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Literature Review

Abstract

The rise in obesity numbers worldwide have led to an increase in associated co morbidities and consequently to a rise in the direct and indirect costs to society. For these reasons it is essential to treat obese and overweight individuals and to prevent the emergence of new cases. However, one of the most widely used approaches for the treatment of obesity, the daily calorie restriction diets, have been shown to be ineffective due to low adherence, becoming urgent to find new methods for control and treat this multifactorial disease. Therefore, intermittent fasting approaches have gained popularity because they have been shown to be effective in reducing body weight and body fat, while preserving lean tissue and, as such, have been seen as a solution to the replacement of traditional daily caloric restriction diets.

Introduction

In England, a report from Health and Social Care Information Centre (2013) shows that the percentage of overweight and obese people increased from 57.6 % to 65.0% in men and from 48.6% to 58.4 % in women since 1993 until 2011. The arising in obesity numbers and consequently in the associated co-morbidities lead to an increase in the direct costs to the NHS from £479.3 million in 1998 (National Audit Office, 2001) to £4.2 billion in 2007 (UK Government's Foresight Programme, 2007). Additionally, the indirect costs of obesity to the economy are much higher, and have been calculated to had been around £18.8 billion in 2007 and estimated to reach £27 billion in 2015 (UK Government's Foresight Programme, 2007).

In order to decrease the related health costs of this epidemic and the mortality rate associated with it (Pischon, et al., 2008), it is required to prevent the increase in the numbers either by treating overweight and obese individuals or by preventing the occurrence of new cases (Sharma, 2007).

Obesity is a multifactorial health issue that combines genetic, metabolic, behavioral, environmental, cultural and socio-economic influences (Sharma, 2007), however, the origin of the problem arises from an imbalance in energy intake since body weight gain is the result of consuming more energy than one expends (Verduin, Agarwal, & Waltman, 2005). Due to this, interventions such as daily calorie restriction (DCR), which are defined as a 15 to 60% reduction in the energy intake required to maintain body weight (Varady, 2011), have been the most widely used strategy (Steyer & Ables, 2009) in order to promote a negative energy balance and, consequently, a reduction in total body weight (Heymsfield, et al., 2007). However, these protocols have a low efficacy (Heymsfield, et al., 2007) due to difficulties in adherence and compliance (Dansinger, et al., 2005; Del Corral, Chandler-Laney, Casazza, Gower, & Hunter, 2009; Heymsfield, et al., 2007), especially when food is not provided (Moreira, Most, Howard, & Ravussin, 2011), which gives rise to an urgent necessity of developing and adopting new and effective energy restriction protocols.

Daily calorie restriction and healthy eating

Although the interventions to prevent and treat obesity can be behavioral, pharmacological, surgical or environmental (Sharma, 2007), this paper will only address the behavioral component, more precisely the aspect of diet and energy intake.

As stated above, DCR approaches have been widely used to promote body weight loss but not always in a successful way. Heymsfield et al. (2007), in an attempt to evaluate the effectiveness of DCR, concluded that body weight loss achieved by the participants in weight loss studies is less than half of that predicted, partly due to difficulties in adherence and metabolic adaptations. As observed in Figure 1, if the participants were completely adherent to DCR, the reductions in body weight would look like the "predicted" weight loss curve rather than the weight loss curve that actually represents the real weigh loss ("Observed curve").

