 2010). A *normal* blood glucose level is between 3.5 – 5.5 mmol/L (Kolset, 2019). Ingestion of carbohydrates increases the glucose concentration in the blood, before it decreases and reaches a *normal level* 2 - 3 h after (Kolset, 2019). *Absorptive state* is occurring up to 4 h after ingesting a meal, and this is an anabolic phase where all tissues use glucose as an energy substrate and all nutrients are absorbed (Ferrier, 2014). After the breakdown of starch, the absorption goes to various tissues, for instance to the skeletal muscles and adipose tissue with the help of GLUT 4 trans-porter (Ferrier, 2014; Koolman & Roehm, 2013). Insulin promotes glucose utilization in these tissues when the blood glucose is high (Kolset, 2019). At rest glucose is the preferred energy substrate, and muscles that use fatty acids (e.g. during fasting) will switch to glucose utilisation when there is increased plasma glucose due to eating (Frayn, 2010).

When the blood glucose is high, some of it will be stored as liver glycogen, and when the blood glucose is low, liver glycogen will break down and increase the blood glucose (Koolman & Roehm, 2013). The regulation of energy stores are carefully modulated through plasma substrates and secretion of pancreatic hormones (Frayn, 2010). The changing levels of blood glucose are regulated to be in a normal range by

the peptide hormones insulin and glucagon (Koolman & Roehm, 2013). Depending on the blood glucose levels this ratio change throughout the day (Kolset, 2019). Insulin is secreted from β-cells when the blood glucose concentration is too high (hyperglycemia), and the hormone will increase glycogen synthesis, and stimulate lipogenesis (Kolset, 2019; Koolman & Roehm, 2013). Insulin activates glycogen synthase and works as an inhibitor in the process of gluconeogenesis (Koolman & Roehm, 2013). Noninsulin-dependent diabetic subjects have a decreased insulin response compared to healthy subjects (Bogardus, Lillioja, Howard, Reaven, & Mott, 1984). Lipogenesis is a metabolic process with the conversion of glucose to fat through pyruvate and acetyl-CoA (Frayn, 1983). Glycerol can form triacylglycerol in the liver through lipogenesis (Ferrier, 2014).

2.4 Catabolism

2.4.1 Glycogenolysis and liver glycogen

The physiological adaptations to fasting starts already with the lack of energy intake after the *absorptive phase* (Ferrier, 2014). The decreasing plasma glucose and insulin implies an adaptation in metabolic substrate, but vital organs still need energy in the form of glucose. Hours after the absorptive phase the blood glucose concentration is low, and the body´s own endogenous energy stores will be broken down to supply glucose-requiring tissues with energy (Jørgensen & Holmquist, 2011). However, people with diabetes are hyperglycaemic (high blood glucose concentration) in a fasted-state (Bogardus et al., 1984; Magnusson et al., 1992).

Glucagon has the reverse effect of insulin: glucagon is produced by the α-cells in the pancreas, secreted at hypoglycaemia (low blood glucose concentration) and it activates glycogenolysis in the liver (Kolset, 2019; Koolman & Roehm, 2013). This process will increase the plasma blood glucose by breaking down stored liver glycogen into glucose through increased glucagon-to-insulin ratio, which sustains the energy metabolism of primarily the brain (Ferrier, 2014). Fasting for 8-10 h, such as an overnight fast, leads to reduction in liver glycogen, and hepatic glycogenolysis is stimulated to supply glucose to the brain (Maughan, 2010). Fasting for 24 h

contributes to emptying of the liver glycogen depots (Nilsson & Hultman, 1973). The liver stores about 100 g of glycogen (Jensen et al., 2011). 1 g of liver glycogen is attached to 2.7 g of water (Drevon, 2019c). In the first days of fasting there will be a considerable weight reduction primarily due to the loss of fluids and depletion of glycogen (Jørgensen & Holmquist, 2011).

2.4.2 Gluconeogenesis and protein metabolism

Gluconeogenesis strikes in when the liver glycogen is depleted, and this process contributes to increased and maintained euglycemia (normal blood glucose concentration) in the early days of fasting (Ferrier, 2014; Nilsson & Hultman, 1973). As the brain continues to need glucose, and the carbohydrate stores are limited, activation of gluconeogenesis will be initiated and increase the glucose production from other energy substrates to supply the brain with newly synthesized glucose, primarily by breaking down amino acids from skeletal muscle protein (Drenick et al., 1964; Frayn, 2010; Koolman & Roehm, 2013). «Amino acids can be oxidized to provide energy or converted to glucose and fatty acids, which can then be oxidized» (Frayn, 2010, p. 238). For every 1 g of glucose made from muscle protein during gluconeogenesis, approximately 1.75 g of muscle protein is broken down (Frayn, 2010). The conversion of amino acids to glucose is not beneficial because skeletal muscle is a valuable tissue, so evolutionary mechanisms contributes to protein sparing (Frayn, 2010). Glucose can also be synthesized from other substrates that does not derive from carbohydrates and amino acids, and those are lactate and glycerol (Ferrier, 2014). Through the Cori cycle the muscle glycogen can break down to lactate, which can be transported to the liver and increase the blood glucose (Jensen et al., 2011). People with diabetes type 2 have an increased rate of gluconeogenesis compared to non-diabetics (Bogardus et al., 1984; Magnusson et al., 1992).

2.4.3 Free fatty acids

The early stages of fasting leads to increased plasma fatty acids from adipose tissue, and the change in substrate availability contributes to tissues like skeletal muscles to utilize energy from fat and spare glucose (Frayn, 2010). Skeletal muscles can use fatty acids and ketone bodies as energy substrates in the first two weeks of fasting (Ferrier, 2014). Fatty acids cannot be converted to carbohydrates (Drevon, 2019c). The brain cannot use free fatty acids as an energy substrate, but free fatty acids are

the main substrate for ketogenesis (and conversion to ketone bodies) (Evans et al., 2017). Increased lipolysis will also contribute to increased fatty acid metabolism and inhibit glucose uptake along with insulin resistance (Qvigstad, Bjerve, & Grill, 2002). Fatty acids are transported in the blood, along with triacylglycerol, or attached to albumin as free fatty acids, or with other lipoproteins (Drevon, 2019c). Seven days of prolonged fasting contributes to a three-fold increase in free fatty acids (from 0.5 to 1.5 mg / 100 ml) compared to a normal fed-state (Cahill, 1970). After a few days of fasting about 75 % of energy comes from the reserves of body fat (Cahill, 1970).

2.4.4 Ketone bodies

During prolonged fasting, a crucial metabolic adaptation occur in the energy metabolism of the brain: Fatty acids can be converted to ketone bodies and this substrate can supply energy to various tissues that normally requires glucose, which is primarily the brain, but also muscles and other organs (Balasse & Fery, 1989; Frayn, 2010; Koolman & Roehm, 2013). This adaptation in the brain will in turn reduce the consumption of glucose, a mechanism to spare body proteins (Cahill, 1970). A reduction of 25 % of glucose substrate used by the brain is reported after 3.5 days of prolonged fasting (Hasselbalch et al., 1994). As glucose decreases during the fast, and the ketone bodies rise, ketone body levels reaches a plateau around day five of prolonged fasting (Balasse & Fery, 1989). From this point on the brain uses primarily this as energy substrate (Jørgensen & Holmquist, 2011). Ketogenesis is the *production* of ketone bodies, and ketolysis is the *breakdown* and *utilization* of these (Evans et al., 2017). The increased glucagon-to-insulin ratio is also favouring keto-genesis (Laffel, 1999). The ketone bodies are produced in the liver, and there are three types: Acetoacetate, acetone and β-hydroxybutyrate (Evans et al., 2017). In one prolonged fasting study of three days the plasma β-hydroxybutyrate concentration was equal to the ketone body oxidation utilized by the brain (Pan, Rothman, Behar, Stein, & Hetherington, 2000). Compared to the post-absorptive phase, seven days of prolonged fasting led to a change in various plasma energy substrates, such as red-uced glucose from 80 to 65 mg / 100 ml, increase the β-hydroxybutyrate by 400-fold (from 0.01 to 4.0 mM), but no change in amino acids, lactate or pyruvate (Cahill, 1970).

2.4.5 T_3 (thyroid hormone metabolite)

In the brain the hypothalamus register stimuli such as stress, and specialized neurons secrete the hormone thyroliberin, which stimulates the production of thyrotropin which regulates the thyroid cells (Koolman & Roehm, 2013). The active metabolite of the thyroxine hormone is T_3 (3,5,3´-triiodothyronine), and it regulates the oxygen uptake in the respiratory system as well as increasing the RMR (Koolman & Roehm, 2013). A reduced level of thyroid hormones depresses the metabolic rate (Frayn, 2010), and hypothyroidism contributes to the feeling of fatigue (Bansal, Kaushik, Singh, Sharma, & Singh, 2015). Many metabolic hormones are regulated rapidly, but the thyroid hormones seem to be acting at a slower pace (Frayn, 2010).

It has been reported a reduction of T_3 concentrations in female athletes who had reduced their caloric intake for four days (Ciloglu et al., 2005). Brufladt (2018) found that T_3 in participants reduced by half after seven days of prolonged fasting. Another study saw a decrease in T_3 levels in healthy men after seven days of fasting, and the levels increased back to baseline with a few days of re-feeding (Palmblad et al., 1977). A decrease in heart rate (HR) was also seen, but the HR did also increase with re-feeding (Palmblad et al., 1977).

2.5 Re-feeding after a prolonged fast

There are not many studies on metabolic regulation associated with eating after a prolonged fast. In healthy, normal-weight participants a rapid increase in body weight is seen in the first few days of eating after ten days of prolonged fasting, due to fluid absorption and normalization of water balance (Consolazio et al., 1967). In another study where overweight patients performed a caloric restriction and exercise leading to an energy deficit of 5000 $kcal \cdot day^{-1}$ for four days, hydration recovered within three days with a normal diet and limited exercise (Calbet et al., 2015).

Filling up the glycogen stores in the liver and in the muscles depend on the time and amount of carbohydrate intake: One study found that participants who ate a carbohydrate-rich diet after 10 days of fasting increased their liver glycogen levels

above baseline (*glycogen supercompensation*) after a few days with re-feeding, and blood glucose was back to normal (Nilsson & Hultman, 1973).

2.6 Indirect calorimetry

2.6.1 Measurement of substrate oxidation

To measure the type of fuel used in human energy expenditure the net oxidation rates from gaseous exchange can be calculated using indirect calorimetry (Frayn, 1983). Péronnet and Massicotte (1991) emphasize that it is not possible to draw solid conclusions on what exactly is the source in nonprotein oxidations, and whether it is e.g. from ingested glucose or from liver glycogen, or in fat, e.g. from adipose tissue or from ingested fatty acids. However, by measuring the ratio between consumption of O_2 and CO_2 it is possible to find to what *extent* the different substrates provides energy at both exercise and at rest (Goran & Astrup, 2002; Jeukendrup & Wallis, 2005). The calorimetric ratio can be used to find the respiratory quotient (RQ) between the gasses, where RQ of fat is $[VCO_2 / VO_2] = 0.7$ and RQ of carbohydrate is $[VCO_2 / VO_2] = 1.0$ (Frayn, 1983; Goran & Astrup, 2002). A glucose molecule and a typical fatty acid molecule are oxidized under normal circumstances by the following two equations, given by Frayn (1983):

$$\text{glucose } (C_6H_{12}O_6) + 6\ O_2 \rightarrow 6\ H_2O + 6\ CO_2$$

and

$$\text{triacylglycerol } (C_{55}H_{104}O_6) + 78\ O_2 \rightarrow 52\ H_2O + 55\ CO_2$$

Protein oxidation can be estimated with nitrogen excreted in urine (Drenick et al., 1964; Frayn, 1983). Calculation of urinary nitrogen loss can be found by multiplying the daily nitrogen excretion with 6.25, as 1 g of urinary nitrogen comes from 6.25 g of protein (Drenick et al., 1964; Frayn, 1983; Goran & Astrup, 2002). In a prolonged fasting study excretion of urinary nitrogen was associated with protein loss from skeletal muscles (Brufladt, 2018).

Table 1. *Overview of RQ and energy of the different oxidized substrates, based on data from Ulmer (1983b).*

	RQ	$kJ \cdot L^{-1} O_2$	$kcal \cdot L^{-1} O_2$
Carbohydrates	1.00	21.1	5.05
Fats	0.70	19.6	4.69
Proteins	0.81	18.8	4.48

Absolutely correct calculations of substrate oxidation are challenging during fasting. Metabolic processes such as lipogenesis, gluconeogenesis, ketogenesis, and great concentrations of lactate may disturb and influence the calculations of the actual substrate oxidations (Frayn, 1983). Brufladt (2018) found a decrease in respiratory exchange ratio (RER) from 0.86 to 0.76 after six days of prolonged fasting, indicating a higher oxidation of fat during fasted-state compared to baseline.

2.6.2 Resting metabolic rate

Indirect calorimetry can be used to discover the metabolic cost and total energy output. Resting metabolic rate (RMR) can be calculated when measuring the oxygen uptake at rest, by using the designated substrate equation above (see *Table 1*.). The equation for RMR is given by Weir (1949):

$$RMR = 3.941 \times VO_2\,(L) + 1.106 \times VCO_2\,(L) - 2.17 \times \text{urinary nitrogen (g)}$$

A 70 kg man with 15 kg of body fat has RMR of about 1 800 $kcal \cdot day^{-1}$ (Cahill, 1970). RMR is usually larger for larger subjects than for people of less weight, and athletes have a higher metabolism than normal individuals (Benedict, 1915). One review that analyzed many hundreds of publications found that women have lower RMR than men, most likely due to less muscle mass (as muscle tissue is the metabolically active tissue), and obese people have the lowest RMR independent of gender (McMurray, Soares, Caspersen, & McCurdy, 2014). One study found no change in RMR after 72 h of fasting (Vendelbo et al., 2012). Another study found no change in RMR from baseline to six days of prolonged fasting even though the participants had lost a large amount of body weight (Brufladt, 2018).

2.7 Body composition

Dual Energy X-Ray Absorptiometry (DXA) has for decades been used to quantify total body composition (Ackland et al., 2012). DXA is one the best tools for assessing muscle mass (Juby, 2014) and body fat (Drevon, 2019a), and it has a low radiation dose compared to other alternatives (Deurenberg, 2002). Quantifying the body composition can somewhat give information of a person´s health status, as it can give information on the risk of comorbidities associated with the body composition (Deurenberg, 2002). Examples of this is that it can be used to detect sarcopenia and overweight (GE Healthcare Lunar, 2016).

On the other side, exercise will improve physical capacity, and result in increased lean mass, and thus enhanced body composition (Venkata Ramana et al., 2004). There are studies that indicate correlations between subjects who have less body fat and a high VO_{2max} (Shete, Bute, & Deshmukh, 2014), and subjects who have increased lean mass and increased VO_{2max} (Venkata Ramana et al., 2004). Lean mass on DXA includes skeletal muscles and organs and previous ingested food in the intestines (Lee et al., 2016). Lean mass has a large constituent of water (GE Healthcare Lunar, 2016; Lohman, Harris, Teixeira, & Weiss, 2000). Water balance reduces as a result of fasting (Consolazio et al., 1967). It is likely that estimates of body composition will be affected by a change in fluid balance, and it is suggested that a 5 % change in water content in lean mass can affect the estimates with a few percent (Lohman et al., 2000).

2.8 Physical capacity

2.8.1 Maximal oxygen uptake

The capability to endure strenuous muscle work over time can be directly linked to *good health* as this parameter will affect us on a daily basis (Anderssen & Jensen, 2019). The maximal oxygen uptake (VO_{2max}) is a parameter which estimates the maximal rate of oxygen consumption during exercise (Lieber, 2010). At submaximal work the oxygen uptake increases with increased load, and when the load is continually increased and the oxygen uptake stabilizes and reaches a plateau, the

person has reached VO_{2max} (Ingjer, Hem, & Leirstein, 2011). VO_{2max} represents endurance capacity and is «determined by the oxygen supply of the blood and by the oxygen consumption of the skeletal muscle» (Schmidt & Prommer, 2010, p. 68). Absolute VO_{2max} is measured as the volume of oxygen consumed per minute, $L \cdot min^{-1}$, and relative VO_{2max} is measured as $ml \cdot kg^{-1} \cdot min^{-1}$, a unit which is consequently weight dependent (Lieber, 2010). Relative VO_{2max} will be affected by changes to a person´s weight.

For Norwegian adults VO_{2max} at 30 - 40 $ml \cdot kg^{-1} \cdot min^{-1}$ is considered quite low, and 60 – 70 $ml \cdot kg^{-1} \cdot min^{-1}$ can be considered quite high, and values in between is what to expect for a healthy, young population (Ingjer et al., 2011). VO_{2max} can increase with systematic training, and athletes have a higher VO_{2max} than other groups (Rezaeimanesh, Farsani, & Saidian, 2011). Both heart rate and VO_2 increased linearly on various exercise intensities (Skinner et al., 2003). With exercise eventually the heart rate will decrease while the same power is maintained (Skinner et al., 2003). In one study competitive male runners who refrained from exercise for 10 days did not reduce their VO_{2max}, but HR_{max} increased with 5 % (Cullinane, Sady, Vadeboncoeur, Burke, & Thompson, 1986). A fitness study showed that women had lower absolute and relative VO_{2max} and lower maximal workload compared to men (Loe, Rognmo, Saltin, & Wisloff, 2013). Physical activity will increase the insulin sensitivity (Anderssen & Jensen, 2019).

2.8.2 Fat oxidation capacity

Fat oxidation (fatox) is important in exercise intensities ≤ 65 % of VO_{2max} (Romijn et al., 1993b). Aerobic exercise will increase the rates of fat oxidation (Achten & Jeukendrup, 2004). One study indicated that athletes who had a $VO_{2max} > 65$ $ml \cdot kg^{-1} \cdot min^{-1}$ had a higher fatox (mean fatox (SD) 0.56 (0.14) $g \cdot min^{-1}$) compared to athletes who have $VO_{2max} < 65$ $ml \cdot kg^{-1} \cdot min^{-1}$ (mean fatox (SD) 0.48 (0.15) $g \cdot min^{-1}$) (Achten & Jeukendrup, 2003). In one study fatox was increased during the sixth day of a prolonged fast compared to at baseline (Brufladt, 2018). It is unknown if fatox is maintained after a prolonged fast along with re-feeding.

2.9 Energy requirements during exercise

In skeletal muscles chemical energy is converted to mechanical muscle contraction (Jensen et al., 2011). The energy expenditure can increase up to 25 times from rest to hard physical activity, and the cardiac output also increases with physical exertion (Anderssen & Jensen, 2019). Many oxidative intracellular processes are occurring during exercise to provide the organism with energy, and oxidation can be from various energy substrates (Hargreaves & Spriet, 2006). Aerobic respiration is when there is sufficient oxygen available to break down carbohydrates (glucose) and fats (palmitic acid) to cover the energy needed (Ingjer et al., 2011). Oxidative phosphorylation contributes with most of the energy supplies needed, all the way from a few min of work, and up to intensities of 90 % of maximal capacity (Ingjer et al., 2011). Most exercise intensities that needs adenosine triphosphate (ATP, energy) are involved by pathways of aerobic metabolism (Hargreaves & Spriet, 2006). With aerobic exercise fat will be broken down to fatty acids, and glycogen will break down to glucose in the skeletal muscles (Anderssen & Jensen, 2019). Most of the oxidation of fatty acids takes place in the mitochondria (Drevon, 2019c).

ATP delivered from anaerobic metabolism occurs when aerobic ATP provision cannot provide sufficient ATP to the body, such as during intense and sprint exercises (Hargreaves & Spriet, 2006). Glucose can be converted to lactate through *anaerobic glycolysis* which produces ATP in tissues without mitochondria (for example red blood cells) or in cells where O_2 is lacking (Ferrier, 2014). During anaerobic activity when the muscle cells does not get enough O_2, the lactate accumulation contributes to a lowering of pH, and the muscles contraction and work intensity reduces (Anderssen & Jensen, 2019).

2.9.1 Muscle glycogen during exercise

Glucose can be stored as muscle glycogen (Jensen et al., 2011). Skeletal muscle glycogen is calculated to be limited to 500 g, which accounts for 80 % of the total body glycogen stores (Jensen et al., 2011). In evolution, skeletal muscle glycogen may have been evolved to serve energy in situations of *fight-or-flight* (Jensen et al., 2011). It is the most important energy substrate supplying the active muscles with energy in high-intensity exercise > 70 % of VO_{2max}, and empty glycogen stores

contributes to fatigue (Jensen et al., 2011). A research review concludes that there are big variations in muscle glycogen content in different people: at rest an athlete has higher glycogen stores than a sedentary person, at physical exhaustion the glycogen stores are very low, and a high-carbohydrate diet will re-fill the glycogen stores (Hearris, Hammond, Fell, & Morton, 2018).

Oxidation of carbohydrates are important in high exercise intensities, and both plasma glucose and muscle glycogen oxidation increased with increasing intensities on ergometer bike in one study (Romijn et al., 1993b). If glycogen stores are first emptied through hard, physical work, glycogen synthesis will increase by using blood glucose to replenish the stores (Jensen et al., 2011). By eating a high-carbohydrates meal the muscle glycogen concentration is seen to increase much (Bergstrom et al., 1967). Glycogen synthesis is less with a diet of much fat and protein after exercise compared to carbohydrates, and changes in diet for the same person can affect the muscle glycogen levels (Bergstrom et al., 1967).

One study found an approximate linear reduction in muscle glycogen in both trained and untrained subjects performing more than 1h interval biking at 77 % of VO_{2max} with 50 rpm, even though the subjects were given carbohydrates prior to the exercise (Hermansen, Hultman, & Saltin, 1967). Blood glucose dropped in the first 20 min of biking, and then remained constant, whereas blood lactate increased rapidly in the first 10-20 min, and then gradually decreased throughout the test (Hermansen et al., 1967). In one study muscle glycogen stores were emptied after biking for 2 h, and when subjects were given carbohydrates after the exercise, concentrations of muscle glycogen, insulin, and blood glucose increased and were maintained above baseline after many hours (Ivy, Lee, Brozinick, & Reed, 1988).Some days with a high-carbohydrate diet improved endurance capacity compared to a high-fat diet on identical loads (Christensen & Hansen, 1939). Increased fat oxidation during exercise when ingesting a low-carbohydrate, high-fat diet is seen (Burke et al., 2017).