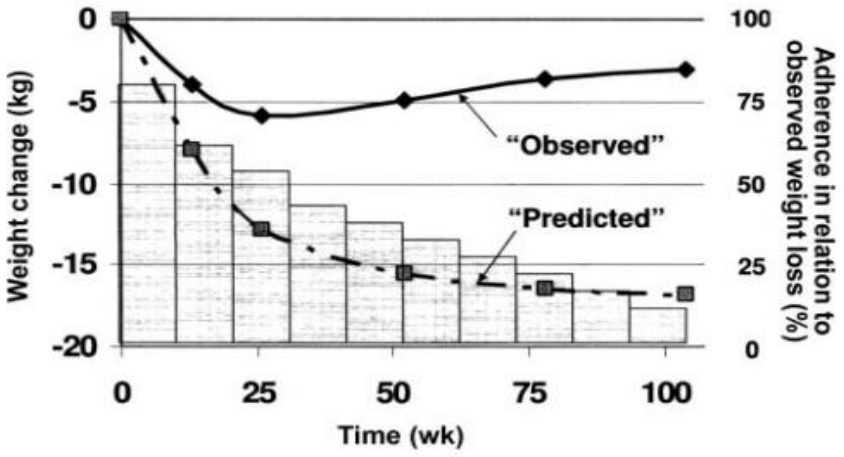

Figure 1: The "Observed" weight loss curve was obtained with a commercial low-caloric diet in the study of Stanley, James, Richard, & Frank (2003). The bars are an estimate of diet adherence based on the study of Dansinger, Gleason, Griffith, Selker and Schaefer (2005), and the "predicted" weight loss curve represents the decrease in body weight that should be seen if participants fully adhere to the prescribed low calorie diet. Source: (Heymsfield, et al., 2007)

However, failure in body weight loss studies may not just be due to lack of compliance and adherence from participants. The reduced body weight loss achieved with DCR diets could be a consequence of a reduction in thermogenesis as a metabolic adaptation to the calorie restriction (Tremblay & Chaput, 2009). This means that a decrease in energy expenditure can be observed (Luke

& Schoeller, 1992; Redman, et al., 2009), which further exceeds the reduction predicted as a consequence of changes in lean tissue and fat mass and, in most cases, it is sufficient to compensate for energy restriction applied (Major, Doucet, Trayhurn, Astrup, & Tremblay, 2007). It was reported that a total body weight loss of 10% in an obese person leads to a decrease by about 8 kcal/kg of lean body mass/day (Leibel, Rosenbaum, & Hirsch, 1995). Additionally to the low effectiveness of the DCR diets, multiple health related institutions and/or organizations have publications regarding healthy eating and dieting in which it is recommend to eat small portions of food distributed in several meals per day. As an example, the British Nutrition Foundation (2012) states that it is necessary to "restrict the amount of high energy snacks" but not ban them completely, since "it will make your diet more difficult to maintain". Also, another publication from the same organization reports that "It is fine to snack … If you feel hungry between meals" (British Nutrition Foundation, 2011). Furthermore, the Food Standards Agency (2007) recommend "four eating occasions, namely breakfast, lunch, evening meal and food consumed between meals (snacks)," while the United States Department of Agriculture (n.d.) advises to "Plan for 3 meals and 1 or 2 snacks each day." Therefore, it is suggested that for a healthy body weight loss and, even to achieve what is considered a healthy eating pattern, it is necessary and recommended to eat small amounts of food distributed for several meals during the day. Despite the majority of the studies being inconclusive to establish a relationship between meal pattern and regulation of body weight (Bellisle, McDevitt, & Prentice, 1997; Egberts, Kymmell, Brown, Brennan, & O'Brien, 2011; La Bounty, et al., 2011; Mattson, 2005), some literature associates a higher meal frequency (≥5 times/day) with a lower energy intake (Farshchi, Taylor, & Macdonald, 2005). However, a research from Popkin and Duffey (2010) revealed that nowadays, the adult population consumes more 280 kcal/day in snacks and an average of 120 kcal/day more during regular meals than 30 years ago. More recently, a study from Ohkawara, Cornier, Kohrt and Melanson (2013), reported that 6 meals per day, in comparison to 3 meals per day, have no significant effect on fat oxidation while, on the other hand, increases hunger and desire to eat. Therefore, it appears that, in fact, a higher meal frequency does not have a positive effect in reducing total energy intake during the day and, despite the lack of evidence regarding the ideal meal frequency, the majority of the health-care professionals and health organizations continue to recommend small portions of food several times per day.

In the last couple of years, a solution for the problems pointed out above has been proposed and implemented through a model that involves intermittent feeding and fasting cycles, called from now on as intermittent fasting (IF). A description of these interventions will be presented in further detail in the next chapters of this paper.

A summary of the information presented above is schematically shown in Figure 2.

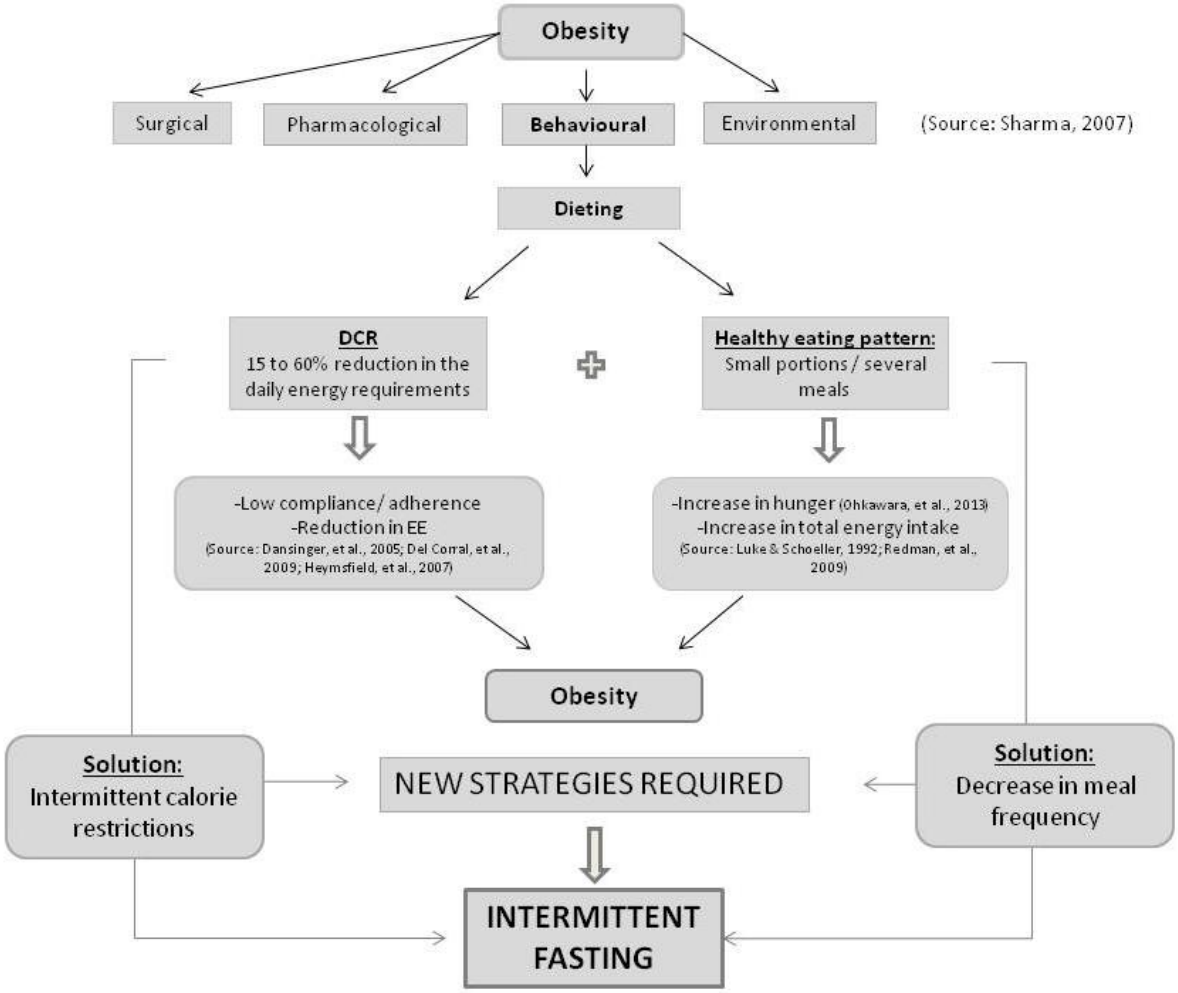

Figure 2: Schematic representation of the problems associated with daily calorie restrictions and healthy eating patterns and a proposed solution to solve them