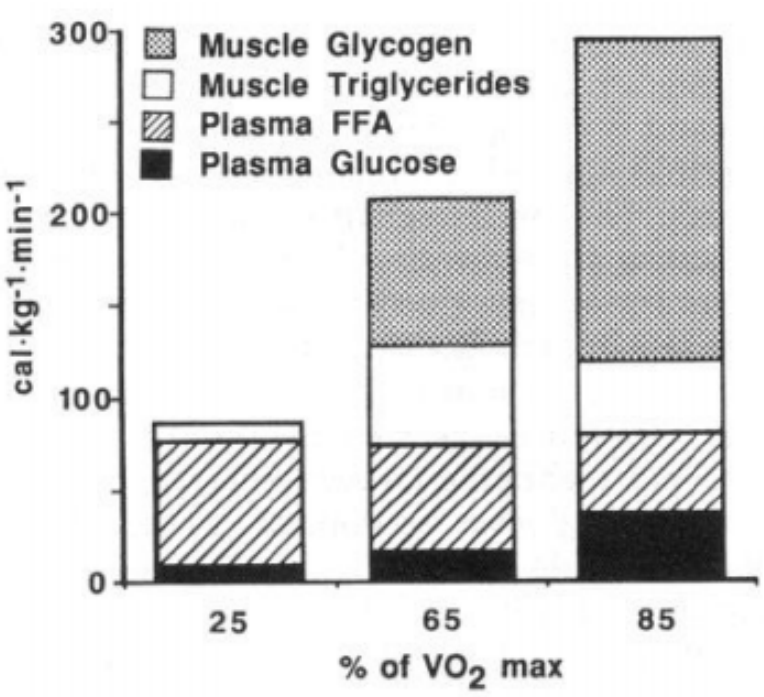


Figure 1: *Overview of energy substrate utilization during three different exercise intensities. From «Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration»* by *Romijn et al. (1993a). Am J Physiol-Endocrinology and Metabolism, 265(3), p.E387. Copyright 1993, The American Physiological Society. Figure reproduction used with permission.*

2.10 Fasting and exercise

Both fasting and exercise are dependent on metabolic regulation to a greater extent than at rest (Frayn, 2010). Fasting will lead to alterations in available energy substrates and the oxidation rate of these. This altered metabolism will affect physical capacity. For instance will both exercise and fasting lead to increased fatty acid uptake by the skeletal muscles, which also spares the glucose utilisation by this tissue (Frayn, 2010). Some athletes incorporate fasting for performance purposes aiming to improve body composition, optimize power-to-weight ratio and achieve less weight and body fat (Ackland et al., 2012; Ferguson et al., 2009). However, physical capacity may decrease during prolonged fasting (Henschel, Taylor, & Keys, 1954).

2.10.1 Prolonged fasting and physical capacity

There has been performed fasting in sports, but only a few studies investigate how prolonged fasting affect fitness in healthy, normal-weight participants: there are research done that support that prolonged fasting for three days increases fat oxidation in skeletal muscles and reduces insulin sensitivity (Vendelbo et al., 2012). It was found increased fat oxidation at submaximal intensity after six days of prolonged fasting in healthy, normal-weight adults, but also a reduction of more than 500 $mL \cdot min^{-1}$ in absolute maximal oxygen uptake (VO_{2max}), most likely due to dehydration (Brufladt, 2018). In the same study there was seen a rise in blood glucose during intense exercise even after six days without energy intake. In another study Henschel et al. (1954) found an 8 % reduction in absolute VO_{2max} in healthy men fasting for five days, but no change in relative VO_{2max} ($ml \cdot kg^{-1} \cdot min^{-1}$). Another study showed that prolonged fasting for 3.5 days did not impair aerobic endurance or anaerobic capacity in healthy men (Knapik, Jones, Meredith, & Evans, 1987). Nilsen (2019) found a reduction in fat mass, a loss of about 1.5 kg of muscle mass, a loss of 3.2 kg muscle mass, but no significant change in lower body strength in subjects after six days of prolonged fasting. One study by (Vendelbo et al., 2012) found no decrease in muscle glycogen after a 72 h fast, whereas Frank, Katz, Andersson, and Sahlin (2013) found a small reduction in another fasting study.

2.10.2 Other fasting strategies and physical capacity

More studies have investigated other fasting variations and fitness: caloric restriction and overnight fast performed by competitive cyclists lead to weight and fat mass reduction, increased lean mass, no change in VO_{2max}, and biking on higher load (Watt) at VO_{2max} (Ferguson et al., 2009). One study found that low-intensity exercise combined caloric restriction for four days contributed to preservation of lean mass and reduction of fat mass in overweight men (Calbet et al., 2017). Changes in physical capacity during Ramadan is also studied (Shephard, 2012), and in one study VO_{2max} at submaximal intensity did not change in sedentary males who performed IF during Ramadan (Ramadan & Barac-Nieto, 2000).

2.11 Fasting and dehydration

In a normal, non-fasted state, dehydration prior to exercise is shown to impair aerobic endurance, reduce plasma volume, and increase heart rate during exercise (Barr, 1999). Prolonged fasting for many days leads to dehydration as there will be fluid loss as the liver glycogen breaks down (Consolazio et al., 1967; Jørgensen & Holmquist, 2011). Dehydration along with fatty acid synthesis is also supported in other literature (Griffin & Cunnane, 2002). Dehydration during fasted-state will also negatively affect physical capacity. Blood contains 55 % of plasma, and the plasma consists of almost only water (The National Health Service, n.d.). One study concluded that dehydration most likely contributed to a decreased VO_{2max} during six days of fasting (Brufladt, 2018). In another study with normal-weight subjects, red blood cell-, blood- and plasma volume decreased during 10 days of prolonged fasting, and the levels remained unchanged even after four days of re-feeding (Consolazio et al., 1967). In this study haematocrit and haemoglobin levels remained unchanged during fasting, but these levels dropped drastically in the days with re-feeding after the fast, «demonstrating tissue hydration due to an increased water retention» (Consolazio et al., 1967, p. 675).

2.12 Blood and VO_{2max}

Haemoglobin (Hb) in red blood cells transports oxygen from the lungs to the cells (Ferrier, 2014). Men has almost 10 % more iron and Hb in the blood compared to females (Cable et al., 2016). Drawing blood (e.g. through blood donation, where about 5.7 dl blood is taken) the iron in Hb is reduced, and it takes a few weeks before the red blood cells are built up again (The National Health Service, n.d.). In a non-fasting state, the water in the blood plasma will be replaced right away through water intake (The National Health Service, n.d.). Furthermore, there is a small positive association in the relationship between relative VO_{2max} and haemoglobin (Hb) concentration ($g \cdot dl^{-1}$), and a large positive correlations between Hb mass ($g \cdot kg^{-1}$) and both absolute and relative VO_{2max} (Schmidt & Prommer, 2010). The same goes for absolute VO_{2max} and blood volume, and VO_{2max} and cardiac output (Schmidt & Prommer, 2010). A change in 1g of Hb mass can change the absolute VO_{2max} by 4 $ml \cdot min^{-1}$ (Schmidt & Prommer, 2010).

3. Methods

The research is conducted and performed by Department of Physical Performance, the Norwegian School of Sport Sciences (NIH). The research is in accordance with the Norwegian Center for Research Data (NSD), World Medical Association Declaration of Helsinki (2018), and The Health Research Act. The primary purpose for the fasting study aimed at investigating how various forms of exercise can reduce muscle loss during seven days of fasting. The research was evaluated by Regional Committees for Medical and Health Research Ethics (REC): they assessed the project to be in the field of exercise physiology, and the application was not processed (see *appendix 1*). The research project was then approved by the Ethics Committee at Norwegian School of Sport Sciences for two separated interventions (see *appendix 2*). Ethical research was carried out throughout the whole intervention, in accordance with the medical principle of *primum non nocere*- first, do no harm (Smith, 2005).

In the first part master students Brufladt (2018) and Nilsen (2019) investigated the effects of six days of fasting on loss of muscle mass, strength, endurance and plasma substrates in 13 healthy, normal-weight individuals. The participants fasted for 7 days, and physical capacity tests were performed on the very last fasting day. This part was the pilot study, and improvements were done based on the experiences gained from that study.

This present thesis is part of the second part, with new participants and an updated test protocol. The data collection in the second part of the fasting study was performed during the period between October 2018 and Mars 2019.

3.1 Subjects

Volunteer participants were recruited through the Norwegian School of Sports Sciences (NIH). Recruitment posters were put up at NIH, at University of Oslo, at Oslo University Hospital and in the city centre. The recruitment information was also shared on social media (Facebook).

3.1.1 Inclusion criteria

Participants were to be (a) between 18 - 45 years and (b) healthy both physically and mentally. (c) Body mass index (BMI) (kg/m^2) was set to between 22-30. Minimum body fat percentage (d) was 12 % for men, and 15 % for women. Participants had to (e) sign the consent form prior to the intervention, along with (f) a declaration of health form. There were no criteria for a certain physical capacity or training status. The participants did not take any kinds of vitamins during the intervention, and they were encouraged to not perform any kind of fasting in the previous weeks before the study. None of the participants had recently performed a prolonged fast.

3.1.2 Exclusion criteria

Participants were excluded if they had (a) any diseases or illnesses, b) any heart- and/or (c) cardiovascular diseases, (d) diabetes or (e) other diseases affecting the metabolism. They were excluded if they were (f) using medications, (g) smoking or using other forms of nicotine.

3.2 Test schedule

Participants met for a total of 13 days during the fasting study over a three-week period. The participants met at the same time each day between 7-10 am. The testing time varied from half an hour to five hours depending on the daily test protocol. All clinical data were collected by qualified and trained research staff. The tests parameters below in this chapter are included in the results. (For an overview of all the methods in the fasting study, see *appendix 7.*) Below are figures describing the test protocol:

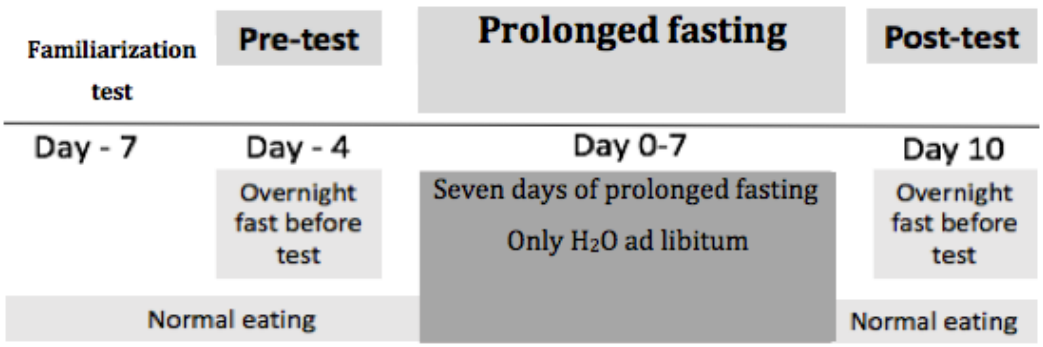
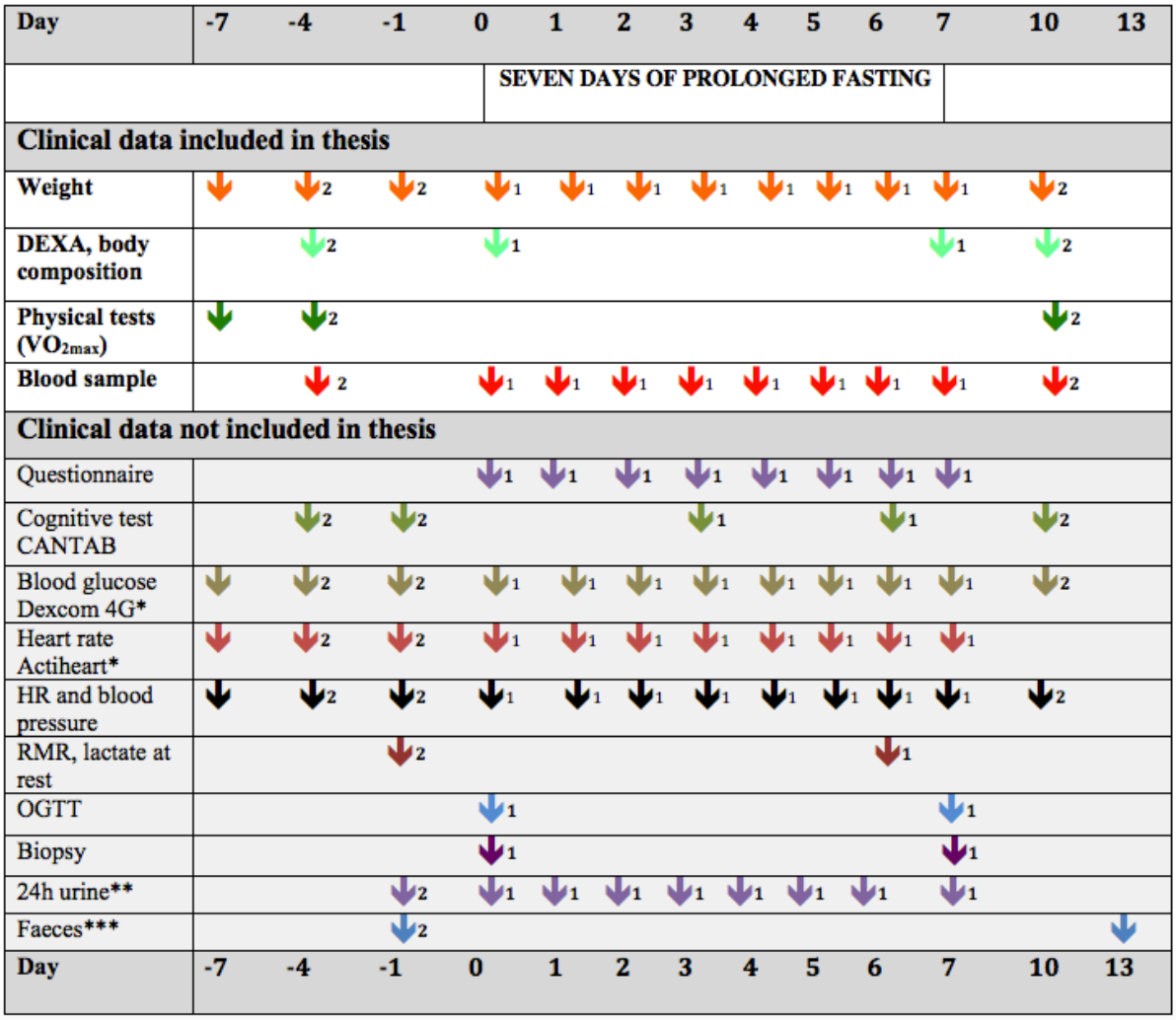


Figure 2. *A simplified representation of the different stages in the fasting study.*



[1] Seven days of fasting
[2] Overnight fast
* Continuous measurement
** 24h urine was handed in
*** Faeces sample was handed in

Figure 3. *A detailed schedule of the complete fasting study protocol. The clinical data on the white background (top) represents the data included in the thesis, the tests on the grey background (bottom) represents the other data collected for the main study. Each coloured arrow indicates the respective test of the day, the colours differentiates between the tests. Day -7 was familiarization test (baseline), day -4 was pre-test, day -1 was baseline, day 0-7 were the seven days of fasting, day 10 was post-test, and day 13 was after intervention.*

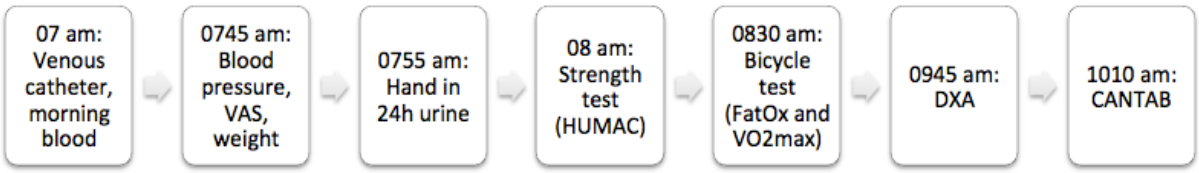


Figure 4. *Time schedule for one participant during pre-test and post-test.*

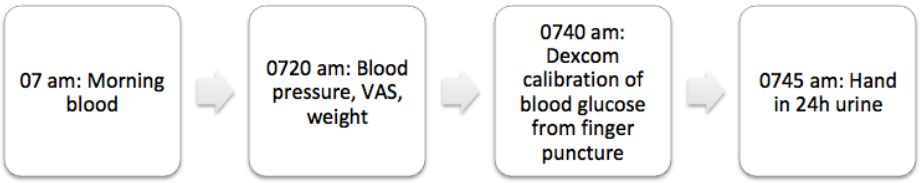


Figure 5. *Time schedule for one participant during the seven days of fasting. (Other tests in addition).*

3.3 Body composition and DXA

3.3.1 Weight

Weight was measured every morning throughout the study using Seca 877 (Seca, Hammer Steindamm 3-25, Hamburg, Germany). Participants wore a minimum of standard clothing for each measurement.

3.3.2 Dual Energy X-ray Absorptiometry (DXA)

Body composition included fat (%), lean mass (kg) and body fat (kg), and this was quantified using DXA (Lunar iDXA, GE Healthcare, Madison, USA). DXA scans were performed four times, and data was analyzed on three occasions: Two baseline scans were performed on day -4 and on day -1 before the fast, and the average of these are included in the baseline data, referred to as pre-test. One scan was performed at the end of the fast, on day 7. The last scan was performed three days after the fast (on day 10, post-test). Height was measured at baseline, and weight was measured before each scan. For scans on pre-test and post-test the participants performed an overnight fast, with the last energy intake no later than 10 PM the night before. The participants wore underwear and removed all objects containing metal (piercings, zipper on clothes, bras etc.). The participants were instructed to lie down on the back with the arms alongside the body in the middle of the scanning table and

keep their position during the scan (a total of 7 min). There was a minor gap between the arms and the upper body. Hands were pointed up with a 45° supination and the legs were strapped at the ankles and knees to restrict movement, in addition to create a slight inwards rotation in the lower extremities for an optimal scan (Nilsen, 2019).

The scans were analyzed and adjusted the same way for each person, and one person analyzed all the DXA scans. All data were analyzed using the associated data program enCORE Software version 17.

3.3.3 Urinary nitrogen

24-hour urine was collected to measure nitrogen loss, which in turn can estimate daily muscle mass loss. Sterilized bottles for collecting 24-hour urine were given to participants. Participants handed in 24-hour urine prior to fasting for assessing baseline. On every day during the fast (day 0-7) the participants were instructed to collect all urine into bottles. The next morning bottles were handed in for lab analyses, and participants were asked if they had collected all 24 h urine. If they failed to collect all urine, they were asked to estimate about how much urine that was not collected, and the volume was kept track of in the daily data logging system. The participants had to collect all 24-hour urine, and they were given up to three bottles every day, which each contained around 2000 g when full. Every bottle was weighed, and all the bottles from the 24-hour urine from one participant was thoroughly mixed into a bigger glass container and pipetted for further analyses. Specific gravidity of mix was calculated by finding the average of 5 samples à 1000 µg. Every day 4 samples à 1 mL urine per person were pipetted into aliquot tubes. 1 x 13 ml urine was pipetted using Pasteur pipettes from each 24-hour urine. All the urine samples were stored in a designated freezer at -20 °C before further analyses. Some samples were analyzed in Domus Medica, University of Oslo, Norway. These samples were analyzed for determination of nitrogen content using the Kjeldahl method, a laboratory technique used to measure urinary nitrogen in fasting studies. Calculation of muscle mass was performed by multiplying the daily urinary nitrogen with 6.25, as 1 g of urinary nitrogen is coming from 6.25 g of protein (Drenick et al., 1964; Frayn, 1983; Goran & Astrup, 2002).

3.4 Physical capacity

The physical capacity tests on bicycle were performed in accordance with the first fasting study by Brufladt (2018). An ergometer bike (Lode Excalibur Sport, Groningen, Nederland) was used for all tests. The bicycle seat was adjusted individually for each person and the same adjustments were used on all tests. Participants used the same designated bicycle shoes according to their size for all tests. Heart rate was measured throughout the whole test using Polar RS800CX with a sender Polar Wearlink, attached to a chest belt Soft Strap (all devices provided by Polar Electro Oy, Kempele, Finland).

3.4.1 Familiarization test

The familiarization test took place one week before the fast and introduced the participants to the physical capacity test protocol. The participants started with an *incremental test* starting with loads at either 75, 100 or 125 Watt depending on their fitness level (as predicted by research staff) (see *figure 6*). The load increased with 25 Watt for every 5 min, in total there were 5 different loads. Participants were instructed to bike on a cadence of 80 rpm (revolution per minute, pedalling rate) at all times during incremental test. After 2.5 min on each load, [VO_2] and [CO_2] was measured. Lactate was taken after 4 min on each load. After the incremental test was a 3 min active recovery where they pedalled on desired load. Then the participants performed a *modified Wingate 10 s* test: They biked on 80 rpm for 10 s, and they were encouraged to bike with maximal effort. The participants then had an active recovery of 3 min on desired load.

The participants then performed a *VO_{2max} test*. This was the last load below *threshold* obtained from *incremental test,* which was defined with an increase in minimum 1.25 mM lactate from the last measured load.

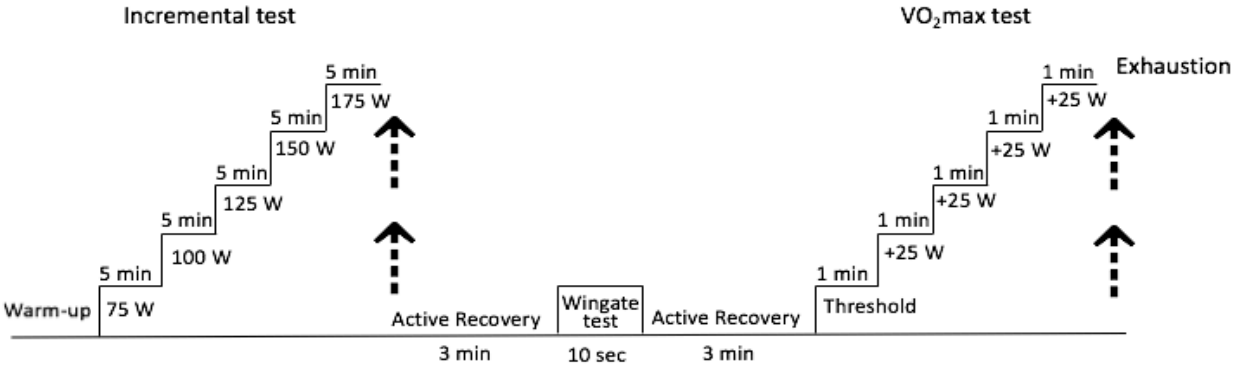


Figure 6. *Overview of the physical capacity tests during familiarization test. Figure redrawn after Brufladt (2018). Used with permission.*

3.4.2 Fat oxidation test

The fat oxidation (fatox) test was performed prior to the VO_{2max} test on pre-test and post-test (see *figure 7*). The results from VO_{2max} at familiarization test were used to predict intensities of fatox for pre-test and post-test (see *figure 6*).