Intermittent Fasting

Characterization of Intermittent Fasting approaches

Fasting is defined as the complete or partial abstinence from nourishment due to a variety of reasons, especially by medical and/or religious beliefs (Adams, et al., 1987). Therefore, intermittent fasting (IF) regimes are characterized by alternations between *ad libitum* feed and fasting periods, (Varady, Bhutani, Church, & Klempel, 2009). However, modified IF protocols, during which the consumption of 15% to 25% of daily energy requirements on fasting periods is allowed (Varady, et al., 2009) have also been used with the same success as true intermittent fasting approaches regarding reduction in fat cell size and lipolysis stimulation in mice (Varady, Roohk, Loe, McEvoy-Hein, & Hellerstein, 2007). Also, modified IF approaches have been used in human trials as a way of increasing adherence and compliance (Varady & Hellerstein, 2007) during body weight loss programmes, and up to now, only one human trial used a true IF protocol (Heilbronn, Smith, Martin, Anton, & Ravussin, 2005).

Why Intermittent Fasting?

The postulated reason for the implementation of IF regimes is that they can mimic the cycles of food abundance and shortage (Neel, 1962) and, consequently, the energy oscillations characteristics of the Paleolithic era (Halberg, et al., 2005), to which we are genetically programmed (Chakravarthy & Booth, 2004; Mattson, 2005; Zimmet & Thomas, 2003). This genetic adaptation was necessary for surviving during shortage food periods since it allowed to spare lipid and glycogen stores as well as lean body tissue and it is believed to be part of a thrifty genotype (Chakravarthy & Booth, 2004; Neel, 1962).

Nowadays in the western countries, there is a surplus of food availability, and the rules of a healthy eating pattern dictate that we should eat frequently as a way of controlling appetite (Speechly & Buffenstein, 1999) and managing body weight (Farshchi, et al., 2005).

Therefore, our eating patterns are much different from our ancestors, which is creating a gap between our environment and the genome to which we are programmed to (Halberg, et al., 2005).

Physiology of fasting

After an overnight fast (~10h) all the macronutrients have been absorbed by the body leading to the beginning of a fasting state (Lanham-New, Mcdonald, & Roche, 2011). This state is characterized by a drop in glucose levels (a little under 5 mmoll/l) and a consequent decrease in insulin (Frayn, 2010), as represented in Figure 3 during the night time and early morning period.

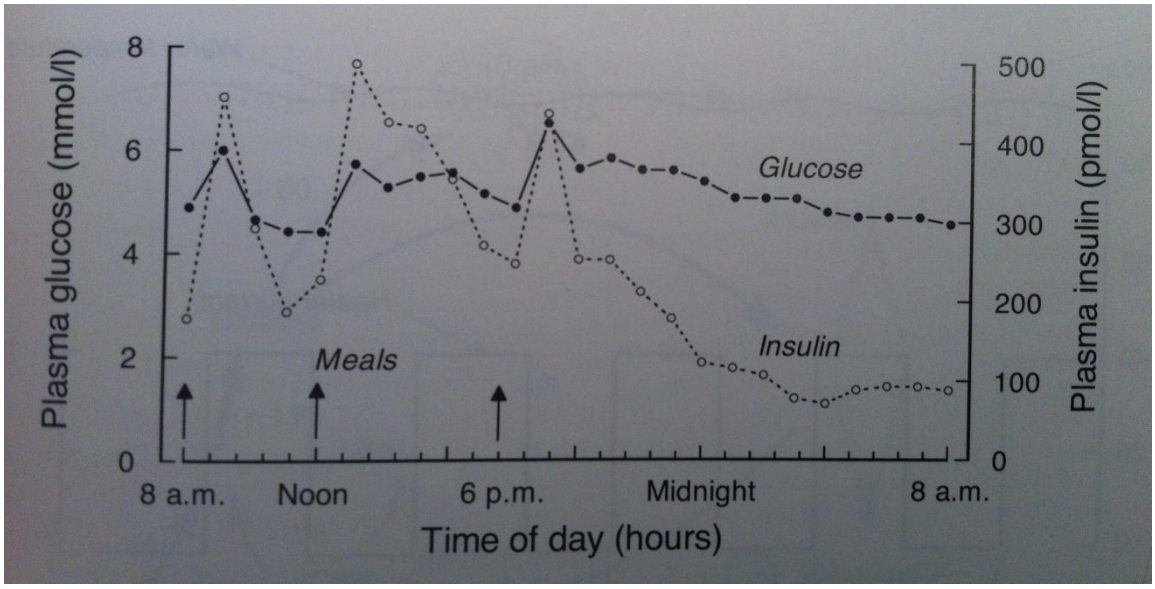

Figure 3: Blood glucose concentrations during the day compared with the variability of plasma insulin concentrations. Source: (Frayn, 2010)

The decrease in insulin levels leads to an increase in glucagon secretion by the α cells of the pancreas in order to mobilize glycogen stores in the liver to avoid hypoglycemia when food is not being supplied (Lanham-New, et al., 2011; Newsholme, Leech, & Board, 2010). Thus, glucagon's main function is to induce glycogen breakdown in the liver in order to produce glucose, and to inhibit glycogen synthesis through activation of cyclic AMP cascade (Berg, Tymoczko, & Stryer, 2002). Glucose arising from glycogen breakdown in the liver is released into the bloodstream and is up taken by muscle and adipose tissue (Berg, et al., 2002). Therefore, hepatic glucose output is regulated by the ratio of insulin to glucagon (insulin/glucagon high – glucose output suppressed; insulin/glucagon low – glucose output increased) (Gibney, Roche, Macdonald, & Nutrition, 2003).

In addition, high levels of glucagon leads to a reduction in the activity of the following enzymes: glucokinase (GK), phosphofructokinase-1 (PFK-1) and pyruvate kinase (PK), and

consequently to a decrease in glycolysis rate (Roach & Benyon, 2003). Furthermore, once pyruvate production is decreased, due to the decrease in glycolysis rate, a consequent reduction in Acetil-CoA carboxylase (ACC) activity occurs (which converts Acetyl CoA into Malonyl CoA) and therefore an inhibition in fatty acid synthesis occurs (Berg, et al., 2002), as represented in Figure 4.

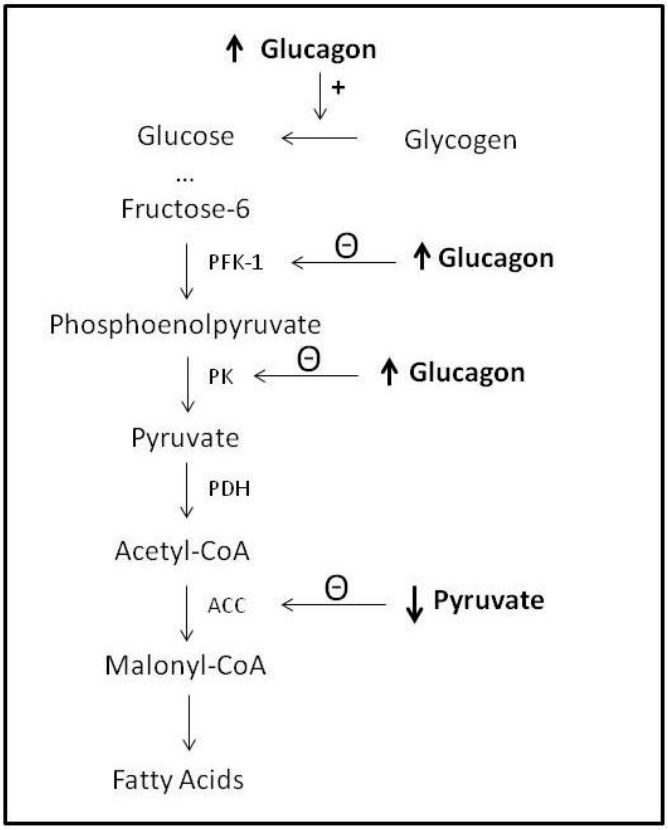

Figure 4: Glucagon effects on glycolysis and lipogenesis

Additionally, the fall in blood glucose levels stimulates the secretion of epinephrine, which will cause the mobilization of stored glycogen and triacylglycerol, contributing to the raise in glucose and non-esterified fatty acids (NEFA) plasma concentrations (Gibney, et al., 2003).