The participants warmed up for 5 min at 25 % of VO_{2max} calculated from the familiarization test. During the fatox test the participants biked on six intensities corresponding to intensities of 30, 40, 50, 60, 70 and 80 % of VO_{2max}. The cadence on all intensities was set to 80 rpm. At every intensity various exercise parameters were tracked. For the last 1 min of fatox test the $[VO_2]$ and $[CO_2]$ was used to calculate fatox. Fatox was predicted with the following equation (Achten & Jeukendrup, 2003; Frayn, 1983):

$$\text{Fatox } (g \cdot min^{-1}) = 1.67 \times VO_2 \ (mL \cdot min^{-1}) \ - 1.67 \times VCO_2 \ (mL \cdot min^{-1})$$

Calculation of absolute maximal fat oxidation, $Fatox_{max}$, and absolute load (Watt) at maximal fat oxidation ($Fatox_{max}$) were analyzed on pre-test and post-test. A third-degree polynomial algebra regression was used for estimation of both parameters based on fatox calculations from Andersson-Hall et al. (2018) and Stisen et al. (2006). Below is a figure showing the physical capacity test for pre-test and post-test:

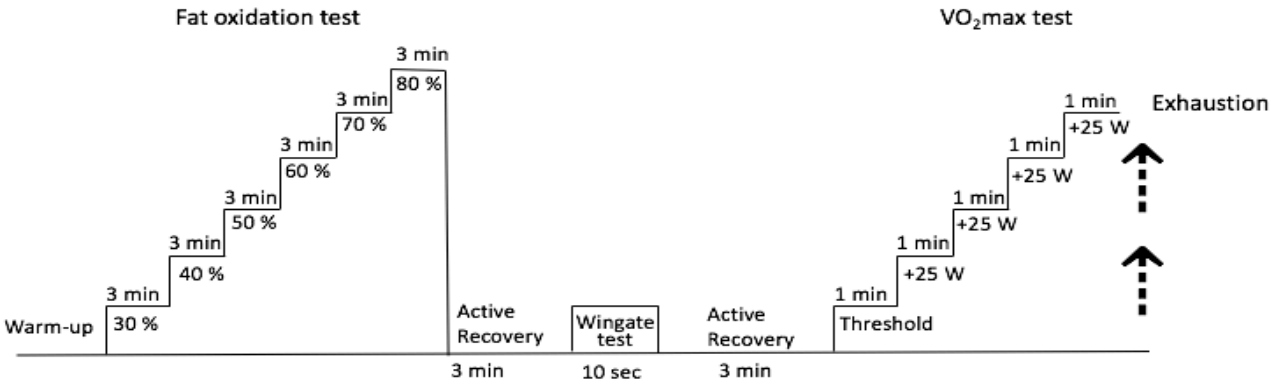


Figure 7: *Overview of the physical capacity tests on pre-test and post-test: Fat oxidation, modified Wingate 10 s and VO_{2max} were performed. Figure redrawn after Brufladt (2018). Used with permission.*

3.4.3 VO_{2max} test

The VO_{2max} test was first performed at familiarization test (see *figure 6*), and the results obtained from this test were used to predict the start load (Watt) and intensities of VO_{2max} for both pre-test and post-test (see *figure 7*). VO_{2max} started with an increase in 25 Watt for every 1 min at threshold until exhaustion. Participants biked with a cadence of minimum 60 rpm throughout the test, and if they couldn´t keep the pedalling rate above 60 rpm, the VO_{2max} test ended, and VO_{2max} was determined (in accordance with the protocol of Brufladt (2018)). The average of the last two measurements for the final 1 min of [VO_2] determined VO_{2max}. Blood samples were taken according to test protocol (see *appendix 6*). The following parameters from the VO_{2max} test were analyzed: Absolute VO_{2max} ($ml \cdot min^{-1}$), relative VO_{2max} ($ml \cdot kg^{-1} \cdot min^{-1}$), maximal load ($Watt_{max}$) at VO_{2max}, respiratory exchange ratio (RER), heart rate (HR, bpm), and lactate.

3.5 Blood samples

Blood samples were taken every day (except on days -1 and 6). The blood was taken before any activity or test. Morning blood was drawn in the morning between 06:45-10:00 am, and participants met for the same time each day. The volume of blood taken for each tube was drawn in the following order: 5 ml for serum with gel, 5 ml Li-Heparin with gel, and 6 ml for EDTA. The blood samples were turned upside down around five times, then the Li-Heparin and EDTA tubes were placed on wet ice

(box with ice and water) immediately before centrifugation. The serum was placed in room temperature to coagulate for 30 min before centrifugation. All blood samples were centrifuged at 4 °C for 10 min at 3500 G. The respective volume of plasma or serum was pipetted into LoBind Eppendorf 1.5 ml tubes on dry ice before placed into a freezer holding -80 °C for storage, before being sent to collaborative institutions for further analyses. Intravenous (IV) catheters were used on days when there were more than three occasions with blood samples, and blood drawn through IVs during OGTT and Exercise test, Posiflush saltwater (NaCl) was injected into the venous catheter every 20 min to prevent coagulation of tube. (*OGTT is described in appendix 2: Blood tests protocol, OGTT*).

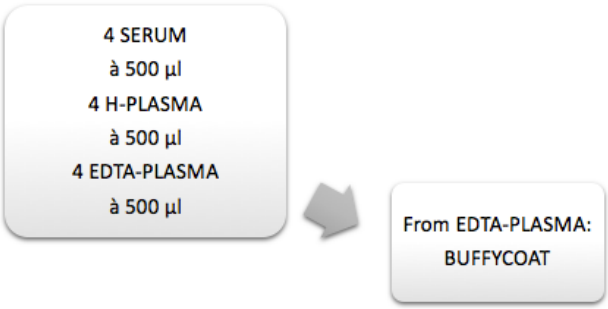


Figure 8. *Blood samples drawn and volume of aliquots taken during fasting morning blood on pre-test, during the seven days of fasting, and on post-test.*

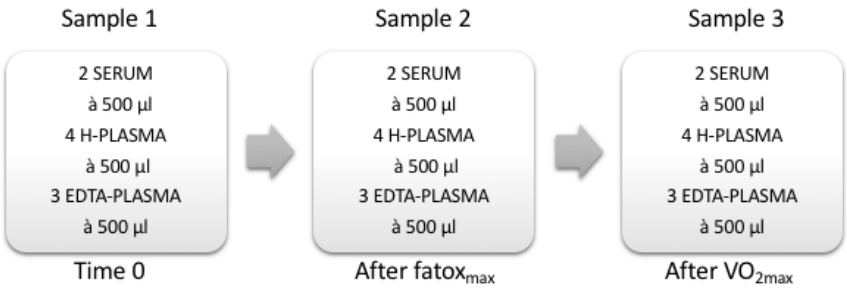


Figure 9. *Blood samples drawn and volume of aliquots taken during Exercise test. Sample 1 Exercise was drawn together with fasting morning blood. Sample 2 and 3 were drawn during the VO_{2max} test.*

4. Statistics

As this thesis is part of a bigger fasting project, the project leader estimated the number of participants (sample size) with a power of 80 % for chances to detect an effect. The power calculation was based on the statistically significant difference in expected nitrogen excretion during intervention: In previous studies urinary nitrogen excretion of 1.6 g / day was found, and an increase to 2 g / day was estimated to be an important change. This sample size estimation was also used in the first fasting study with 13 subjects (Brufladt, 2018; Nilsen, 2019).

4.1 Investigating the data distribution

First the pre-test variables were investigated to see if they were normally distributed. Then the change values between paired groups (pre-test and post-test) were investigated to see if they were normally distributed. (Examples of normal-distribution in histograms, see *appendix 5*). For evaluation of data distribution, the continuous data were investigated following these three steps (Pallant, 2010):

1. Histograms with a normal-distribution line, and a confidence interval (CI) of 95 % (Pallant, 2010).
2. QQ-plot and the proximity between the line and the data (Pallant, 2010).
3. The central tendency can give an indication of the data distribution. In normal-distributed data the mean and median will be similar (Whitley & Ball, 2002).

4.2 Presenting the data

Variables that are normal-distributed are presented in mean ± standard deviation (SD) for the parametric tests. Variables that are not normal-distributed are presented as median and interquartile range (IQR) at respectively 25 % and 75 % levels for the non-parametric tests. Statistical significance level described with probability value of difference between groups is set to alpha level of $p < 0.05$: A p-value less than 0.05 shows statistically significant difference, whereas a p-value above this shows a non-statistically significant change (Pallant, 2010).

4.3 Statistical analyses

The purpose of the statistical tests in the present study is:

1. To test for a statistically significant change between two paired groups (change from pre-test and post-test) (Pallant, 2010). The two possibilities for comparing related variables for one group at two occasions (Pallant, 2010) are:

A) Parametric test: Student T-test for data that were normal-distributed.

B) Non-parametric test: Wilcoxon Signed Rank Test for data that were not normal-distributed.

2. To test for statistically significant changes between related groups at three occasions (changes between pre-test, day 7 of the fast, and post-test) (Laerd statistics, 2018):

A) one-way repeated measures ANOVA to test for variance and an interaction effect for data that is normal-distributed (Pallant, 2010). *Mauchly´s Test of Sphericity* shows whether or not the assumptions are violated, and significance level $p > 0.05$ tells that the assumptions are met (Pallant, 2010). This test is presented with the following equation and p-value (Laerd statistics, n.d.):

$$X^2 \text{ (df)} = \text{Approx. Chi-Square, } p = \text{Sig.}$$

The "overall test", *Test of Within-Subjects Effects*, tested for statistically significant change between the three means (SPSS Tutorials, 2020b). *Post hoc – analysis* with *Bonferroni* was performed, and *pairwise comparisons* show statistical significance between the groups (Pallant, 2010).

B) Friedman test for data that is not normally distributed (Pallant, 2010). The "overall test", *within-subjects rank*, was performed to test for a statistically significant change between the groups (SPSS Tutorials, 2020a). *Mean ranks* are presented, and results of the Friedman test are presented with the following equation and probability value (SPSS Tutorials, 2020a):

X^2 (df) = Chi-Square, p = Asymp. Sig.

Post hoc - analysis with *Wilcoxon* was performed to find statistical significance between the groups (Pallant, 2010).

All statistics are analyzed using IBM SPSS Statistics 25 (Statistical Package for the Social Sciences). All graphs and figures presented in the *Statistics* chapter are made with GraphPad Prism 8 for macOS (2018 GraphPad Software, LLC, version 8.3.0 (328)). (Also see *appendix 5*.)

5. Results

5.1 Study participants

14 subjects signed up for the study. They were all recruited through Norwegian School of Sport Sciences (NIH). There were two dropouts in the early phases of the study, one independent of fasting, and these data have not been included in the analysis. The remaining 12 subjects completed the fasting study. Table 2 shows the overview of the participants:

Table 2. *Age and anthropometric baseline characteristics of the participants in the fasting study. Number of subjects are given in parentheses.*

	Group (n=12)	Women (n=5)	Men (n=7)
Median age, years (IQR)	26 (22, 37)	24 (23, 33)	28 (20, 39)
Mean weight, kg (SD)	77.4 (15.70)	64.8 (6.42)	86.6 (13.83)
Median height, cm (IQR)	177 (170, 181)	169 (162, 177)	181 (176, 182)
Mean BMI (SD)	24 (2.6)	23 (1.3)	26 (2.6)
Mean fat % (SD)	25.6 (7.2)	25.8 (4)	25.4 (9.2)

5.1.1 Outlier

As BMI alone can only give indications of health as it categorizes a person as healthy-, under- or overweight or obese, BMI is not sufficient to account for body composition or metabolic status, and it has limitations (Juby, 2014): In accordance with the project leader one participant with a BMI ≥ 30 kg/m^2 was included in the study, as the primary focus on inclusion was fulfilling the requirement of minimum body fat. For that reason this person stands out from the rest of the group when it comes to BMI ≤ 30, as this participant is *obese (Expert Panel on the Identification, Evaluation, and Treatment of Overweight in Adults, 1998).* This participant also had a higher fat percentage and weight compared to the rest of the group. However, this person was considered healthy and all its data is accounted for and included in every variable.

5.2 Weight and body composition

5.2.1 Weight (kg)

The mean (SD) weight on pre-test was 77.4 kg (15.77). On day 7 weight was decreased to 71.8 kg (15.18). On post-test weight was increased to 74.3 kg (15.55). Mauchly´s Test of Sphericity: X^2 (2) = 0.939, (p = 0.625). Test of Within-Subjects Effects showed the following difference between the occasions: F (2, 22) = 172.43, (p < 0.001). All participants reduced his or her body weight by ≥ -1.9 kg from pre-test to post-test.

Post hoc- analysis:

The change from pre-test to day 7 was -5.6 kg (1.11), (p < 0.001).

The change from pre-test to post-test was -3.2 kg (0.68), (p < 0.001).

The change from day 7 to post-test was 2.4 kg (1.03), (p < 0.001).

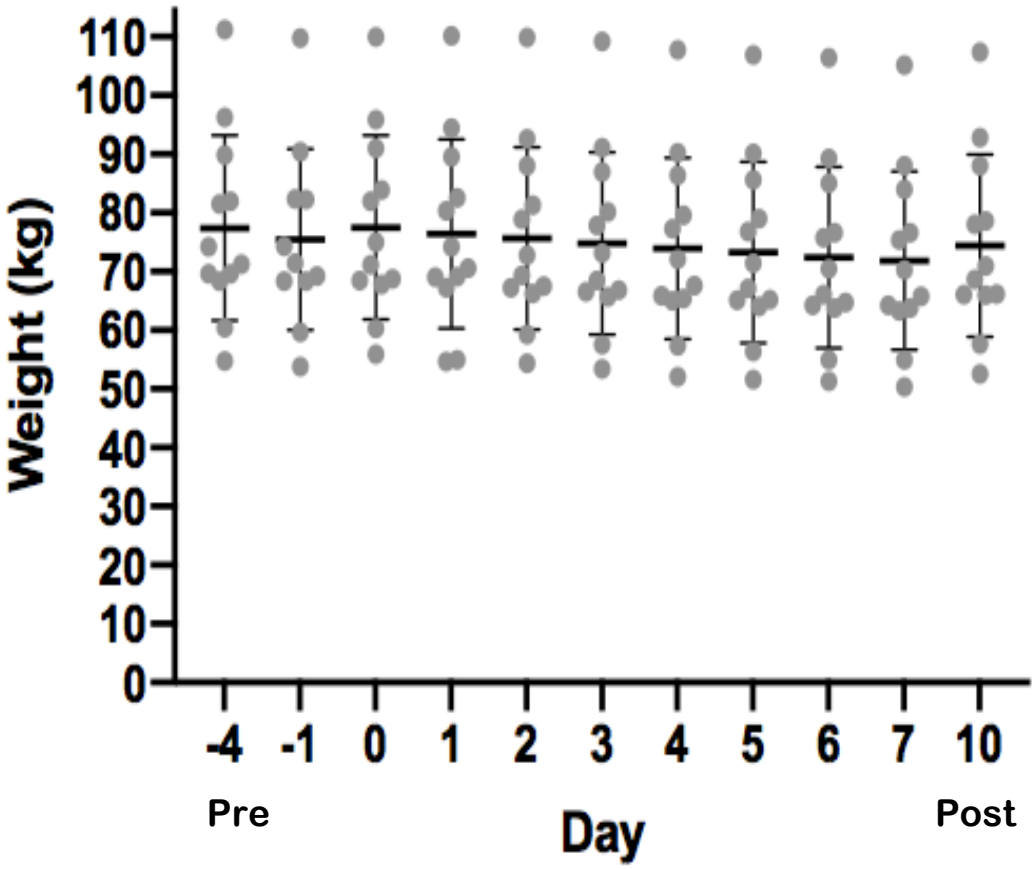


Figure 10. *Mean body weight in the days before (pre-test), during and three days after (post-test) the seven days of fasting (n=12). The dots are individual values. Vertical bars represent the group mean (bold line) and SD (thin line) values.*

5.2.2 Lean mass (kg)

The median (IQR) lean mass on pre-test was 55.9 kg (45.88, 63.41). On day 7 lean mas decreased to 52.5 kg (42.70, 59.46). On post-test lean mass increased to 54.6 kg (45.30, 63.18). Friedman test showed X^2 (2) = 18.67, ($p < 0.001$).

Table 3. *Friedman Test Ranks lean mass.*

	Mean rank
Pre-test	2.67
Day 7	1.00
Post-test	2.33

(n=12)

Post hoc- analysis:

The change from pre-test to day 7 was -3.7 kg (-4.18, -3.15), ($p = 0.002$).

The change from pre-test to post-test was -0.8 kg (-1.23, 0.11), ($p = 0.023$).

The change from day 7 to post-test was 3 kg (1.94, 3.64), ($p = 0.002$).

5.2.3 Urinary nitrogen (g)

The mean urinary nitrogen (SD) during the seven days of prolonged fasting was estimated to be 75.9 g (17.56) (n=12). The nitrogen loss was multiplied with the constant 6.25, and the mean protein loss (SD) was calculated to be 474.8 g (105.10). This equals a loss of 2.4 kg (0.57) mean muscle mass (SD) from day 0 to day 7.

5.2.4 Body fat (kg)

The mean (SD) body fat on pre-test was 19.6 kg (9.45). On day 7 body fat decreased to 18.0 kg (9.43). On post-test body fat decreased to 17.8 kg (9.45). Mauchly´s Test of Sphericity: X^2 (2) = 4.214, ($p = 0.122$). Test of Within-Subjects Effects showed the following difference between the occasions: F (2, 22) = 115.39, ($p < 0.001$).

Post hoc- analysis

The change from pre-test to day 7 was -1.6 kg (0.46), ($p < 0.001$).

The change from pre-test to post-test was -1.8 kg (0.54), ($p < 0.001$).

The change from day 7 to post-test was -0.2 g (0.30), ($p = 0.054$).

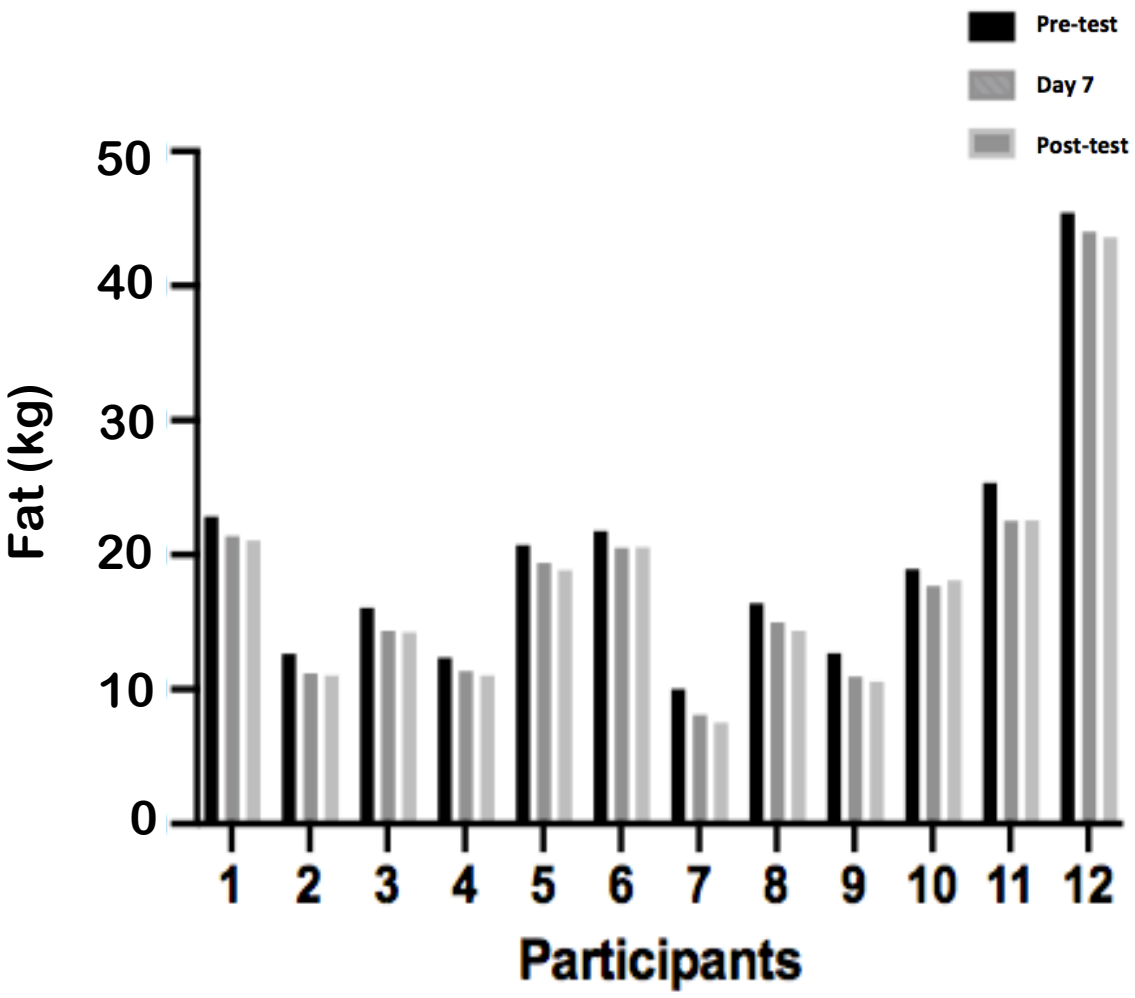


Figure 11. *Mean body fat on pre-test, on day 7 of the fast, and on post-test for each individual participant (n=12).*

5.3 Physical capacity

5.3.1 Absolute VO_{2max} ($ml \cdot min^{-1}$)

The median (IQR) VO_{2max} on pre-test was 3227 $ml \cdot min^{-1}$ (2895.9, 4113.5). On post-test absolute VO_{2max} decreased to 3116 $ml \cdot min^{-1}$ (2554.5, 3828.0). The change in median values from pre-test to post-test was -245 $ml \cdot min^{-1}$ (-383.1, -85.0), ($p = 0.002$; Figure 12). Three subjects did not reduce their VO_{2max} (average percent change < -1 %). Two subjects experienced a relatively large decline in VO_{2max} (-649 and -701 $ml \cdot min^{-1}$, average percent change -18 %). By excluding the two extreme values, the VO_{2max} reduced with -196 $ml \cdot min^{-1}$ (average percent change -6 %), (n=10). Figure 12 shows the individual values in absolute VO_{2max} on pre-test and post-test:

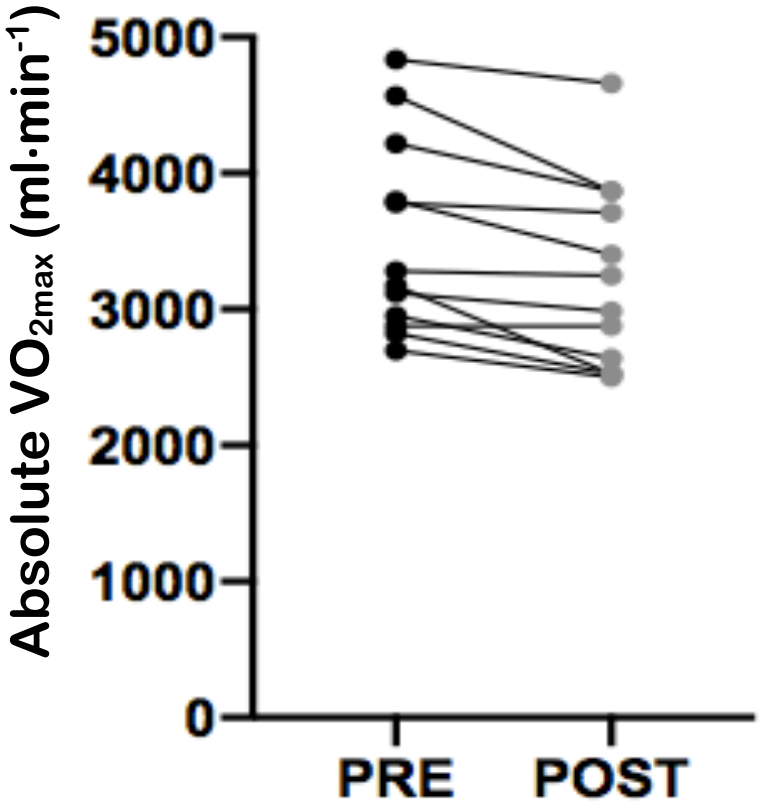


Figure 12. *Median absolute maximal oxygen uptake on pre-test and post-test and within-subject change (n=12).*

5.3.2 Relative VO_{2max} ($ml \cdot kg^{-1} \cdot min^{-1}$)

The mean (SD) VO_{2max} related to body weight on pre-test was 46.2 $ml \cdot kg^{-1} \cdot min^{-1}$ (9.13). On post-test relative VO_{2max} was 44.4 $ml \cdot kg^{-1} \cdot min^{-1}$ (8.61). The change in mean values from pre-test to post-test was -1,8 $ml \cdot kg^{-1} \cdot min^{-1}$ (3.03), ($p = 0.067$), (average percent change -4 %), (see *figure 13*). Two subjects scored the same on both tests. Three subjects improved their VO_{2max} with > 1,7 $ml \cdot kg^{-1} \cdot min^{-1}$ (average percent change 4 %). The same two subjects who experienced a large decline in absolute VO_{2max} (see chapter 4.3) also had a large decline in relative VO_{2max} (average reduction of -6.8 $ml \cdot kg^{-1} \cdot min^{-1}$, average percent change -14 %). By excluding the two extreme values, the VO_{2max} was reduced with -0.8 $ml \cdot kg^{-1} \cdot min^{-1}$ (average percent change -2 %), (n=10). Figure 13 shows the individual values in relative VO_{2max} on pre-test and post-test:

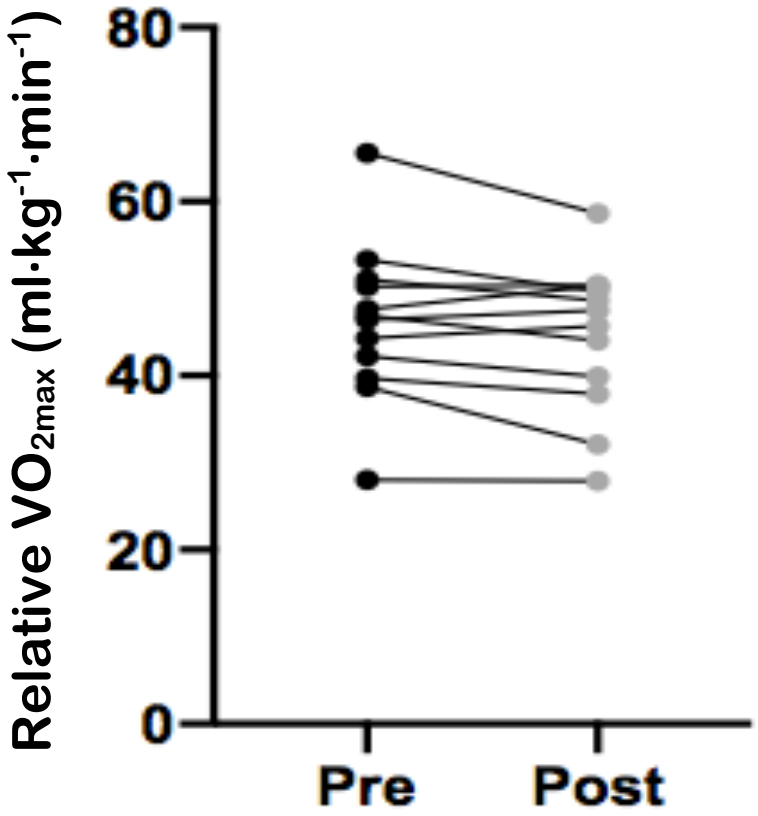


Figure 13. *Mean relative maximal oxygen uptake on pre-test and post-test and within-subject change (n=12).*

5.3.3 Maximal load ($Watt_{max}$) at the VO_{2max} test

The mean maximal load ($Watt_{max}$) (SD) at the VO_{2max} test on pre-test was 294 Watt (58.5). On post-test maximal load was 273 Watt (57.9). The change in mean values from pre-test to post-test was -21 Watt (14), ($p < 0.001$), (average percent change -7 %), (see figure 14). Three subjects biked on the same load on both tests. One subject reduced with 50 Watt (percent change – 20 %); This person was one of the two whom had the largest decline in absolute VO_{2max}. All others reduced with 25 Watt (average percent change – 8 %), (n=8). By excluding the one extreme value, the participants reduced with -18 Watt (average percent change -6 %), (n=11). Figure 14 shows the individual values in maximal load ($Watt_{max}$) at the VO_{2max} test on pre-test and post-test:

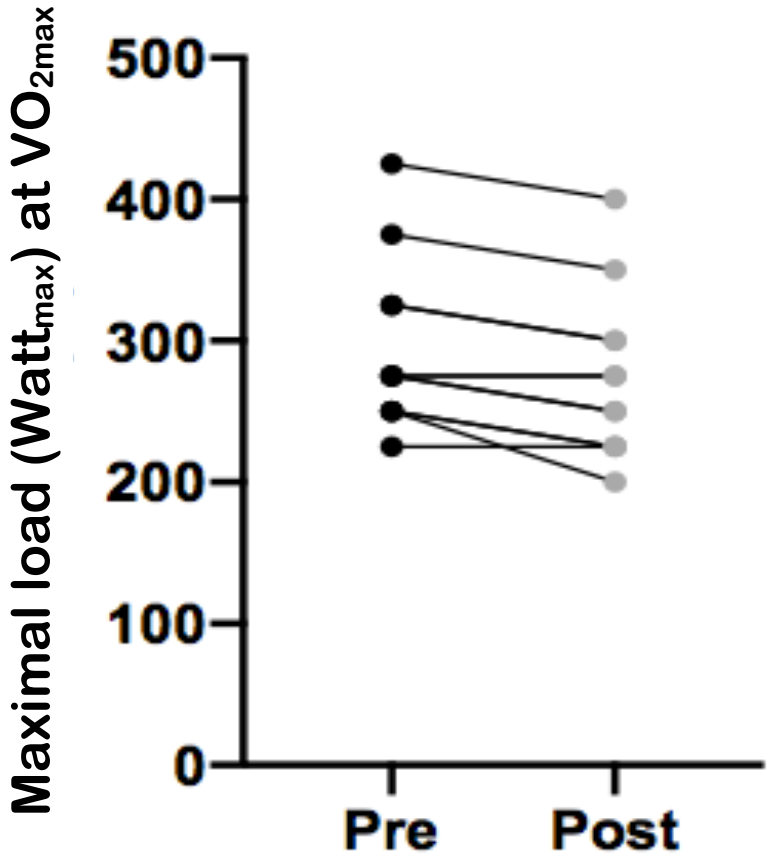


Figure 14. *Mean maximal load at the VO_{2max} test on pre-test and post-test and within-subject change (n=12).*

5.3.4 Heart rate, lactate and RER at the VO_{2max} test

Associated parameters during the VO_{2max} test showed that the mean heart rate increased significantly. Both mean lactate acid concentration and mean respiratory exchange ratio were unchanged from pre-test to post-test (see *table 4*).

Table 4. *Other parameters during maximal oxygen uptake.*

Parameter	Pre-test	Post-test	Change	p-value
Mean heart rate (bpm) (SD)	180 (9.0)	185 (9.0)	5 (4.0)	0.008
Mean lactate (mM) (SD)	11.6 (1.70)	11.6 (1.80)	0.0 (1.80)	0.957
Mean RER [VCO_2/VO_2] (SD)	1.1 (0.05)	1.1 (0.05)	0.0 (0.02)	0.781

(n=12)

5.3.5 Fat oxidation (fatox) test

There was a tendency towards reduced mean fat oxidation ($fatox_{max}$), but this was not a statistically significant change. There was a statistically significant decrease in mean absolute load (Watt) at $fatox_{max}$ from pre-test to post-test (see *table 5*).

Table 5. *Changes in work at submaximal intensities.*

Parameter	Pre-test	Post-test	Change	p-value
Mean $fatox_{max}$ [$g \cdot min^{-1}$] (SD)	0.46 (0.228)	0.39 (0.177)	-0.07 (0.160)	0.162
Mean absolute load at $Fatox_{max}$ (Watt) (SD)	96 (33.0)	83 (28.0)	-13 (11.0)	0.002

(n=12)

5.4 Metabolic markers in blood

5.4.1 Blood glucose (mmol/L)

The median (IQR) blood glucose on pre-test was 5.1 mmol/L (4.90, 5.36), (n=11). On day 7 blood glucose decreased to 3.7 mmol/L (3.54, 3.82). On post-test blood glucose increased to 5.0 mmol/L (4.83, 5.19), (n=11). Friedman test showed X^2 (2) = 16.545, ($p < 0.001$).

Table 6. *Friedman Test Ranks blood glucose.*

	Mean rank
Pre-test	2.45
Day 7	1.00
Post-test	2.55

(n=11)

Post hoc- analysis:

The change from pre-test to day 7 was -1.3 mmol/L (-1.73, -1.18), ($p = 0.003$).

The change from pre- to post- test was 0.0 mmol/L (-0.24, 0.21), ($p = 0.894$).

The change from day 7 to post-test was 1.4 mmol/L (0.99, 1.77), ($p = 0.003$).

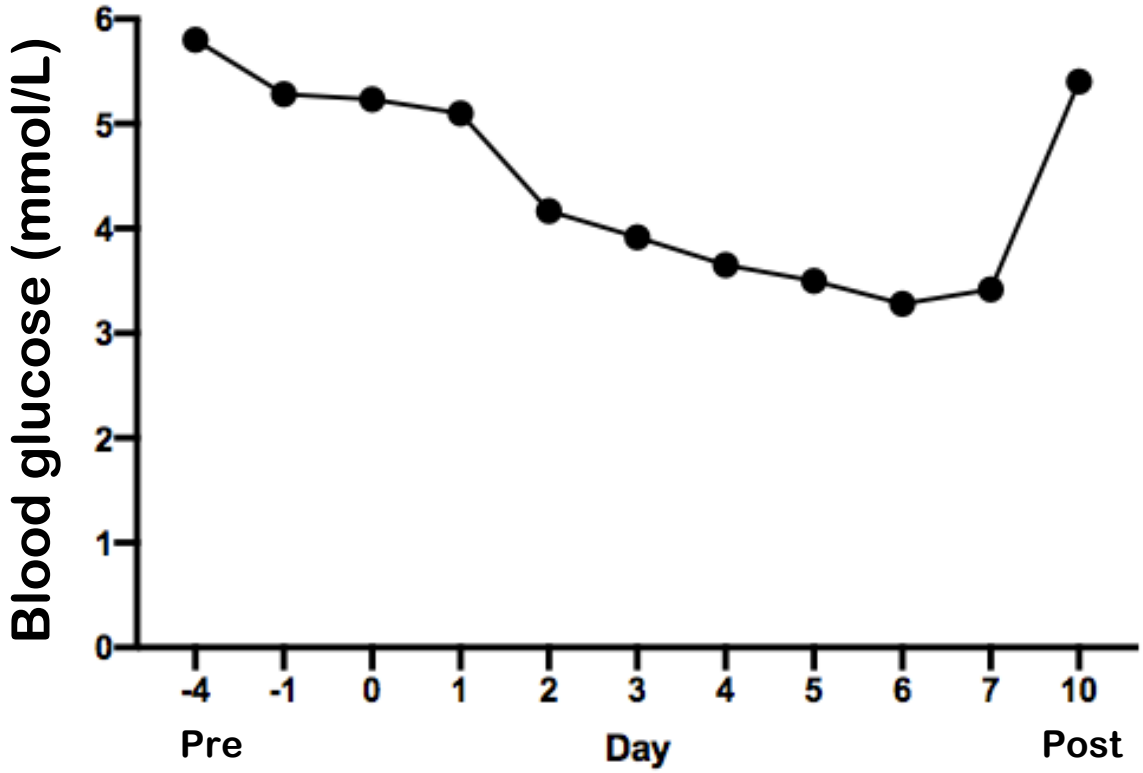


Figure 15. *Mean blood glucose in the days before (pre-test) (n=11), during (n=12) and after (post-test) (n=11) the seven days of fasting.*

5.4.2 Triacylglycerols (mmol/L)

The mean (SD) triacylglycerols on pre-test was 0.88 mmol/L (0.488) (n=11). On day 7 triacylglycerols increased to 1.16 mmol/L (0.304) (n=11). On post-test triacylglycerols decreased to 0.91 mmol/L (0.312) (n=11). Mauchly´s Test of Sphericity: X^2 (2) = 1.114, (p = 0.573). Test of Within-Subjects Effects showed the following difference between the occasions: F (2, 20) = 2.167, (p = 0.141).

Post hoc- analysis:

The change from pre-test to day 7 was 0.28 mmol/L (0.558), (p = 0.385).

The change from pre- to post- test was 0.04 mmol/L (0.458), (p = 1.000).

The change from day 7 to post-test was -0.24 mmol/L (0.424), (p = 0.260).

5.4.3 Free Fatty Acids (mmol/L)

The mean (SD) free fatty acids on pre-test was 0.39 mmol/L (0.191). On day 7 fatty acids increased to 1.30 mmol/L (0.290). On post-test fatty acids decreased to 0.33 mmol/L (0.226). Mauchly´s Test of Sphericity: X^2 (2) = 2.129, (p = 0.345). Test of Within-Subjects Effects showed the following difference between the occasions: F (2, 20) = 55.481, (p < 0.001).

Post hoc- analysis:

The change from pre-test to day 7 was 0.91 mmol/L (0.333), (p < 0.001).

The change from pre-test to post-test was -0.06 mmol/L (0.269), (p = 1.00).

The change from day 7 to post-test was -0.96 mmol/L (0.405), (p < 0.001).

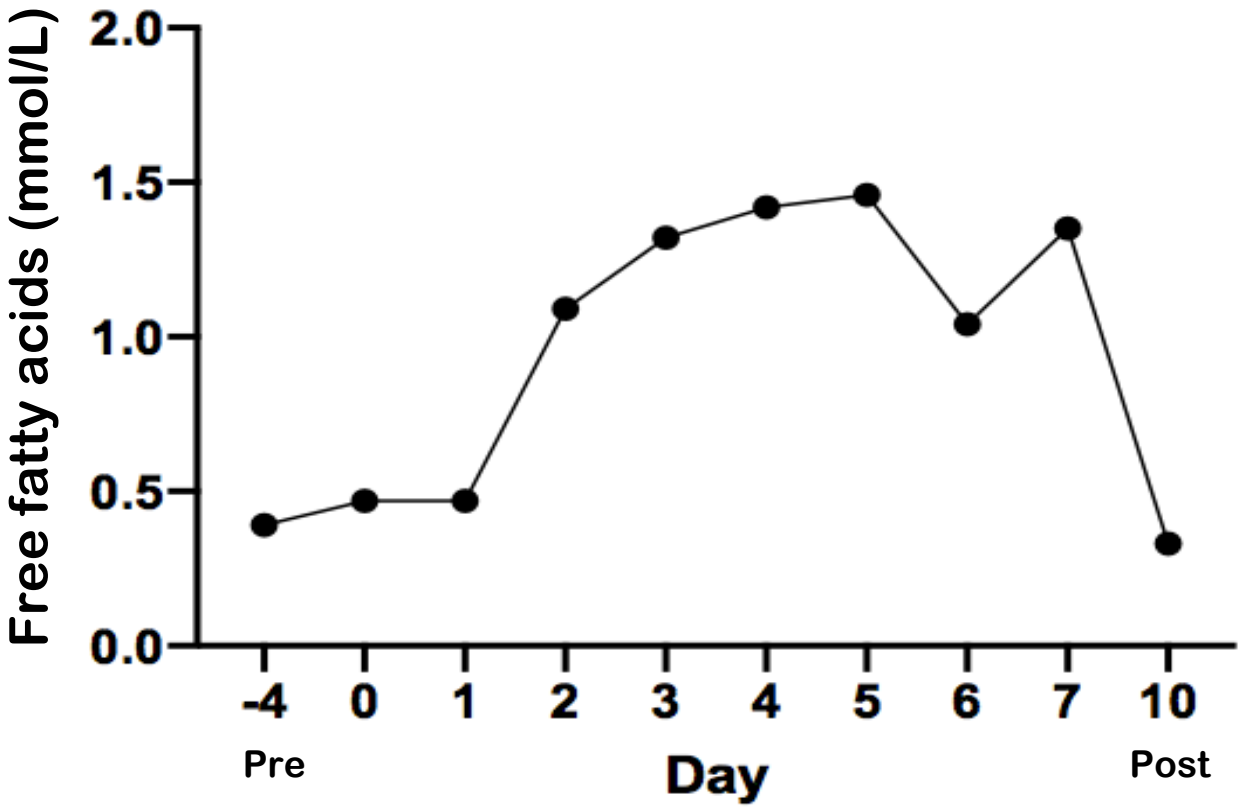


Figure 16. *Mean free fatty acids in the days before (pre-test), during and after (post-test) the seven days of fasting (n=12).*

5.4.4 β-hydroxybutyrate (mmol/L)

The median (IQR) β-hydroxybutyrate on pre-test was 0.04 mmol/L (0.020, 0.080). On day 7 β-hydroxybutyrate increased to 4.42 mmol/L (3.683, 5.028). On post-test

β-hydroxybutyrate decreased to 0.12 mmol/L (0.110, 0.140). Friedman test showed X^2 (2) = 20.17, (p < 0.001).

Table 7. *Friedman Test Ranks β-hydroxybutyrate.*

	Mean rank
Pre-test	1.08
Day 7	2.92
Post-test	2.00

(n=12)

Post hoc- analysis:

The change from pre-test to day 7 was 4.21 mmol/L (3.633, 5.098), (p = 0.002).

The change from pre-test to post-test was 0.09 mmol/L (0.040, 0.100), (p = 0.05).

The change from day 7 to post-test was -4.10 mmol/L (-4.97, 3.50), (p = 0.003).

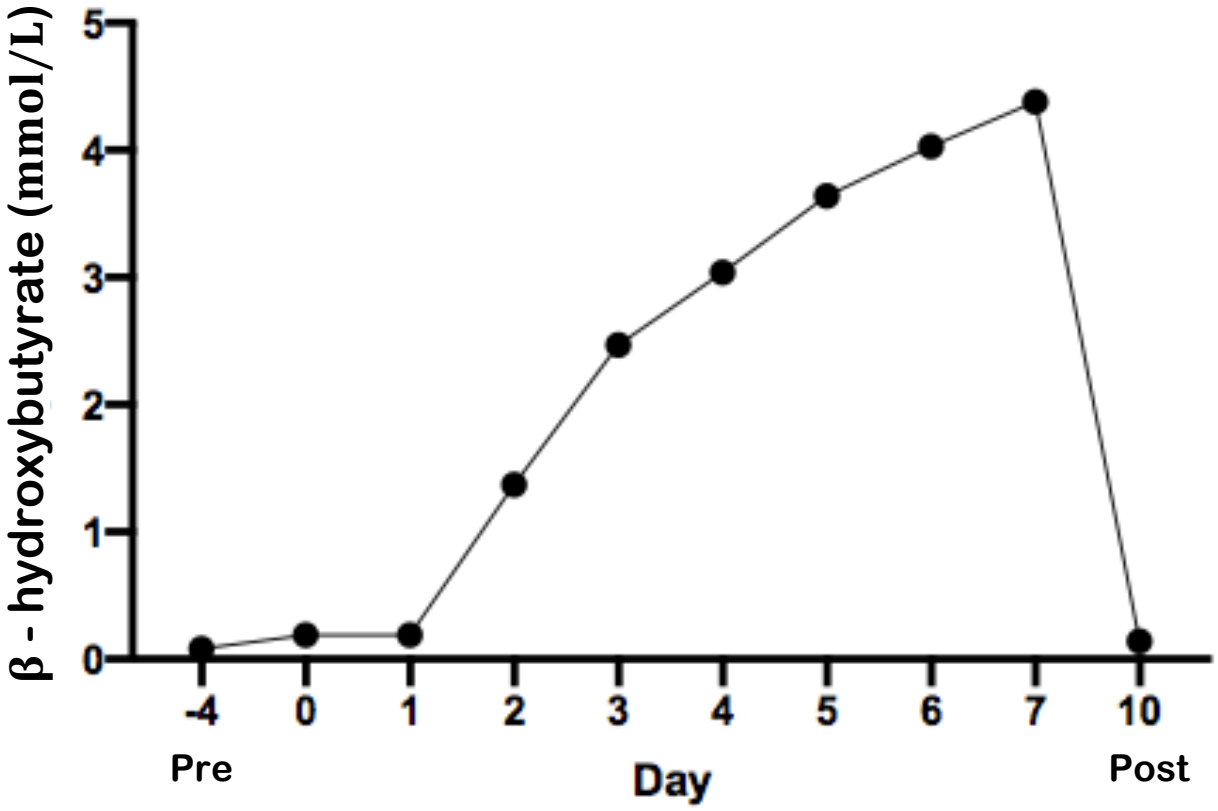


Figure 17. *Median β-hydroxybutyrate in the days before (pre-test), during and after (post-test) the seven days of fasting (n=12).*

5.4.5 T_3 (thyroid hormone metabolite) (pM)

The median (IQR) T_3 on pre-test was 4.80 pM (3.450, 5.500). On day 7 T_3 reduced to 2.90 pM (2.500, 3.00) (n=11). On post-test T_3 increased to 4.50 pM (4.125, 4.625), (n=6). Friedman test showed X^2 (2) = 12.000, (p = 0.002).