At this stage, and because the brain and the red blood cells are mainly dependent on glucose (Lanham-New, et al., 2011), this substrate needs to continue being produced, mainly from two processes: glycogenolysis (muscle glycogen stores cannot be breakdown and released into the bloodstream due to the absence of glucose-6-phosphatase but can contribute with lactate to be used by the liver for gluconeogenesis (Frayn, 2010)), and from gluconeogenesis (Konig, Bulik, & Holzhutter, 2012).

Therefore, the substrates for gluconeogenesis are lactate and pyruvate (from red blood cells muscle and adipose tissue), alanine (release from muscle and adipose tissue) and glycerol (release from adipose tissue as a result of lipolysis) (Frayn, 2010; Gibney, et al., 2003), as exemplified in Figure 5. At this stage, protein breakdown is stimulated by low insulin levels and by noradrenaline and cortisol and noradrenaline also activates hydrolysis of triacylglycerol to release glycerol (Roach & Benyon, 2003).

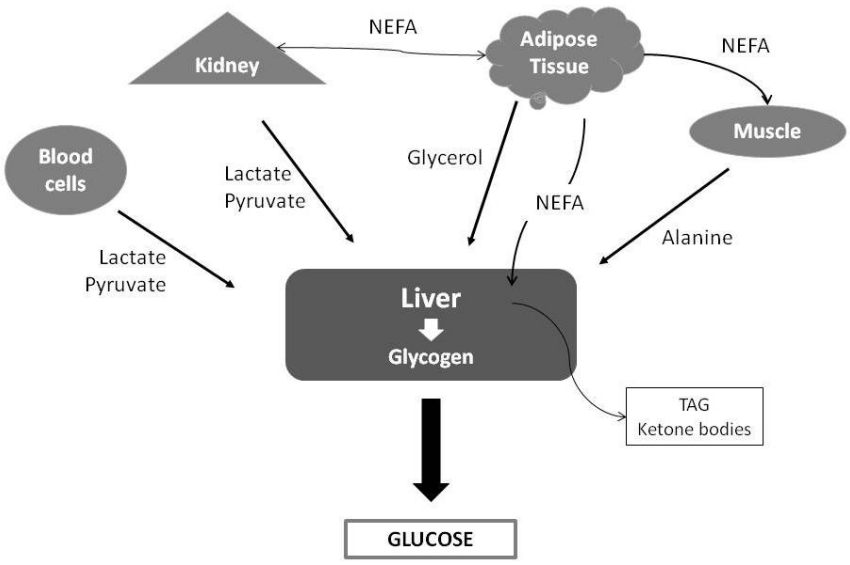

Figure 5: Metabolism after an overnight fast. Adapted from: Frayn (2010)

During the fasting state, red blood cells play an important role in the production of glucose through the Cori Cycle, due to the fact that they do not fully oxidize glucose, being the majority of it released as lactate, which allows the conversion of lactate into glucose by gluconeogenesis, as represented in Figure 6. The Cori cycle works in parallel with the glucose-alanine cycle, which converts the lactate and the amino acid, alanine, released by the muscle into glucose.

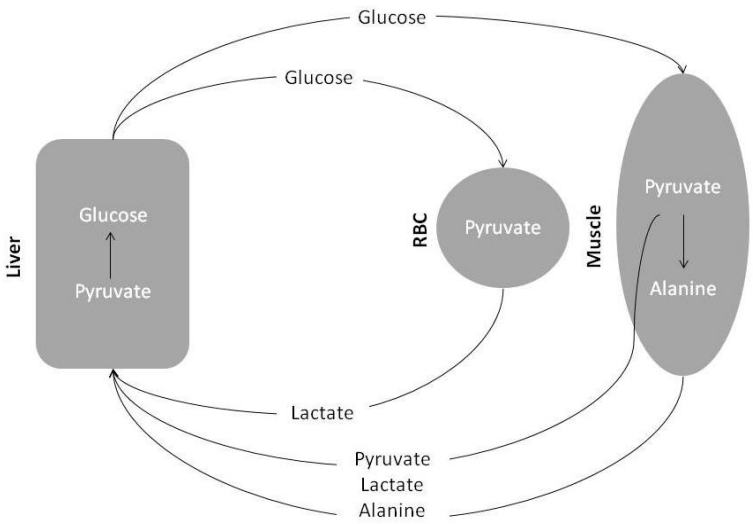

Figure 6: Cori cycle (glucose-lactate) and Glucose-alanine cycle

Glucose can also be produced by protein breakdown, which provides carbon skeletons that can be metabolized into intermediates of the Krebs cycle and enter into gluconeogenesis (Berg, et al., 2002; Roach & Benyon, 2003).