Table 8. *Friedman Test Ranks T_3.*

	Mean rank
Pre-test	3.00
Day 7	1.00
Post-test	2.00

(n=6)

Post hoc- analysis:

The change from pre-test to day 7 was -1.40 pM (-3.100, -0.300), (p = 0.008).

The change from pre-test to post-test was -0.55 pM (-1.200, -0.475), (p = 0.027).

The change from day 7 to post-test was 1.65 pM (0.975, 2.075), (p = 0.028).

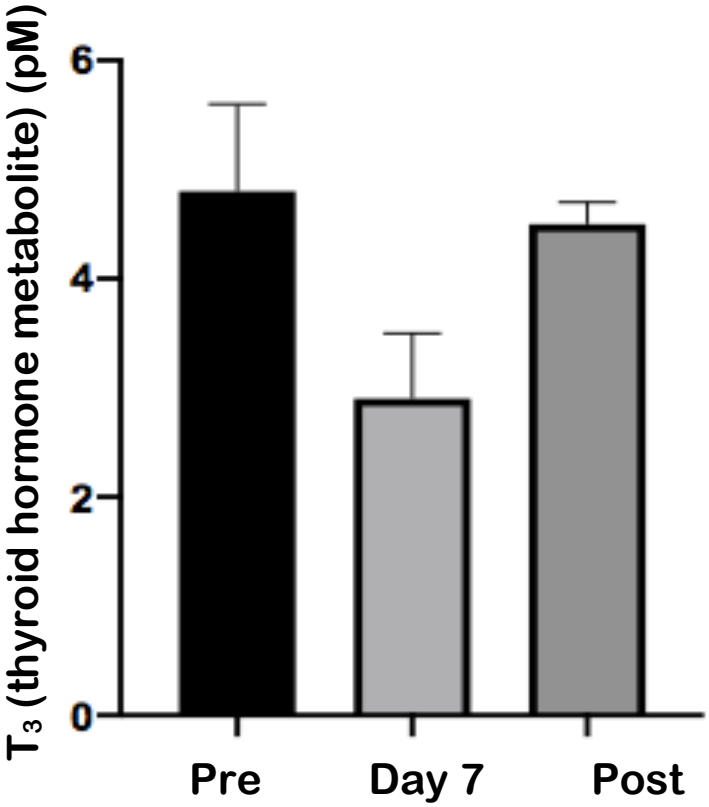


Figure 18. *Median T_3 on pre-test (n=12), on day 7 of the fast (n=12) and on post-test (n=6) after the seven days of fasting.*

6. Discussion

Prolonged fasting causes major metabolic changes of which some are considered beneficial, while others are deteriorating to health. However, there is little knowledge about the metabolic adaptation when eating starts again after a fast. If prolonged fasting is performed and/or repeated as a strategy to control body weight, it is important that harmful metabolic states do not occur when food is re-introduced. The aims of the thesis were to investigate the effects of seven days of prolonged fasting followed by three days with re-feeding on body composition, physical capacity and metabolic health in healthy, normal-weight subjects. The main findings in this thesis were that the participants significantly reduced their body weight, lean mass and fat mass during seven days of the prolonged fast. Weight increased from day 7 to post-test three days later as a result of re-feeding, due to a significant increase in lean mass. Replenishment of glycogen stores and bound water could explain an increase in lean mass (Consolazio et al., 1967), in addition to content in intestines. There was a tendency towards a reduced fat mass even after re-feeding. On physical tests, absolute VO_{2max} and loads (Watt) at both VO_{2max} and $fatox_{max}$ were decreased. This indicates reduced physical capacity. However, the energy substrate utilization at $fatox_{max}$ during exercise was not reduced significantly. Most of the blood concentrations of hormones and metabolites considered markers of metabolic health changed during the seven days of fasting. Three days of re-feeding was sufficient time to normalize most of these metabolic blood markers. A prolonged fast of seven days followed by three days of re-feeding is not harmful in normal, healthy-weight subjects, but a large proportion of muscle protein will be lost during the fast.

6.1 Body composition changes

6.1.1 Body composition changes from pre-test to day 7

There is reported a great weight-loss in the first week of a prolonged fast with overweight subjects (Thomson et al., 1966). Our study with normal-weight subjects found a reduction of 5.6 kg in mean body weight after seven days of prolonged fasting ($p < 0.001$). In the previous fasting study at NIH a reduction of 5.3 kg was found after seven days of prolonged fasting (Brufladt, 2018; Nilsen, 2019). These two

studies are highly comparable as they had the same inclusion and exclusion criteria, but with different subjects. Furthermore, Nilsen (2019) reported of a daily weight loss of 0.8 kg, which is equivalent to the findings in this study. Both lean mass and fat mass reduced in that fasting study of Nilsen (2019) and Brufladt (2018), which is also in line with our findings. In this thesis the daily weight is calculated from a stationary scale, and both lean mass and fat mass are calculated from DXA: for this reason, the numbers cannot be compared, the numbers can only be used as indicators.

In this study most of the weight loss occurring from pre-test to day 7 was due to a reduction in lean mass, as detected by DXA. The median lean mass (IQR) reduced with 3.7 kg ($p = 0.002$). The reduction in lean mass is most likely due to increased breakdown of proteins in the early phase of the fast, in addition to fasting-induced dehydration. Consolazio et al. (1967) found substantial dehydration in participants during prolonged fasting. The majority of reduction in lean mass comes from the loss of fluids and glycogen breakdown (Jørgensen & Holmquist, 2011). Since glycogen binds water, reduction in glycogen will cause loss of water. As the liver stores about 100 g of glycogen (Jensen et al., 2011), and 1 g of liver glycogen is attached to 2.7 g of water (Drevon, 2019c), we can estimate that the breakdown of liver glycogen would account for ≈ 270 g of lean mass loss. With the same calculation, as muscle glycogen is limited to 500 g (Jensen et al., 2011), it could potentially account for ≈ 1.4 kg of lean mass loss. In total, the carbohydrate stores would make up for ≈ 1.6 kg of lean mass loss. In addition, empty content in intestines after refraining from food for seven days also accounts for a big proportion of the reduction in lean mass from pre-test to day 7.

During the 7 days of prolonged fasting the loss of mean urinary nitrogen (SD) was estimated to be 75.9 g (17.56) for the participants, which is equal to a loss of 2.4 kg (0.57) of mean muscle mass (SD). Brufladt (2018) found a reduction of 2.6 kg of muscle mass after seven days of prolonged fasting, whereas Nilsen (2019) found a reduction of 3.2 kg of muscle mass, both calculated from urinary nitrogen excretion. Because muscles contain 75 % of water (Sawka, 1992), it can be assumed that a great majority of the reduction in lean mass also comes from water in skeletal muscles. Most of the protein oxidation may have occurred in the first few days of the fast,

during gluconeogenesis (Drenick et al., 1964; Frayn, 2010; Koolman & Roehm, 2013), but this was not analysed.

The total reduction in median lean mass from pre-test to day 7 includes the loss of fluids, most likely empty carbohydrate stores (1.6 kg), and the loss of muscle mass (2.4 kg). In addition, lean mass on DXA also includes the weight of content in intestines. An average stool can vary from ≈ 100 - 500 g per day (Cummings, Bingham, Heaton, & Eastwood, 1992). As the participants did not eat from day 0 to day 7 during the fast, we must account for additional weight loss from the empty intestines with no digested food during the fast. In this thesis this will be estimated to 800 g. In total this will be an average of 4.8 kg lean mass loss from pre-test to day 7. As the lean mass reduction of 3.7 kg in this thesis is analyzed with median, this comparison is only estimated as it cannot be compared.

The participants lost on average 1.6 kg of body fat ($p < 0.001$) from pre-test to day 7. This is 200 g more compared to the studies of Nilsen (2019) and Brufladt (2018) where the participants reduced their fat mass with 1.4 kg. Since the participants in this study lost a little more body fat compared to the study of Brufladt (2018), and the initial weight of the participants were respectively 77.4 kg and 79.4 kg, this can indicate that the participants in this present study had a slightly increased rate of fat oxidation compared to the other NIH study. As the total weight loss from pre-test to day 7 for the participants in the present study were 5.6 kg, whereas in the studies of Nilsen (2019) and Brufladt (2018) the participants reduced their body weight with 5.3 kg, that makes a difference of 300 g between the two NIH fasting studies, of which minimum 200 g can be directly related to fat mass. The fat oxidation can best be described when looking at the figure of free fatty acids (see *figure 16*), where the peak was at day 5.

6.1.2 Body composition changes from day 7 to post-test

Mean weight increased with 2.4 kg ($p < 0.001$) from day 7 to post-test. The weight gain was due to re-feeding, and the median lean mass increased with 3 kg. The weight gain was most likely intestines filling up and absorption of fluids after fasting-induced dehydration, and not necessarily due to an increase in muscle mass. Consolazio et al. (1967) reported of a rapid weight gain in the first days of eating

after a prolonged fast due to increased fluid absorption when the hydration balance normalizes. Calbet et al. (2015) also found recovered normohydration within three days with a normal diet after severe caloric restriction. In this present study we did not measure if the subjects drank sufficient amounts of water and continued to be dehydrated even after re-feeding, but most likely they achieved normohydration within three re-feeding days. Besides water absorption and fluids, the gain in lean mass weight came from content in intestines. It is safe to say that ingested food in intestines increased the weight and lean mass during the three days of re-feeding. The latter could account for almost 1 kg of the total of 3 kg (1.94, 3.64) increase in median lean mass from day 7 to post-test.

The replenishing of liver glycogen may also have contributed to increased weight gain in this study. One study reported on replenished liver glycogen levels above baseline (glycogen supercompensation) after days with re-feeding after a fast (Nilsson & Hultman, 1973). The glycogen increase would potentially increase the weight in this tissue on post-test compared to pre-test. Replenishment of liver glycogen depends on the diet after a fast, as days with a low-carbohydrate diet maintains low liver glycogen levels, whereas a high-carbohydrate diet contributes to the supercompensation (Nilsson & Hultman, 1973). If the glycogen stores are emptied as a result of exercise, a diet of fat and protein will contribute to a lower rate of glycogen synthesis (Bergstrom et al., 1967). The m. glycogen stores are also affected by changes in diet (Bergstrom et al., 1967). As data from continuous glucose measurement (Dexcom) was not analysed in this thesis, it is unsure what macronutrients the participants ate after the fast. It is also unknow if their glycogen stores were sufficiently filled up, as liver biopsies were not performed.

Rather interestingly, fat mass continued to decrease with 0.2 kg even after re-feeding, but the decrease was not statistically significant ($p = 0.054$). The fat mass loss during re-feeding may indicate that the body continues to metabolize fat until glycogen stores are completely replenished after a prolonged fast. It may be that the adaptation to reverse a maximal fat oxidation takes more than three days, even though carbohydrates are available thought diet. Accordingly, even though the participants had an increased ability to break down fats (see *figure 16*) up until day 7, when they

did eat carbohydrates during re-feeding this may have been the preferred energy substrate by various tissues.

6.1.3 Body composition changes from pre-test to post-test

The weight change from pre-test to post-test after three days with re-feeding was -3.2 kg ($p < 0.001$), of which 1.8 kg was fat mass ($p < 0.001$). The loss in median lean mass by -0.8 kg of from pre-test to post-test is most likely muscle mass, as hydration levels and full intestines were normalized, and most likely glycogen stores as well.

6.2 Physical capacity changes

6.2.1 VO_{2max} changes

In this study we found a statistically significant decrease in median absolute VO_{2max} with -245 $ml \cdot min^{-1}$ ($p = 0.002$) (see figure 12). As absolute VO_{2max} is synonymous with metabolic capacity (Schmidt & Prommer, 2010), a decrease in volume indicates a disadvantage in exercise physiology. A reduction in absolute VO_{2max} after five days of prolonged fasting is also found by Henschel et al. (1954), where heathy men reduced their physical capacity by 8 %. A reduction in absolute VO_{2max} as a result of prolonged fasting is also in accordance with the study of Brufladt (2018): the participants reduced their VO_{2max} by almost 500 $ml \cdot min^{-1}$, but this was on the sixth day during a prolonged fast, and the participants were most likely dehydrated. In this present study the participants were most likely not dehydrated after three days with re-feeding. Two subjects experienced a large decline of -18 % (respectively -649 and -701 $ml \cdot min^{-1}$). Removing these two outliers did not contribute to a very different result as the parameter was presented in median. Three subjects did not reduce their VO_{2max}, which can indicate different biological variations and adaptations to fasting and re-feeding.

Relative VO_{2max} indicates how many milliliters of oxygen the body needs per kilo per minute. Relative VO_{2max} is a weight-dependent parameter and may be affected by changes in the mass. The participants in this study reduced their weight by -3.2 kg from pre-test to post-test. Furthermore, there was a tendency towards reduced relative

VO_{2max} from pre-test (46.2 $ml \cdot kg^{-1} \cdot min^{-1}$) to post-test (44.4 $ml \cdot kg^{-1} \cdot min^{-1}$), but this was not a statistically significant decrease. Five subjects slightly improved or maintained the relative VO_{2max} from pre-test to post-test. The same two participants who had a large decline in absolute VO_{2max} also had a large decline in relative VO_{2max} with -14 %. By excluding the two extreme outlier values, the mean relative VO_{2max} was -0.8 $ml \cdot kg^{-1} \cdot min^{-1}$ (average percent change -2 %) (n=10). Brufladt (2018) found a reduction in relative VO_{2max} on the sixth day of a prolonged fast, from 47.9 to 44.4 $ml \cdot kg^{-1} \cdot min^{-1}$. Henschel et al. (1954) found no change in relative VO_{2max} at 45 % of VO_{2max} after five days of a prolonged fast, but the absolute VO_{2max} was reduced. These latter results are in line with this present study.

Other research states that an improved body composition could promote physical capacity (Venkata Ramana et al., 2004), and a reduction in body fat is beneficial for people partaking in sports (Ackland et al., 2012). The participants in this study lost more lean mass compared to fat mass, and this could have contributed to less metabolic active tissue and thus reduced VO_{2max}. It could also be that if the participants did not have fully replenished glycogen stores after three days with re-feeding. As empty glycogen stores contribute to fatigue (Jensen et al., 2011), not fully replenished carbohydrate stores could have led to an accelerated state of fatigue in this study. However, supercompensation in liver glycogen could also have occurred after empty glycogen stores (Nilsson & Hultman, 1973), but then this energy substrate could potentially have contributed to an increased VO_{2max}. However, RER during VO_{2max} was at 1.1 on both pre-test and post-test, indicating only carbohydrate metabolism, and a normalization of this parameter after three days with re-feeding. The study of Brufladt (2018) however did report of an increased fat metabolism during VO_{2max} during fasted-state.

Along with a decrease in VO_{2max} there was also a significant reduction in maximal load ($Watt_{max}$) at the VO_{2max} test, supporting that seven days of fasting measured after three days with re-feeding reduced the physical capacity. An *improved* physical capacity would result in maintained and/or increased absolute VO_{2max} and $Watt_{max}$ on pre-test and post-test. RER and lactate during VO_{2max} remained unchanged, supporting that the subjects obtained maximal effort during both tests. As RER indicates oxidation of substrates, it is assumed that the same ratio of plasma energy

substrates was utilized on both pre-test and post-test. However, as metabolic processes such as lipogenesis and ketogenesis are disturbing RQ calculations (Frayn, 1983), the RER data may not be completely correct. The oxidation of the respective substrates may have changed after the fast, while the total sum of RER was unchanged. Unchanged lactate may indicate that carbohydrates from lactate was providing the same amount of energy on pre-test and post-test.

In the present study the heart rate (HR) increased with 5 bpm during VO_{2max} from pre-test to post-test ($p= 0.008$). As the absolute VO_{2max} decreased and HR increased, this can indicate reduced physical capacity (Skinner et al., 2003). At submaximal intensities during fasted-state an increase in HR is found in the studies of Knapik et al. (1987) and Brufladt (2018). However, Brufladt (2018) found a reduced HR during VO_{2max} during fasted-state, most likely due to dehydration, and an early on-set of fatigue.

In another study red blood cells, blood volume and plasma volume decreased during ten days of prolonged fasting, and the levels remained decreased even after days with re-feeding (Consolazio et al., 1967). As the plasma volume takes times to build up and might have been replaced by water instead of other components in the days of re-feeding, this could have contributed to a changed ratio of plasma and red blood cells in this present study. Reduced plasma and blood volume from either taking blood and from dehydration could potentially explain the increased heart rate at VO_{2max} and reduced aerobic endurance (Barr, 1999).

During the fasting intervention there was taken blood samples equivalent to one blood donation (see *appendix 6*). It is unknown if the total amount of drawn blood contributed to clinically reduced hemoglobin (Hb). As Hb and iron levels affect the oxygen transport capacity (The National Health Service, n.d.), reduced Hb and blood volume might have contributed to a reduced VO_{2max} for all subjects (Schmidt & Prommer, 2010). A greater reduction might have been in females as men have more Hb in blood, and the same amount of blood was drawn for both genders. However, it is unknown if the women experienced a greater reduction in VO_{2max} compared to men, because the sample size in this thesis would have been too low to get valid results if dividing into sub-groups.

6.2.2 Fat oxidation changes

In the present study there was a tendency towards decreased maximal fat oxidation ($fatox_{max}$) from pre-test (0.46 $g \cdot min^{-1}$) to post-test (0.39 $g \cdot min^{-1}$) (p=0.162). The slight reduction still indicates that the energy substrates utilization during sub-maximal intensity were normalized from pre-test to post-test. Mean absolute load (Watt) at $fatox_{max}$ was reduced by 13 Watt. The statistically significant reduction in load during fatox from pre-test to post-test suggests that biking was more exhausting on post-test (p=0.002). A similar result can be interpreted from the study of Brufladt (2018), where 2 of 13 participants experienced fatigue during the fat oxidation test. However, for the remaining 11 participants, Brufladt (2018) found an increased fat oxidation during fasted-state. In this present study the participants continued to decrease their fat mass during the three days with re-feeding, but this was not expressed as increased fat substrate utilization during fatox test.

In another study prolonged fasting for 3.5 days did not impair physical capacity at submaximal intensity in healthy men (Knapik et al., 1987). Brufladt (2018) found an increased HR during the fatox test, again indicating dehydration during fasting-state.

The conclusion is that seven days of prolonged fasting followed by three days of re-feeding contributed to a decline in physical performance. A reduction in VO_{2max}, loads (Watt) at both VO_{2max} and during $fatox_{max}$ indicates that the physical capacity was reduced. The relative VO_{2max} is less crucial for determining the change in physical capacity, but also this parameter was slightly reduced, though not statistically significant.

6.3 Metabolic marker changes

6.3.1 Glucose changes

Plasma glucose declines rapidly during a prolonged fast (Frayn, 2010). In the present study plasma glucose decreased steadily during the seven days of the prolonged fast and it was normalized after three days with re-feeding (see figure 15). The early fasting adaptations maintained euglycemia through glycogenolysis from day 0 to day 1.

As the liver glycogen only lasts about 24 h, glucose production through gluconeogenesis then continued to maintain the blood glucose levels (Ferrier, 2014; Nilsson & Hultman, 1973). The most striking plasma glucose drop occurred from day 1 to day 2. This is according to other literature describing the metabolic shift from glucose to the increased breakdown of fat tissue (Cahill, 1970). As the protein sparing mechanism sets in after some days of prolonged fasting (Frayn, 2010), and as fat metabolism increases and plateaus around day 5 (Balasse & Fery, 1989), the results in the present study shows a plateau of blood glucose around day 6. There was a statistically significant decrease in median blood glucose from pre-test to day 7. There was a statistically significant increase in blood glucose from day 7 to post-test as a result of re-feeding. In another study eating sufficient carbohydrates after a fast contributed to a replenishment of the glycogen stores and blood glucose within a few days (Nilsson & Hultman, 1973). The same course of events must have occurred in the participants in this study: as the blood glucose normalized along with re-feeding with no change from pre-test to post-test, this indicates a normalization in carbohydrate metabolism. Since the blood samples were measured either during fasted-state or after an overnight fast, the source of the blood glucose must be primarily from liver glycogen through glycogenolysis (Maughan, 2010). The normalization in glucose metabolism on post-test can also be seen in the RER during VO_{2max}, as the ratio is at 1.1 on both pre-test and post-test. This indicates that primary carbohydrates were the utilized energy substrate during exercise (Ulmer, 1983a).

6.3.2 Triacylglycerol changes

This study showed that there was no statistically significant change in mean triacylglycerols from pre-test, day 7 and post-test, and no statistically significant change between the means. However, there was a slight increase in mean triacylglycerols from pre-test to day 7, and a slight decrease from day 7 to post-test, but the triacylglycerols were normalized on post-test. Interestingly, as there was no statistically significant change from day 7 to post-test, this can mean that there was no clinical change in this parameter, and that it is difficult to detect increased fat oxidation by looking at triacylglycerols. It can also mean that there is in reality a change, but it was not detected with the statistical tests.

6.3.3 Free fatty acid changes

In this study free fatty acids increased significantly during the fast, and the levels were normalized after three days with re-feeding. Increased fatty acids is an indicator of fasting-induced lipolysis (Qvigstad et al., 2002). The highest increase occurred from day 1 to day 2, occurring at the same time as there was a major drop in blood glucose. This is in harmony with other literature stating that increasing levels of fatty acids will inhibit glucose uptake and glucose metabolism (Qvigstad et al., 2002). The free fatty acids continued to increase, and there was a drop on day 6 before it increased again on day 7. There was a statistically significant decrease from day 7 to post-test three days after re-feeding due to normal carbohydrate metabolism along with energy intake. There was no statistically significant change from pre-test to post-test. This can indicate that the subjects were no longer under lipolysis after re-feeding (Qvigstad et al., 2002).

6.3.4 β-hydroxybutyrate changes

In this present study β-hydroxybutyrate increased 110-fold, from 0.04 mmol/L on pre-test to 4.42 mmol/L on day 7. A 400-fold increased (from 0.01 to 4.0 mmol/L) in β-hydroxybutyrate after seven days of prolonged fasting is reported by Cahill (1970). The increasing levels in β-hydroxybutyrate during fasting state indicated the shift in energy substrate utilization to the brain occurring during fasting and ketolysis (Evans et al., 2017; Jørgensen & Holmquist, 2011). Ketone bodies are reported to reach a peak and plateau around day 5 during a prolonged fast (Balasse & Fery, 1989). In our study a rapid increase occurred from day 1 to day 3, and the plasma β-hydroxy-butyrate concentration raised steadily until the end of the fast on day 7. There was a statistically significant decrease from day 7 to post-test three days after re-feeding. The levels dropped tremendously due to re-feeding. This may be explained by participants eating sufficient glucose during re-feeding, and glucose is the preferred energy substrate utilized by the brain (Frayn, 2010). When there is increased plasma glucose various tissues will use carbohydrate (Frayn, 2010). Even though an overnight fast will reduce the glucose levels, chances are high that the glycogen stores were considerably replenished during the days of re-feeding as compared to fasted-state (Nilsson & Hultman, 1973). This in turn could increase the plasma glucose concentration during hypoglycaemia and glycogenolysis (Kolset, 2019;

Koolman & Roehm, 2013), and this would lower the utilization of β-hydroxy-butyrate. With re-feeding the levels normalized, but there was a tendency towards increased level in β-hydroxybutyrate, but no statistically significant change from pre-test to post-test.

6.3.5. T_3 changes

In the present study there was a statistically significant decrease in median T_3 from pre-test to day 7. A reduction during prolonged fasted-state is also found in other studies (Brufladt, 2018; Palmblad et al., 1977). A reduction in T_3 is also found in one study where the participants had a caloric restriction diet (Ciloglu et al., 2005). Low levels of thyroid hormones impair the metabolic rate (Frayn, 2010), and contributes to a feeling of fatigue (Bansal et al., 2015).

Furthermore, there was a statistically significant increase in T_3 from day 7 to post-test due to re-feeding. However, the levels were statistically significant reduced from pre-test to post-test. As T_3 was not fully normalized on post-test, this might indicate that the body was still under a certain amount of stress from the fast. As these hormones seem to act at a slower pace than other hormones (Frayn, 2010), it could be that T_3 takes more than three days to normalize after a fast. This result is in contrast to the study of Palmblad et al. (1977) which reported of a normalization in T_3 after a few days of re-feeding. Why the levels did not normalize in this study, is unknown.

6.4 Statistical and methodological considerations

6.4.1 Power

The power of 80 % was calculated from the chances of detecting a clinical difference in *urinary nitrogen loss,* which was the primary endpoint for the main fasting studies. However, the sample size in this thesis can question the chances of detecting an *actual clinical difference* in other endpoints presented in this thesis. The number of subjects in this thesis is also equivalent to the other NIH fasting study by Brufladt (2018) and Nilsen (2019) with n=13.

Both power and type 2 error can be influenced by a small number of participants (and falsely rejecting a "true" H_0), in addition to limit and restrict the choice of statistical tests (Pallant, 2010). Too few subjects can also influence the p-value, and make it challenging to discover big changes in the intervention (Skovlund, 2001). A larger sample size in the present study could have contributed to increased *power* and strength, especially in non-significant results (Pallant, 2010).

6.4.2 Research ethics

It was important to avoid recruiting unnecessary *"extra"* subjects to avoid unnecessary harm in the sake of health research: The research was carried out to avoid any harm on the participants, and the research followed the medical principle of *primum non nocere* (Smith, 2005). Despite the invasive test protocol, the small sample size allowed thorough follow-up of each subject.

The Ethics Committee had one remark in their approval letter: they recommended the project leader to put mental disorders as one exclusion criteriae, especially considering *eating disorders.* All participants appeared to be healthy, and they were included or excluded based on the criteria. The participants signed a letter of consent, and they filled out a decleration of health form. Participation in the project was voluntary, and the participants were at any given time allowed to withdraw their consent and exit the study. Throughout the intervention and every day during the fast the participants were in daily contact with the research staff on-site. Various physiological functions such as heart rate and blood pressure, in addition to a daily questionnaire, were recorded daily. There were also doctors available during the intervention. The project leader was responsible to stop or pause the project if unforeseen events occurred, or if the intervention was harmful for any participant (see *appendix 2).*

Every participant was given a unique ID to secure privacy and confidentiality both during the data collection and data processing. Upon request all individual data was given the respective participant.

6.4.3 Drop-outs

In this study only data from participants who completed the whole intervention were analyzed. The baseline data from the two dropouts who withdrew independent of the fast were not analyzed, only *per protocol* (participants who completed the whole study) were analyzed, and this can lead to bias (Skovlund, 2001). Not every physiological adaptation to fasting is known, but there is likely some individuals who are responding poorly to prolonged fasting, and will be at a certain risk when undergoing a fasting protocol (Stewart & Fleming, 1973). A potential scenario could be that the two dropouts happened to be subjects who responded poorly to fasting and / or had a low tolerability to fasting adaptations as there are biological variability within individuals (Stewart & Fleming, 1973). If the two drop-outs completed the intervention, we might have had other results. However, as 86 % of the recruited participants who signed the letter of consent completed the whole fasting study, this emphasize a low drop-out rate, which may add quality to the study (Lindbæk & Skovlund, 2002).

6.4.4 Criticism regarding statistical tests

Statistical tests were selected after investigating normal distribution in the data sets, and this has been *subjectively* interpreted. If the data distribution allowed it, operating with only parametric tests could have made the statistical differences more "robust", as non-parametric tests are less sensitive in finding a difference in a data set than para-metric tests (Pallant, 2010). For the Friedman Tests, post-hoc tests were performed without Bonferroni adjusted alpha value (0.05 / 3), and this might lead to more type 1 error (Pallant, 2010). Multiple endpoints without correcting for Bonferroni contributes to increased chances of *false positive findings*, which can give one in twenty tests an incorrect low p-value in every statistical test when the significance level is 5 % (Skovlund & Vatn, 2008).

In this thesis the hypotheses did include many variables, and that was a challenging part. Even though it is possible to combine many endpoints to describe an overall *goal* (Skovlund & Vatn, 2008), fewer endpoints would have made it easier to draw conclusions, and less bias.

6.4.5 Statistical versus clinical change

Statistical significance is not synonymous with a clinical significance (Lindbæk & Skovlund, 2002). A statistically significant change is not a final *proof* that there exist changes or effects after an intervention (Skovlund & Vatn, 2008). In addition, publishing only statistically significant results and not non-statistical findings will contribute to increased *false positive results* (Skovlund & Vatn, 2008).

As there have been many various parameters and tests in this thesis, I have not succeeded to find clinical reductions for all parameters. The parameters were selected before any statistical analysis were performed, and none of the parameters has been excluded after analysis.

6.4.6 Generalizability

External validity could be strengthened if we had more participants, as the results derived from the study are representing the similar population: because of this, the data cannot necessarily be put to use in another group or population, e.g. overweight subjects or patients with other metabolic risks wanting to perform therapeutic prolonged fasting. This indicates low generalizability (Lindbæk & Skovlund, 2002) (Skovlund, 2001). Even though there does not exist much data on healthy, normal-weight subjects performing prolonged fasts, this study has a low generalizability to other groups besides the subjects that were tested. This can question if the results from this study are transferable to other populations who potentially were rejected from partaking in the study.

6.4.7 Sub-groups

For results and statistics, dividing the participants into sub-groups by gender could have been performed, as the physiology usually differs in terms of body composition and physical capacity. However, this would have led to even smaller sample size than n=12, and contribute to less credible and reliable data: As smaller sample sizes leads to reduced power which might not detect important changes in effect, and many statistical tests in many potential sub-groups will lead to more p-values, this would have increased the chances of *false positive findings* (Skovlund & Vatn, 2008).

6.5.8 Outliers

The aim of the inclusion and exclusion criteria were to screen for a healthy, heterogenerous group. One subject who had a higher BMI and was heavier than the others, was included (see *figure 10* and *figure 11*). This person was considered healthy and its body adapted in the same way as the rest of the participants, but its result may have influenced various endpoints in this study: This subject was on the upper percentile on body weight, fat mass and fat percentage, which contributed to an increase in the mean for certain normally distributed data variables. Its data could have influenced the choice in statistical tests as this person could have contributed to not normally distributed data. Accordingly, in non-parametric distributions the median was not too affected by this outlier.

However, the body composition and physical capacity scores for the group were quite spread, which raises the question if group was in reality heterogenous *enough.* By having an inclusion criterion for scores on physical capacity, the participants could potentially have been more unison, but recruitment would be more time consuming and surpass the planned time schedule for testing. This would also affect the generalizability. Accordingly, two subjects had a relatively large decline in physical capacity as a result of the fast, and their reduction contributed to an overall reduction in the group: Median absolute VO_{2max} was not too sensitive to the outlier results, but the outliers influenced much of relative VO_{2max} presented in mean.

6.4.9 Missing data

For certain blood variables there are unfortunately missing data in one or more data sets. The reason for this was either problems taking enough blood samples at the respective time point. For T_3 only 6 samples were analyzed on post-test. This was a human mistake because the blood tests were not requested. In addition, Friedman test doesn't include variables with missing data: the test only calculates with the minimum number of missing data, and the full sample size is not included in the statistical analysis even though the sample size is more on other time points. This was the case with T_3, as it only had six participants included in the statistics because of the Friedman Test. Low power on this parameter could also contribute to a skewed result if perhaps one or more participants responded poorly to fasting, and this led to an overall reduction on post-test.

5 of the baseline scans on DXA turned out to be “estimated”, meaning that the person wasn´t laying correct on the scanning table within the reference line, and a full body scan was not performed properly. In turn the program “estimated” mass and data by looking at the other half of the body which had been fully measured. 7 participants had two successful baseline scans. For the 5 participants with only one successful baseline scan, only the correct scan was included in baseline data for pre-test. This should be considered as a source to bias.

6.4.10 Validity of the instruments

The results from *familiarization test* was calculated for intensities for VO_{2max} for both pre- and post-tests. As familiarization test was calculated at baseline, and there was no familiarization test *prior* to the post-test, operating with identical loads and intensities of VO_{2max} calculated at *baseline* can question the validity of the post-results. Using the same loads and estimates for VO_{2max} on both tests could have been more critically considered, and relative loads and intensities could have been performed. In reality the physical capacity test protocol could potentially be *more exhausting* on the post-test if the participants reduced their fitness levels. If the participants started at a higher intensity on the second test compared to the first test, fatigue could have occurred earlier. A reduction in maximal load during fat oxidation test and $Watt_{max}$ suggests that biking was more exhausting on the post-test, perhaps due to over-estimated baseline values. However, using the same data could potentially contribute to greater compliance.

The daily weight and body composition parameters were measured with different instruments. Therefore, the results could not be directly matched as the numbers are not accurate.

Another bias can be that the research team consisted of a few staff members. Rotations in working-days could have influenced variations in verbal encouragement on the physical tests and this is potentially a source to bias: adaptations to the familiarization test and learning of the test protocol were needed as the first test at baseline was believed to contribute to improvement due to adaptation, but e.g. cheering on one test and less encouraging on the other test could have led to skewed results.

6.4.11 Data not included in the thesis

Many relevant parameters from the complete fasting protocol have not been included in the thesis. As the Dexcom data were not analyzed it is unknown if participant ingested in carbohydrates during the fasting period, as this would be detected by a rise in blood glucose. Furthermore, Actiheart data were not analyzed in this thesis. The intervention could have contributed to a reduced overall activity level for some participants who tried to avoid reinforced fatigue and increased energy expenditure, which in turn could have contributed to a decline on the physical capacity test(s). The research staff encouraged normal activity during the intervention, except a restriction of low-moderate activity two days prior to the physical tests as this would reduce the liver glycogen and influence the physical capacity test. It is unknown if the participants complied to this restriction of low-moderate activity before the pre-test and post-test.

6.5 Conclusion

6.5.1 Answering of the hypotheses

The first hypothesis: Body weight, lean mass and fat mass changes from pre-test, to day 7, to post-test. All the post hoc-analyses showed statistically significant changes between each occasion ($p < 0.05$), except for body fat from day 7 to post-test. All the body composition parameters showed statistically significant changes between the three occasions ($p < 0.001$). The results from this study support this hypothesis.

The second hypothesis: VO_{2max} and $fatox_{max}$ changes from pre-test to post-test. Absolute VO_{2max} showed statistically significant reduction ($p = 0.002$). There was a tendency towards reduced relative VO_{2max} and $fatox_{max}$, but these changes were not statistically significant ($p > 0.05$). The results from this study partially support this hypothesis.

The third hypothesis was that blood concentrations of hormones and metabolites considered markers of metabolic health changes from pre-test, to day 7, to post-test.

Blood glucose, free fatty acids, β-hydroxybutyrate and T_3 showed statistically significant changes between the three occasions ($p < 0.05$), but there was no statistically significant change in triacylglycerols between the three occasions ($p > 0.05$). The post hoc-analyses for blood glucose, triacylglycerols, free fatty acids and β-hydroxybutyrate showed normalization (no statistically significant change) from pre-test to post-test three days after re-feeding ($p > 0.05$). Only T_3 metabolite remained statistically significant reduced from pre-test to post-test ($p=0.027$). The results from this study mainly support this hypothesis.

6.5.2 Summary

This study has contributed to more knowledge on how the body responds to a prolonged fast followed by three days with re-feeding in healthy, normal-weight participants. The fast led to reductions in all body composition parameters. A large proportion of lean mass was lost, increasing the risk of sarcopenia. Three days with re-feeding contributed to an increase in weight and lean mass due to fluid absorption and content in intestines, whereas body fat continued to slightly decrease along with re-feeding. The body weight was reduced by 4 % after three days with re-feeding. Physical capacity was reduced. The fast contributed to changes in most of the blood markers, and most of these markers normalized on post-test due to three days with re-feeding. These results may indicate that a prolonged fast followed by re-feeding does not contribute to harmful adaptations in normal-weight subjects. A prolonged fast may be one preventative strategy to overcome the overweight epidemic as it reduces body weight and body fat in normal-weight people. However, a substantial amount of muscle mass will be lost. The fasting intervention was well tolerated in the participants, but the strategy might not be beneficial for the metabolic health.

6.5.3 Implications for future studies

Data already collected from the two fasting studies will be analyzed. The health sector may benefit from gaining more knowledge on various strategies and tools which can help to prevent and reduce the risk of overweight in the population. In normal-weight participants more long-term consequences of prolonged fasting on metabolic health may be investigated, especially the adaptation in fat mass. Thus, more fasting studies will lead to more knowledge on fasting physiology and how the body works in healthy, normal-weight people.

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8. Appendices

Appendix 1. Ethics committee REC evaluation

REK
REGIONALE KOMITEER FOR MEDISINSK OG HELSEFAGLIG FORSKNINGSETIKK

Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK sør-øst	Mariann Glenna Davidsen	22845526	29.06.2017	2017/1052 REK sør-øst B
			Deres dato:	**Deres referanse:**
			09.05.2017	

Vår referanse må oppgis ved alle henvendelser

Jørgen Jensen
Norges idrettshøgskole

2017/1052 Effekt av trening på tap av muskelprotein under faste

Vi viser til søknad om forhåndsgodkjenning av ovennevnte forskningsprosjekt. Søknaden ble behandlet av Regional komité for medisinsk og helsefaglig forskningsetikk (REK sør-øst) i møtet 07.06.2017. Vurderingen er gjort med hjemmel i helseforskningsloven (hfl.) § 10.

Forskningsansvarlig: Norges idrettshøgskole
Prosjektleder: Jørgen Jensen

Prosjektleders prosjektbeskrivelse
"*Prosjektet er designet for å se om ulike treningsformer under syv dager faste kan redusere tap av muskelmasse. For å måle tapet av muskelprotein vil all urin bli oppsamlet i fasteperioden og analysert for nitrogen og andre metabolitter. Prosjektdesignet er randomisert kontrollert studie, med to intervensjonsgrupper og én kontrollgruppe. Det er vist at man taper muskelmasse ved faste, og det er også vist at trening kan opprettholde muskelmasse ved vektreduksjon. Derfor er det interessant å se om trening kan ha en effekt på muskelmassen ved faste. De fleste studier har fastet forsøkspersonene i opptil tre dager, denne studien kan derfor gi ny kunnskap om effekten av syv dagers faste på både muskelmasse og vektreduksjon.*"

Komiteens vurdering
Formålet med denne studien å undersøke om «*ulike treningsformer under syv dager faste kan redusere tap av muskelmasse*». Prosjektet skal rekruttere friske deltakere, og hensikten med studien fremstår som idrettsfysiologi.

Helseforskningsloven gjelder for medisinsk og helsefaglig forskning, det vil si «*virksomhet som utføres med vitenskapelig metodikk for å skaffe til veie ny kunnskap om helse og sykdom*», jf. helseforskningsloven § 2, jf. § 4. Komiteen anser ikke at prosjektets formål omfattes av helseforskningslovens virkeområde, og kan derfor ikke ta stilling til hvorvidt de fire spørsmålene skal være med i prosjektet eller ikke. Det kreves ingen forhåndsgodkjenning fra REK for å gjennomføre prosjektet.

Vedtak
Etter søknaden fremstår prosjektet ikke som medisinsk eller helsefaglig forskning, og det faller derfor utenfor helseforskningslovens virkeområde, jf. § 2.

Klageadgang
Du kan klage på komiteens vedtak, jf. forvaltningslovens § 28 flg. Klagen sendes til REK sør-øst B.

Besøksadresse: Gullhaugveien 1-3, 0484 Oslo
Telefon: 22845511
E-post: post@helseforskning.etikkom.no
Web: http://helseforskning.etikkom.no/

Klagefristen er tre uker fra du mottar dette brevet. Dersom vedtaket opprettholdes av REK sør-øst B, sendes klagen videre til Den nasjonale forskningsetiske komité for medisin og helsefag for endelig vurdering.

Komiteens avgjørelse var enstemmig.

Med vennlig hilsen

Grete Dyb
professor, dr. med.
leder REK sør-øst B

Mariann Glenna Davidsen
rådgiver

Kopi til:
- *Norges idrettshøgskole ved øverste administrative ledelse*
- *Avdelingsleder Turid Sjøstedt, Norges idrettshøgskole*

Appendix 2. Ethics committee NIH approval

Jørgen Jensen
Seksjon for fysisk prestasjon

OSLO 28. august 2017

Søknad 15-220817 – Effekt av trening på tap av muskelprotein

Vi viser til søknad, prosjektbeskrivelse, informasjonsskriv og innsendt søknad til NSD.

I henhold til retningslinjer for behandling av søknad til etisk komite for idrettsvitenskapelig forskning på mennesker, ble det i komiteens møte av 22. august 2017 konkludert med følgende:

Vedtak

På bakgrunn av forelagte dokumentasjon finner komiteen at prosjektet er forsvarlig og at det kan gjennomføres innenfor rammene av anerkjente etiske forskningsetiske normer nedfelt i NIHs retningslinjer. Til vedtaket har komiteen lagt følgende forutsetning til grunn:

- *At NSD godkjenner prosjektet og at eventuelle vilkår fra NSD følges*
- *At det etableres nødvendige avtaler med samarbeidende forskningsinstitusjoner.*

Ta kontakt med Avdeling for forskning og bibliotek for bistand med avtaler.

Prosjektet innebærer en tydelig inngripen i prosjektdeltakernes liv. Komiteen vil anmode prosjektleder å vurdere å ta inn psykiske lidelser som eksklusjonskriterium, bla mht spiseforstyrrelse, og understreker prosjektleders ansvar for å ivareta forskningsetiske forhold i hele prosjektperioden. Som prosjektleder har du også ansvar for eventuelt å stoppe prosjektet helt eller delvis dersom det oppstår situasjoner som har eller kan ha betydelige skadepotensial for forskningsdeltakerne.

Komiteen gjør videre oppmerksom på at vedtaket er avgrenset i tråd med fremlagte dokumentasjon. Dersom det gjøres vesentlige endringer i prosjektet som kan ha betydning for deltakernes helse og sikkerhet, skal dette legges fram for komiteen før eventuelle endringer kan iverksettes.

Med vennlig hilsen
Professor Sigmund Loland
Leder, Etisk komite, Norges idrettshøgskole

NIH NORGES IDRETTSHØGSKOLE
Besøksadresse: Sognsveien 220, Oslo
Postadresse: Pb 4014 Ullevål Stadion, 0806 Oslo
Telefon: +47 23 26 20 00, postmottak@nih.no
www.nih.no

Appendix 3. Letter of consent

NIH NORGES IDRETTSHØGSKOLE

Forespørsel om deltagelse som forsøksperson

Effekt av faste på muskelmasse, styrke og utholdenhet

Bakgrunn og hensikt
Dette er et spørsmål til deg om å delta i et forskningsprosjekt som skal undersøke hvorvidt syv dagers faste vil påvirke muskelmasse, muskelstyrke og utholdenhet.

Overvekt og fedme er et økende problem både i Norge og på verdensbasis. Overvekt oppstår når kroppens fettlagre er blitt for store som følge av for høyt energiinntak over lang tid. For å gå ned i vekt er det nødvendig å redusere matinntaket slik at kroppen benytter seg av disse fettlagrene som energi. For å redusere energiinntaket, er det utviklet flere strategier som kan innebære perioder med faste av ulik lengde (f.eks. 5:2 dietten, eller intermittent fasting). Ved vektnedgang som følge av faste, vil både kroppens fettlagre og muskelmasse reduseres. I de første dagene av faste vil kroppen bruke opp glykogenlagrene i lever og bryte ned muskelproteiner, samtidig som noe fett oksideres. Etter ca. to-tre dager vil kroppen i hovedsak bruke fett som energi. Det er få studier som undersøker hvorvidt faste av lengre varighet påvirker fysisk prestasjon og tap av muskelmasse. Formålet med prosjektet er derfor å undersøke hvorvidt langvarig faste vil påvirke muskelmasse, muskelstyrke og utholdenhet.

Hva innebærer studien?
Dersom du ønsker å delta kreves det at du faster i syv dager i strekk. Det er kun tillatt å innta vann. Du skal møte opp på Norges idrettshøgskole hver dag under fasten for blodprøver og andre tester. I tillegg må du møte opp til noen fysiske tester før og etter studien. Det vil bli tett oppfølging under hele perioden.