After an overnight fast, the hormone-sensitive lipase is activated due to the low levels of insulin, which allows the release of NEFA from adipose tissue into the bloodstream. In addition, the release of epinephrine also contributes to this process (Gibney, et al., 2003).

The contribution of the gluconeogenesis and glycogenolysis for the production of glucose changes with the prolonging of the fasting period, with the gluconeogenesis assuming an increasing importance as the glycogen reserves become depleted (Konig, et al., 2012). The Figure 7 is a schematic representation of the human metabolism after an overnight fast.

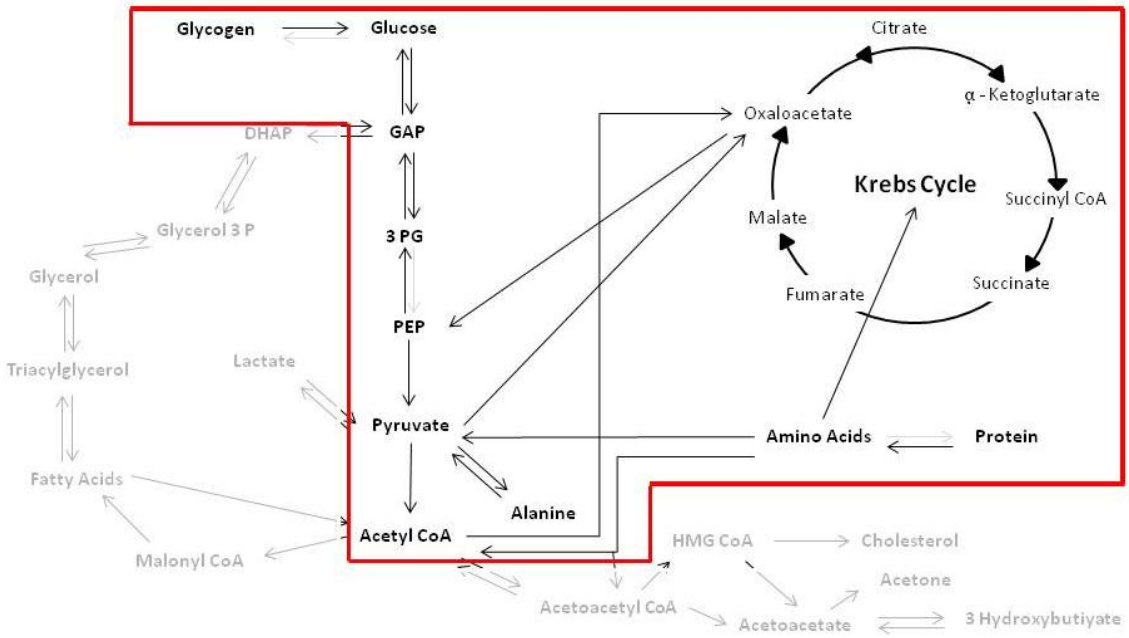

Figure 7: Integration of metabolism after an overnight fast

As a way of adaptation to the further fall in blood glucose concentrations, an increase in lipolysis and a decrease in gluconeogenesis rate and, consequently, in protein breakdown, as a way of sparing muscle and attempted survival can be observed (Berg, et al., 2002; Lanham-New, et al., 2011) (Figure 8). According to Klein, Sakurai, Romijn and Carroll (1993), this shift to the predominant use of lipids as substrate, appears to happen between 18 and 24 hours of fasting in young adult men. However, whole-body adaptations to fast periods appears to be dependent on obesity-associated insulin resistance in women (Horowitz, Coppack, & Klein, 2001), which suggests that because of the higher hepatic glycogen content in insulin-resistant obese individuals, the switching of to fatty acid oxidation takes much longer in these participants relative to a lean person (Muller, et al., 1997).