Testing
Testene som vil bli gjennomført i løpet av perioden er test av hvilemetabolisme, kroppssammensetning (DEXA), muskel- og fettbiopsi, blodprøver, reaksjonstest, fettoksideringskapasitet, maksimal styrke, maksimalt oksygenopptak (VO2maks), oral glukosetest, samt innsamling av urin.

Intervensjon
Når testene før fasteperioden (pretest) er gjennomført, starter fasten som varer i syv

dager. Posttest blir på slutten av fasten. Som deltaker vil du bli oppfordret til å holde et så normalt trenings- og aktivitetsmønster som mulig. Hver morgen skal du møte på NIH for blodprøver.

Det vil også bli gjennomført tester på reaksjonsevne, samt utfylling av et spørreskjema. I tillegg skal du samle opp all urin i fasteperioden. Som forsøksperson skal du til enhver tid under fasten bære en aktivitetsmåler og en glukosemåler.

Mulige fordeler og ulemper
Som deltaker vil du oppleve sultfølelse, spesielt de første 24 timer. Det er imidlertid tidligere rapportert om lite til ingen sultfølelse under faste av lengre varighet. Sultfølelsen avtar vanligvis etter ca. 36 timer. Du vil troligvis få relativt lavt blodsukker etter et par dager, noe som kan gjøre deg periodevis sliten, trøtt og ukonsentrert. Dette kan også gå på bekostning av humør og energinivå. Aktivitet og trening kan oppleves som tung som følge av lavt energiinntak. Det vil bli tatt muskel- og fettbiopsier, laktatprøver og blodprøver, noe som kan gi ubehag og smerte i området rundt penetrering. Testene av maksimalt oksygenopptak, power og muskelstyrke er til utmattelse, og vil oppleves som maksimalt anstrengende.

Som forsøksperson vil du få detaljert informasjon om dine fysiologiske forutsetninger og kvaliteter. Du vil få vite ditt maksimale oksygenopptak, din evne til å oksidere fett, din maksimale power og maksimal kraftproduksjon i muskulatur, hvilemetabolisme, og kroppssammensetning. Forsøkspersonene vil bli tett fulgt opp. I tillegg kan det ordnes veiledet trening av instruktører ved NIH under prosjektperioden. Du vil få innblikk i hvordan et forskningsprosjekt gjennomføres.

Frivillig deltakelse
Dersom du ønsker å delta signerer du på vedlagt samtykkeskjema. Du kan når som helst trekke deg fra studien uten å oppgi grunn, og uten konsekvenser. Du kan også kreve at all informasjon om deg blir slettet, med mindre informasjonen allerede er brukt i analyser eller publisert i vitenskapelige artikler. Dersom du har spørsmål vedrørende studien kan du kontakte professor Jørgen Jensen jorgen.jensen@nih.no, masterstudent Victoria Frivold 94486863 / s.v.frivold@studmed.uio.no eller Steffen J. Brufladt 92622429 / steffen.brufladt@gmail.com .

Hva skjer med informasjonen om deg?
Informasjonen som registreres vil bli behandlet uten navn og fødselsnummer, eller andre direkte gjenkjennende opplysninger. Det vil brukes nummererte koder i stedet for navnet ditt. Alle data vil bli behandlet anonymt og ingen bortsett fra deg og testlederne kan knytte dataene tilbake til deg. Det vil derfor ikke være mulig å identifisere deg i resultatene i studien når disse publiseres.

Ytterligere informasjon om studien finnes i kapittel *A* – *utdypende forklaring av hva studien innebærer*.

Ytterligere informasjon om biobank, personvern og forsikring finnes i kapittel *B* *– personvern, biobank, økonomi og forsikring*.

Samtykkeerklæring følger etter kapittel B.

Kapittel A – Utdypende forklaring om hva studien innebærer

Kriterier for deltakelse i studien

A. Inklusjonskriterier:

- 18-45 år
- BMI mellom 22-30
- Minimum 12% kroppsfett for menn, minimum 15% kroppsfett for kvinner
- Fysisk og psykisk friske personer

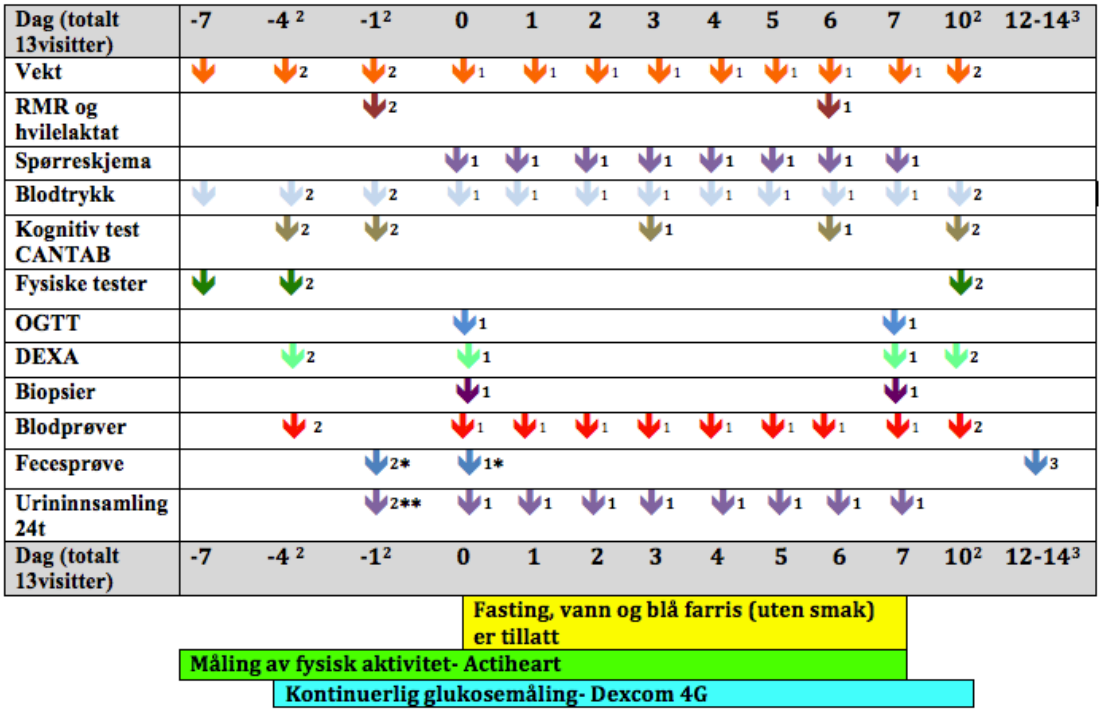
B. Eksklusjonskriterier:

- Ingen sykdommer, som f.eks. hjerte- og karsykdom, diabetes og andre sykdommer som har innvirkning på metabolismen
- Røyker
- Bruk av medisiner

Bakgrunn og hensikt med studien – utdyping av variabler som skal måles
Mange ønsker å gå ned i vekt av helsemessige grunner. Flere metoder for vektnedgang innebærer perioder med fasting av ulik lengde (f.eks. 5:2-dietten, intermittent fasting, osv.). Ved lengre perioder med fasting (syv dager) vil man de første dagene miste en del muskelprotein, men etterhvert vil kroppen i hovedsak gå over til å oksidere fett. Dersom fasten varer i kortere perioder (5:2-dietten), vil kroppen i større grad bryte ned muskelprotein under fasten.

Få studier undersøker hvorvidt langvarig faste påvirker fysisk prestasjon og tap av muskelmasse. Formålet med prosjektet er derfor å undersøke hvordan faste vil påvirke muskelmasse, muskelstyrke og utholdenhet.

Det skal gjennomføres syv dager med sammenhengende faste. Det er kun lov å drikke vann og farris uten smak under fasten. Én uke før fasten må man gjennom tilvenningstester, der det også blir delt ut aktivitets- og glukosemåler. Aktivitets- og glukosemåler skal bæres av forsøkspersonene under hele prosjektet. Det gjennomføres pre-tester før fasten starter. Etter fasten gjennomføres det post-tester. Forsøkspersonene *må* møte på Norges idrettshøgskole hver dag for blodprøvetaking og andre tester, se figur for detaljert informasjon:



Figur 1: Oversikt over fastestudien med 13 visitter. Hver pil indikerer hvilken test som skal tas den respektive dagen. Fargekoder er ment for å skille ulike tester. Dag 0-7 er fasten, dag -7 til -1 er pre-intervensjon, og dag 10 og 12-14 er post-intervensjon.

[1] Fasteintervensjon
[2] Forsøksperson møter fastende for pre- og post-tester
[3] Én prøve leveres 12-14 dager etter dag 0, dvs. ca. én uke etter fasteslutt
* Bare én fecesprøve er tilstrekkelig før fasten starter på dag 0
** 24t urin tas fra morgen før visitt og medbringes til NIH på dag-1

Tilvenningstest:

Det vil bli gjennomført tilvenning av power-, styrke-, fettoksideringskapasitet- og VO_{2maks}- test én uke før fasten starter. Gjennomkjøring av testene har til hensikt å gjøre forsøkspersonene kjent med testene som skal gjennomføres senere.

Hvilemetabolisme

Med hvilemetabolisme menes hvor mye energi kroppen forbruker i hvile. Forsøksperson må møte opp fastende om morgenen, og skal bevege seg så lite som mulig før testen. Testen gjennomføres ved at forsøkspersonen ligger helt i ro på en madrass i ca. 20 min, med en oksygenmaske plassert over nese og munn. Hvilemetabolismen estimeres ved at man leser av oksygenopptaket under fullstendig hvile, og deretter regner ut energiforbruket.

Kroppssammensetning
Måling av kroppssammensetning ved hjelp av en Dual Energy X-ray Absorptiometry (DEXA). Maskinen kan skille mellom beinmasse, fettmasse og fettfri masse. Det vil bli gjennomført to målinger før faste og to målinger ved slutten av fasten. DEXA brukes for å gi detaljerte antropometriske data.

Biopsi
Vevsprøve tas av lårmuskelen (vastus lateralis) og subkutant fett i mageregionen. Biopsi tas før fasten begynner, og etter syv dagers faste. I muskelbiopsiene vil det bli målt glykogen, samt transkripsjon og ekspresjon av proteiner involvert i metabolisme av ketonlegemer. I fettbiopsiene vil det bli målt ekspresjon av proteiner involvert i metabolisme av fett. Ca. 150 mg muskelvev innhentes ved hver biopsi. Biopsiene fryses i flytende og lagres i en fryser som holder -80°C.

Styrke
Test av maksimal isokinetisk kraftutvikling i lårmuskulatur (quadriceps) ved tre forskjellige vinkelhastigheter (60 °/s, 120 °/s og 180 °/s), og maksimal isometrisk kraftutvikling ved 60 ° i dynamometer. 5-10 min oppvarming på ergometersykkel gjøres før test. I tillegg vil det bli tre oppvarmingsrepetisjoner før hver vinkelhastighet, samt to isometriske oppvarmingsrepetisjoner før maksimal isometrisk test.

Fettoksideringskapasitet og maksimalt oksygenopptak (VO2maks)
Forsøkspersonene skal sykle på ergometersykkel i 5 min på 25% av VO_{2maks} som oppvarming. Deretter følger økning i belastning hvert 3 minutt tilsvarende 30 %, 40 %, 50 %, 60 % 70 % og 80 % av VO_{2maks}. Fettoksideringstesten er oppvarming til power-testen (beskrevet under) og VO_{2maks}-testen. Under VO_{2maks}-testen starter forsøkspersonene på terskelbelastning og øker med omtrent 25 W hvert minutt til utmattelse. Det tas tre blodprøver i forbindelse med fettoksidasjonstestene.

Sprint (power)
Test av maksimal power (Watt) over en periode på 10 s. Gjennomføres sittende på ergometersykkel. Det skal gjøres en maksimal spurt på 10 s. Testen gjøres rett etter fettoksideringstesten og før VO_{2maks}-testen.

Blodprøver
Blodprøver (4-5 rør) tas hver dag. Formålet er å se på forskjellige hormoner, proteiner og metabolitter som påvirkes av faste.

Nitrogenbalanse i urin
All urin under fasteperioden samles inn og leveres når man ankommer på morgenen for blodprøver. Formålet med innsamlingen av urin er å få et mål på proteinnedbrytning under fasten.

Feces

Avføringsprøve leveres før og etter faste. Formålet er å se på tarmbakterier og tarmflora.

Aktivitetsmåler
Forsøkspersoner skal gå med aktivitetsmåler over en periode på syv dager før fasten, og gjennom hele fasten. Formålet er å registrere om aktivitetsmønsteret til forsøkspersonene endres gjennom fasten.

Glukosemåling
Forsøkspersoner skal bære en sensor (Dexcom G4) som kontinuerlig måler blodglukosekonsentrasjonen.

Oral glukosetoleransetest
Gjennomføres før fasten starter, og rett før fasten avsluttes på dag syv av fasten. 75 g glukose blandet i vann blir inntatt oralt. Blodprøver tas før inntak, og 15, 30, 60, 90, 120, 180 min etter inntak for å måle glukose, insulin og andre hormoner.

Reaksjonstest
Omtrent annenhver dag skal forsøkspersonene gjennom en kort reaksjonstest. Denne testen skal kartlegge kognitiv funksjon og reaksjonsevne under fasteperioden.

Visuell analog skala (VAS) spørreskjema
Hver dag under fasten må det fylles ut et spørreskjema med omtrent 12 enkle spørsmål. Hensikten er å overvåke blant annet motivasjon og sultfølelse hos forsøkspersonene.

Kapittel B - Personvern, biobank, økonomi og forsikring

Personvern
Opplysninger som registreres om deg er kroppssammensetning, vekt, høyde, maksimal styrke, alder, og resultater fra de fysiologiske testene.

Professor Jørgen Jensen er daglig ansvarlig for prosjektet, og Norges idrettshøgskole ved administrerende direktør er databehandlingsansvarlig. Datamaterialet vil bli benyttet av forskere og masterstudenter ved Norges Idrettshøgskole. I blodprøver vil det bli analysert forskjellige hormoner, peptider og metabolitter. Biopsier vil bli analysert for glykogen, mRNA, og ekspresjon og aktivitet av proteiner. Analyser vil bli foretatt i Norge og av samarbeidspartnere i Danmark og Storbritannia.

Muskelbiopsier vil bli analysert ved University of Sydney, Australia etter godkjent avtale (Standardkontrakt art.46). Kontrakten vil være tilgjengelig ved kontakt av prosjektleder Jørgen Jensen.

Prosjektslutt er 31.08.2027. Data oppbevares til 31.08.2032 av

dokumentasjonshensyn.

Biobank
Blodprøvene som blir tatt og informasjonen utledet av dette materialet vil bli lagret i en forskningsbiobank ved Norges idrettshøgskole. Hvis du sier ja til å delta i studien, gir du også samtykke til at det biologiske materialet og analyseresultater inngår i biobanken. Professor Jørgen Jensen er ansvarshavende for forskningsbiobanken. Etter publikasjon av vitenskapelige artikler vil materiale destruert etter interne retningslinjer.

Rett til innsyn og sletting av opplysninger om deg og sletting av prøver
Dine rettigheter
Så lenge du kan identifiseres i datamaterialet, har du rett til:

- innsyn i hvilke personopplysninger som er registrert om deg,
- å få rettet personopplysninger om deg,
- få utlevert en kopi av dine personopplysninger (dataportabilitet), og
- å sende klage til personvernombudet eller Datatilsynet om behandlingen av dine personopplysninger

Hva gir oss rett til å behandle personopplysninger om deg?

Vi behandler opplysninger om deg basert på ditt samtykke.

På oppdrag fra Norges Idrettshøgskole har NSD- Norsk senter for forskningsdata AS vurdert at behandlingen av personopplysninger i dette prosjektet er i samsvar med personvernregelverket.
Studien og biobanken er finansiert gjennom forskningsmidler fra Norges idrettshøgskole.

Samtykke til deltakelse i studien

"Effekten av faste på muskelmasse, styrke og utholdenhet"

Jeg er villig til å delta i studien

(Signert av prosjektdeltaker, dato)

Jeg bekrefter å ha gitt informasjon om studien

(Signert, rolle i studien, dato)

Appendix 4. Declaration of health form

Egenerklæring for forsøkspersoner

Etternavn:	Fornavn:
Fødselsdato:	
E-post:	
Tlf.:	
FP nr.	
Idrettsbakgrunn (angi omtrent hvor mange timer du trener per uke):	

Takk for at du vurderer å delta som forsøksperson ved Norges idrettshøgskole! Før du kan delta, må vi imidlertid kartlegge om din deltakelse kan medføre noen form for helserisiko. Vær snill å lese gjennom alle spørsmålene nøye og svar ærlig ved å krysse av for JA eller NEI. Hvis du er i tvil, bør du be om å få snakke med legen som er ansvarlig for forsøket.

Hvis du krysser av for JA på ett eller flere av disse spørsmålene, må du gjennomgå en legeundersøkelse før forsøksstart.

Spørsmål	JA	NEI
1. Kjenner du til at du har en hjertesjukdom?		
2. Hender det du får brystsmerter i hvile eller i forbindelse med fysisk aktivitet?		
3. Kjenner du til at du har høyt blodtrykk?		
4. Bruker du for tiden medisiner for høyt blodtrykk eller hjertesjukdom? (f.eks. vanndrivende midler)?		
5. Har noen av dine foreldre, søsken eller barn fått hjerteinfarkt eller dødd plutselig (før fylte 55 år for menn og 65 år for kvinner)?		
6. Røyker du?		
7. Har du besvimt i løpet av de siste seks månedene?		
8. Hender det du mister balansen på grunn av svimmelhet?		
9. Har du sukkersjuke (diabetes)?		
10. Får du allergiske eller hypersensitive reaksjoner av bedøvelse?		
11. Kjenner du til noen annen grunn til at din deltakelse i prosjektet kan medføre helse- eller skaderisiko?		

Gi beskjed straks dersom din helsesituasjon forandrer seg fra nå og til undersøkelsen er ferdig, f.eks. ved at du blir forkjølet eller får feber.

_____

Sted – dato

_____

Underskrift

Appendix 5. Rationale statistical tests. Histograms for two selected variables

1) Absolute VO_{2max} ($ml \cdot min^{-1}$)

The baseline data for absolute VO_{2max} was assumed to not be normal-distributed. The change from pre-test to post- test was assumed to not be normal-distributed either. The variable did not qualify for using a parametric test, and Wilcoxon Signed Rank Test was used to investigate the change at the two occasions.

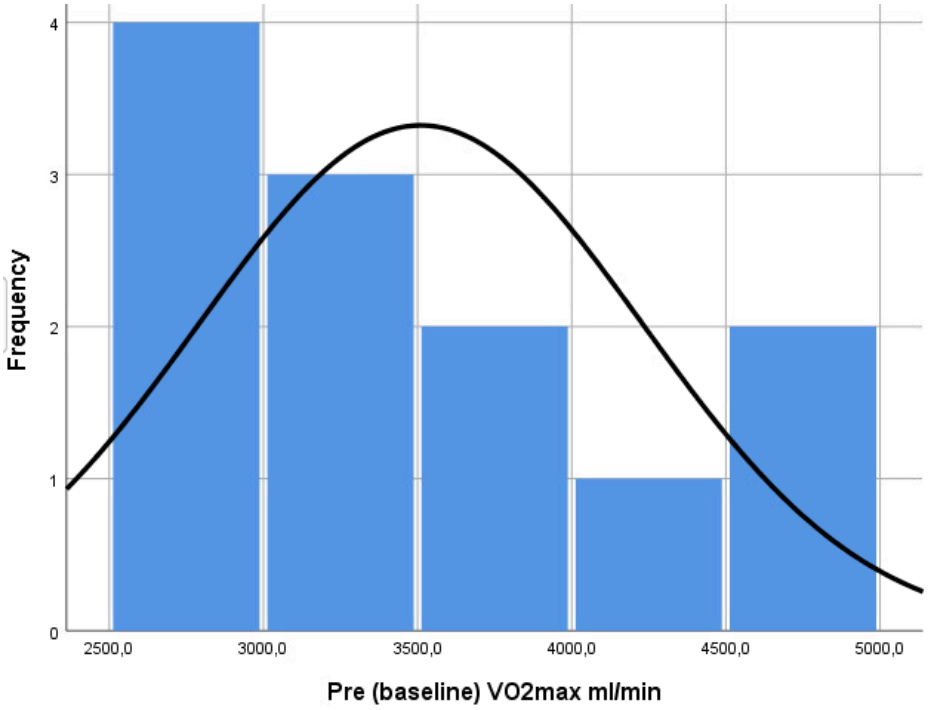


Figure 1. *The histogram shows VO_{2max} ($ml \cdot min^{-1}$) at baseline. The y-axis represents the number of participants, and the x-axis describes the scores on VO_{2max} (n=12).*

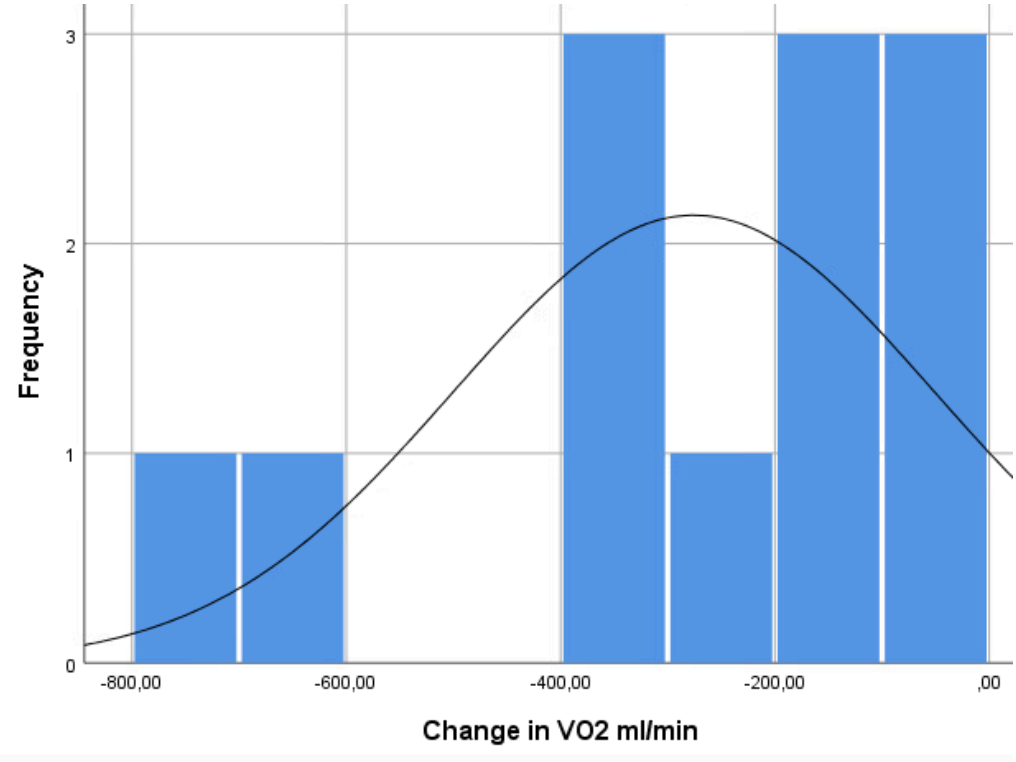


Figure 2. *The histogram shows the change in VO_{2max} ($ml \cdot min^{-1}$) from pre-test to post-test. The y-axis represents the number of participants, and the x-axis describes the change scores in VO_{2max} (n=12).*

2) Relative VO_{2max} ($ml \cdot kg^{-1} \cdot min^{-1}$)

The baseline data for relative VO_{2max} was assumed to be normal-distributed. The change from pre- to post- test was assumed to be normal-distributed: Mean change (-1,78 $ml \cdot kg^{-1} \cdot min^{-1}$) and median change (-2,05 $ml \cdot kg^{-1} \cdot min^{-1}$) were compared, in addition to investigating the scores on the QQ-plot. The variable did qualify for using a parametric test, and Student T-test was used to investigate the change at the two occasions.

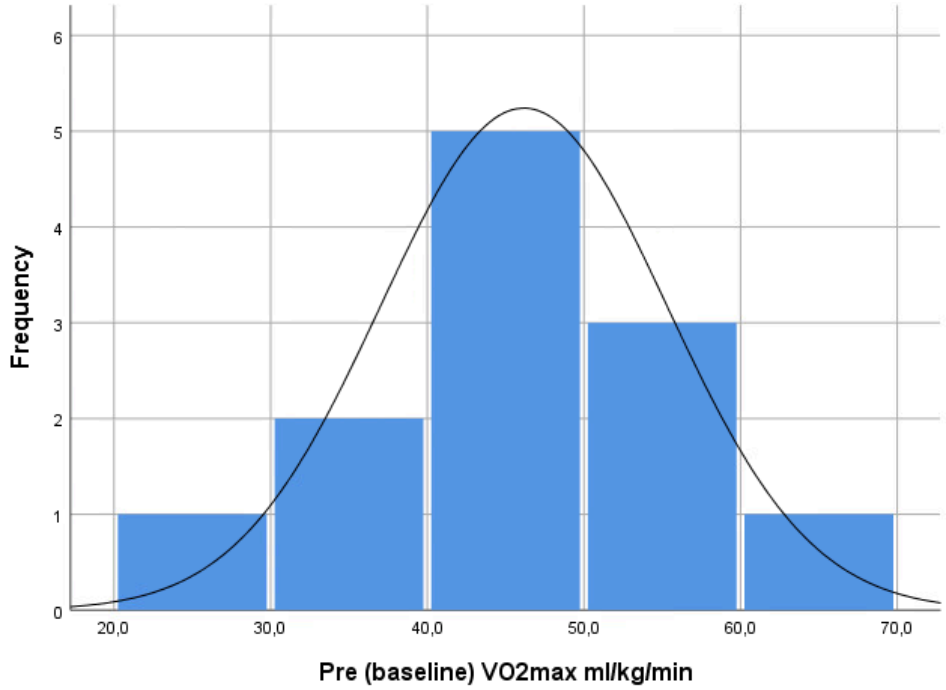


Figure 3. *The histogram shows VO_{2max} ($ml \cdot kg^{-1} \cdot min^{-1}$) at baseline. The y-axis represents the number of participants and the x-axis describes the scores on VO_{2max} (n=12).*

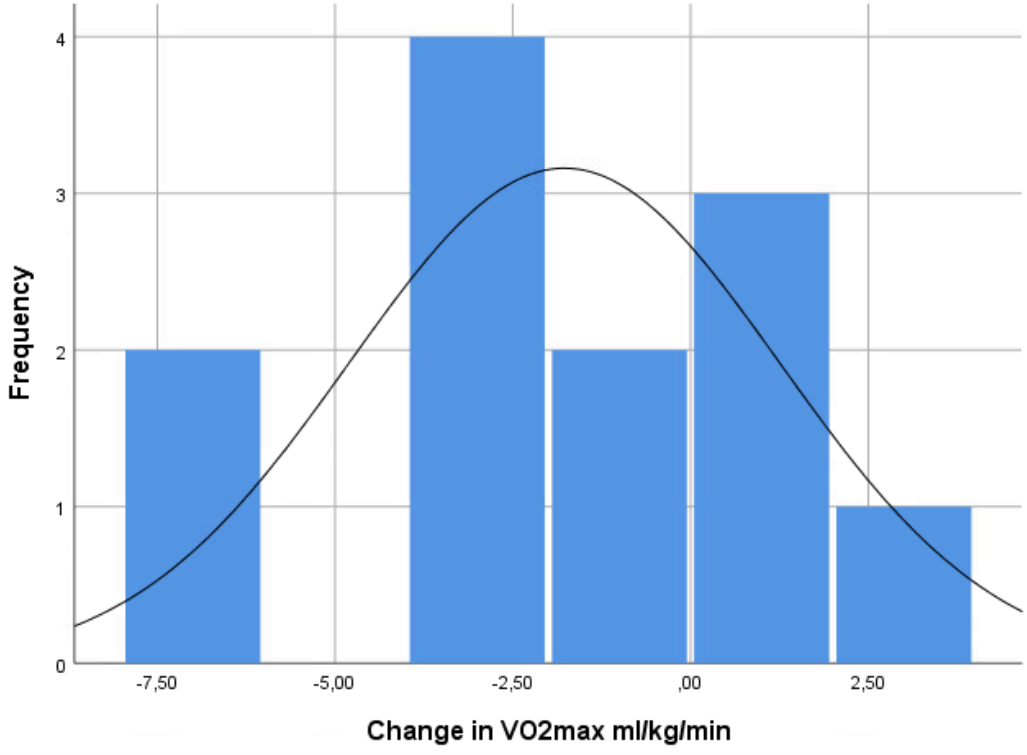


Figure 4. *The histogram shows the change in VO_{2max} ($ml \cdot kg^{-1} \cdot min^{-1}$) from pre-test to post-test. The y-axis represents the number of participants, and the x-axis describes the change scores in VO_{2max} (n=12).*

Appendix 6. Blood tests protocol

1) Morning blood

ID:
Dato/klokkeslett:

Rekkefølge rør	Alikvoter plasma/serum	Kommentarer
5 ml Serum m/gel	4 x 500 µl	Koaguleres 30 min i romtemp
5 ml Heparin m/gel	4 x 500 µl	**Plasseres på is**, sentrifugeres med en gang.
6 ml EDTA	4 x 500 µl	**Plasseres på is**, sentrifugeres med en gang. Buffycoat pipetteres av til 1,5 ml eppendorf rør

Dato/sign:

1. Klokkeslett for prøvetaking **skal** skrives opp
2. Jobb på is hele tiden
3. Etter sentrifugering plasseres prøverør i rack på tørris.
4. Hold rør til alikvoter i rack på tørris

Oversikt over alikvoter

Alikvote #	Type	Comments
1	**Serum 500 µl**	
2	**Serum 500 µl**	
3	**Serum 500 µl**	
4	**Serum 500 µl**	
5	**H-Plasma 500 µl**	
6	**H-Plasma 500 µl**	
7	**H-Plasma 500 µl**	
8	**H-Plasma 500 µl**	
9	**EDTA-Pla 500 µl**	
10	**EDTA-Pla 500 µl**	
11	**EDTA-Pla 500 µl**	
12	**EDTA-Pla 500 µl**	
13	**Buffycoat**	

Dato/sign:

2) Oral glucose tolerance test

ID:
Dato/klokkeslett:

Rekkefølge rør	Alikvoter	Kommentarer
5 ml Serum m/gel	2 x 600 µl	**KUN VED 180 MIN**. Koaguleres 30 min i romtemp
5 ml Heparin m/gel	3 x 600µl	**Plasseres på is**, sentrifugeres med en gang.
6 ml EDTA	2 x 600µl	**Plasseres på is**, sentrifugeres med en gang.

1. 0 prøve kan taes sammen med fasteprøvene
2. Klokkeslett for inntak av glukosedrikk **skal** skrives ned
3. Prøverørene skal holdes på is hele tiden.
4. Sentrifuger prøverørene ved 4°C
5. Hold rør til alikvoter i rack på tørris

0 min	Heparin + EDTA. Prøven taes sammen med de fastende prøvene
15 min	Heparin + EDTA.
30 min	Heparin + EDTA.
60 min	Heparin + EDTA.
90 min	Heparin + EDTA.
120 min	Heparin + EDTA.
180 min	Serum + Heparin + EDTA.

Dato/sign:

Oversikt over alikvoter

OGTT	Alikvote #	Sample #	Type	Kommentarer
0	1	1	H-Plasma 600 µl	
	2		H-Plasma 600 µl	
	3		H-Plasma 600 µl	
	4		EDTA-Pla 600 µl	
	5		EDTA-Pla 600 µl	
15	6	2	H-Plasma 600 µl	

	7		H-Plasma 600 µl	
	8		H-Plasma 600 µl	
	9		EDTA-Pla 600 µl	
	10		EDTA-Pla 600 µl	
30	11	3	H-Plasma 600 µl	
	12		H-Plasma 600 µl	
	13		H-Plasma 600 µl	
	14		EDTA-Pla 600 µl	
	15		EDTA-Pla 600 µl	
60	16	4	H-Plasma 600 µl	
	17		H-Plasma 600 µl	
	18		H-Plasma 600 µl	
	19		EDTA-Pla 600 µl	
	20		EDTA-Pla 600 µl	
90	21	5	H-Plasma 600 µl	
	22		H-Plasma 600 µl	
	23		H-Plasma 600 µl	
	24		EDTA-Pla 600 µl	
	25		EDTA-Pla 600 µl	
120	26	6	H-Plasma 600 µl	
	27		H-Plasma 600 µl	
	28		H-Plasma 600 µl	
	29		EDTA-Pla 600 µl	
	30		EDTA-Pla 600 µl	
180	31	7	H-Plasma 600 µl	
	32		H-Plasma 600 µl	
	33		H-Plasma 600 µl	
	34		EDTA-Pla 600 µl	
	35		EDTA-Pla 600 µl	
	36		Serum 600 µl	
	37		Serum 600 µl	

Dato/sign:

3) Exercise

ID:
Dato/klokkeslett:

Rekkefølge rør	Alikvoter plasma/serum	Kommentarer
5 ml Serum m/gel	2 x 500 µl	Koaguleres 30 min i romtemp
5 ml Heparin m/gel	4 x 500 µl	**Plasseres på is**, sentrifugeres med en gang.
6 ml EDTA	3 x 500 µl	**Plasseres på is**, sentrifugeres med en gang.

1. Klokkeslett for start og slutt sykkeltest **skal** skrives ned
2. Jobb på is hele tiden
3. Etter sentrifugering plasseres prøverør i rack på tørris.
4. Hold rør til alikvoter i rack på tørris

Dato/sign:

Oversikt over alikvoter

Tidspunkt	Alikvote #	Sample #	Type	Kommentar
0	1	1	H-Plasma 500µl	
	2		H-Plasma 500µl	
	3		H-Plasma 500µl	
	4		H-Plasma 500µl	
	5		EDTA-Pla 500µl	
	6		EDTA-Pla 500µl	
	7		EDTA-Pla 500µl	
	8		Serum 500µl	
	9		Serum 500µl	
Etter FATmax	10	2	H-Plasma 500µl	
	11		H-Plasma 500µl	
	12		H-Plasma 500µl	
	13		H-Plasma 500µl	
	14		EDTA-Pla 500µl	
	15		EDTA-Pla 500µl	
	16		EDTA-Pla 500µl	
	17		Serum 500µl	
	18		Serum 500µl	
Etter VO2max	19	3	H-Plasma 500µl	
	20		H-Plasma 500µl	
	21		H-Plasma 500µl	

	22		H-Plasma 500µl	
	23		EDTA-Pla 500µl	
	24		EDTA-Pla 500µl	
	25		EDTA-Pla 500µl	
	26		Serum 500µl	
	27		Serum 500µl	

Dato/sign:

Appendix 7. Other tests from the fasting study protocol

Many tests were performed in the fasting study. The following methods will only be briefly described, and the results are not included in this thesis.

3.1 Continuous blood glucose and finger puncture

Dexcom glucose measurement (Dexcom G4 Platinum, Dexcom, Inc., San Diego, CA, USA) measured continuous blood glucose concentration in subcutaneous stomach fat tissue during the fasting period. The Dexcom was also used to ensure that the participants did not ingest any carbohydrates during the fast and thus remained in a fasted state (Brufladt, 2018).

The site of application was thoroughly cleaned with antibacterial wipes to reduce infection risk and make the instrument with adhesive tape stick to the skin. The instrument was placed on either side of the lower abdomen with a small sensor causing a thin probe to enter just beneath the skin. A receiver was attached to the probe to track glucose ever 5 min with a short delay of actual blood glucose levels.

HemoCue Glucose 201 RT System (HemoCue AB) was used to analyse blood glucose from fingertip every day and to calibrate the Dexcom. A single-use needle was used to puncture fingertip and the blood drop was collected in micro cuvettes. To start a new session (for a new participant) two glucose calibrations from finger were made and the concentration was plotted in the Dexcom G4. An average of two samples from finger puncture were recorded in the morning by the research staff, and the participants made oncalibration were made in the evening by themselves. The numbers were plotted in by the participants on the device. The receiver was blinded, and participants did not know if their blood glucose reached below 3,1 mmol / L.

3.2 Blood pressure and heart rate

Blood pressure (mm Hg) and heart rate (bpm) were recorded daily using a blood pressure monitor with arm cuff (WelchAllyn Spot Vital Signs LXi 0297, Skeneateles Falls, NY, USA). Participants were instructed to sit for 5 min before the test. The arm cuff was placed on either arm. Blood pressure and heart rate were measured three times, and the average of these measurement were calculated.

3.3 Actiheart

Activity levels were tracked continuously with a heart rate gauge (Actiheart, Cambridge, Neurotechnology Ltd, Papworth, UK). Electrodes on the chest were put on one week prior to the fasting intervention to calculate activity and heart rate on a *normal* week (baseline). The electrodes were removed a few days before being put back on again for the fasting week. The results were analyzed with a collaborative institution.

3.4 Strength test

Strength tests were performed prior to the physical capacity tests on bicycle. The strength tests consisted of variations of knee extensions tests using Humac (Humac Norm, HUMAC2009, CMSi Medical Solutions, Stoughton, MA, USA). The participants warmed up on an ergometer bike for 10 min at desired speed. The Humac was individually adjusted to fit each participant´s morphologic leg structure and range of motion (ROM) by adjusting the monorail, seat, height of the dynamometer and so on. The adjustments on Humac were recorded and replicated throughout the intervention for each participant. Participants were instructed to fully flex and extend their knee to detect ROM at each test.

The isokinetic strength tests were performed at three different angular velocities (°/s); 60 °/s, 120 °/s and 180 °/s. Peak torque and maximal force (N·m), average power per repetition (Watt) and time to peak torque (s) were recorded. There were 30 s rest in between each test, and 60 s rest between each angular speed. The highest maximal force and peak torque in the set of three knee extensions at each angular velocity was recorded.

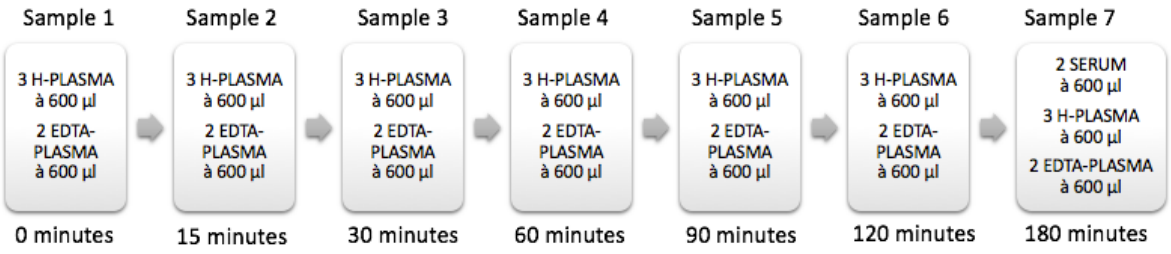
For the isometric (static) strength tests participants were instructed to have the knee at a 60° angle (Humac calculated this angle individually in accordance with the given ROM). Participants warmed up performing one isometric extension lasting 3 s with desired force. There was 60 s of rest before participants were given 3 sets to produce maximal power. There was 60 s rest in between each set à 3 s. Maximal peak power of the three isometric tests was saved in Humac.

3.5 Oral glucose tolerance tests (OGTT)

Oral glucose tolerance test was performed at the beginning (day 0) and at the end

(day 7) of the fasting week. 75 g of sugar was mixed with 3 dl of water the day before and stored in the fridge overnight. Prior to the OGTT the participants had an intravenous (IV) catheter for reducing the puncture on the veins. Morning blood was taken before proceeding to drink the glucose mixture. Participants had to ingest the mixture within 20 s. Individual stop watches was put in front of each participant during the test, and this gave the research staff enough time to prepare for taking blood from the IV according to protocol. Blood was drawn and pipetted according to protocol, and blood glucose from every sample was recorded in the journal.

Figure 1. *Blood samples and volume of aliquots taken during oral glucose tolerance test (OGTT) day 0 and 7.*



*Sample 1 OGTT was drawn together with fasting morning blood

3.6 Resting metabolic rate (RMR)

RMR was measured twice in the fasting study, one in the morning at baseline (day -1), and one in the morning at the end of the fast (day 6). The participants were instructed to avoid physical activity as much as possible prior to the mornings of RMR. Lactate at rest from finger puncture was performed before measuring oxygen uptake with a VO_2 mask (Hans Rudolph Instr., USA) covering both the mouth and the nose. The participants were instructed to lie still on their back on a mat for about 20 min. During the last 5 min of the test the following parameters were tracked every 30 s: RER, HR and VO_2. The mean score of the last ten measurements were recorded.

3.7 Muscle biopsies

Muscle biopsies were performed at the beginning (day 0) and at the end (day 7) of the fasting period. Tissue samples were taken from m. vastus lateralis and from subcutaneous fat from the stomach for electron microscope (EM). The areas of surgery were first disinfected and injected with local anaesthesia (xylocaine without

adrenaline) before a small sample was removed from the respective area. About 150 milligrams / 1mm^2 was taken from each biopsy, in total four (two muscle- and two fat) biopsies. After the tissue samples the wound was covered by bandage.

3.8 Cognitive function tests (CANTAB)

Cambridge Neuropsychological Test Automated Battery (CANTAB) (Cambridge Cognition, Cambridge, UK) was performed on iPad Air (Apple Inc., Cupertino, CA, USA) before, during and after the fasting period to test cognitive function.

3.9 Visual Analogue Scale (VAS)

A VAS questionnaire was given to the participants every morning before measuring blood pressure and weight. The questionnaire was intended to keep track of desiring foods and the experience of hunger. For each question on the VAS they were instructed to put a vertical line on every horizontal line measured to 10 cm.

3.10 Faeces samples

Faeces samples were taken to measure microbiota and bacteria levels. One sample was taken before the fast, and one was taken after three days with re-feeding after the fast. The participants were given home-kits to perform the procedure as close up to the fast as possible. The samples were placed in designated freezing bags and stored in the home freezer before being brought to the test lab freezer. The samples were stored in -80 °C before being sent to collaborative institutions.

Appendix 8. Recruitment poster

NIH NORGES IDRETTSHØGSKOLE

Vil du delta i forskning?

Forskningsprosjekt om effekt av faste på muskelmasse, styrke og utholdenhet

Vi søker forsøkspersoner til et prosjekt som skal undersøke effekten av syv dagers faste på utholdenhet, styrke og muskelmasse

Hva:
- Faste i syv dager
- Oppmøte hver morgen for blodprøver (varighet ca. én time)
- Fysiske tester før og etter fasten

Hvem:
- Menn og kvinner mellom 18-45 år
- Over 12% kroppsfett for menn, over 15% for kvinner
- Fysisk og psykisk friske personer

Hva skal testes:
- Kroppssammensetning (DEXA)
- Muskelstyrke- og funksjon
- Maksimalt oksygenopptak
- Hvilemetabolisme
- Evne til å oksidere fett
- Nitrogenbalanse (i urin)
- Hormoner og metabolitter som påvirker metabolismen (fett- og muskelbiopsi, blodprøver

Fordeler:
- Gratis testing - fysiologiske tester som i utgangspunktet er dyre
- Detaljert informasjon om ulike fysiologiske parametere
- Innblikk i gjennomføring av et forskningsprosjekt
- Tett oppfølging

Ulemper:
- Man kan oppleve sult, spesielt de første dagene av fasten
- Periodevis føle seg sliten og trøtt
- Lavt blodsukker - tidvis ukonsentrert

All testing gjennomføres på Norges Idrettshøgskole. **Oppstart snarest!** Forsøk kjøres gjennom høst og vinter. Dersom dette er av interesse, kontakt prosjektansvarlig professor Jørgen Jensen jorgen.jensen@nih.no, masterstudent Victoria Frivold 94486863 / s.v.frivold@studmed.uio.no eller Steffen Brufladt 92622429 / steffen.brufladt@gmail.com

Appendix 9. VAS

Spørreskjema VAS ID:_____ Date:_____

Vekt: **All urin samlet?:**

Hvor sulten føler du deg?
Ikke sulten Ekstremt sulten

Hvor sterkt er ønsket om å spise?
Ingen ønske Ekstremt sterkt

Hvilke(t) matprodukter har du mest lyst på akkurat nå?

_____

Hvor sterkt er ønsket om å spise dette produktet nå?
Ingen ønske Ekstremt sterkt

Hvor sterkt er ønsket om å avbryte fasten?
Ingen ønske Ekstremt sterkt

Hvor fysisk sliten føler du deg?
Ikke sliten Ekstremt sliten

Hvor psykisk sliten føler du deg?
Ikke sliten Ekstremt sliten

Hvor motivert er du til å fortsette med fasten?
Ikke motivert Ekstremt motivert

Ønsker du å spise noe søtt?
Ikke i det hele tatt Ekstremt stort ønske

Ønsker du å spise noe salt?
Ikke i det hele tatt Ekstremt stort ønske

Ønsker du å spise noe velsmakende?
Ikke i det hele tatt Ekstremt stort ønske

Ønsker du å spise noe fett?
Ikke i det hele tatt Ekstremt stort ønske

Søvn:

Kommentarer:

Appendix 10. Permission to use figure

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