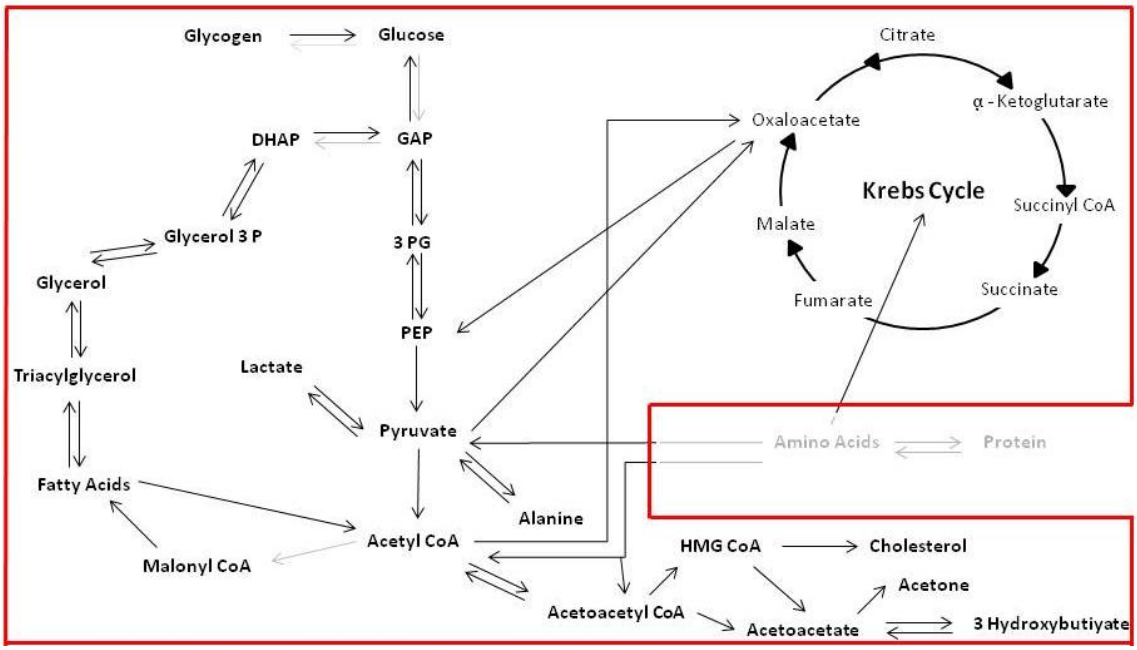

Figure 8: Integration of metabolism after 18/24h of fasting

Pyruvate dehydrogenase (PDH) activity, which catalyzes the irreversible conversion of pyruvate into acetyl-coA, is suppressed during fasting via phosphorylation of the E1 component of the enzyme catalyzed by pyruvate dehydrogenase kinase 4 (PDK4) (Bowker-Kinley, Davis, Wu, Harris, & Popov, 1998) as a way of controlling the amount of glucose that is lost through the body, and thus, the majority of the glucose used by tissues can be converted into lactate, pyruvate and alanine (Lanham-New, et al., 2011). Regarding this issue, Pilegaard, Saltin and Neufe (2003) showed a significant increase in PDK4 and lipoprotein lipase transcription after 20h hours of fasting, which suggests that short-term fasting develops an adaptive response in skeletal muscle that leads to an increased expression of lipid metabolism genes as a way of minimize glucose utilization.

As a consequence of the triacylglycerols breakdown, three fatty acids and one glycerol becomes available to participate in other pathways, thus the glycerol portion can be converted into glucose by gluconeogenesis. However, the glycerol portions become more important only after 24 hours of fasting, when all the glucose is produced by gluconeogenesis (Jensen, Ekberg, & Landau, 2001), becoming essential to spare protein stores (Bortz, Paul, Haff, & Holmes, 1972). In the study of Zauner et al. (2000), after 12 and 36 hours of fasting, the fatty acids increased from 240 ± 191 µmol/L to 616 ± 225 µmol/L, respectively.

The oxidation of the fatty acids originates Acetyl CoA, which normally combines with oxaloacetate in Krebs cycle to form citrate. However, oxaloacetate is diverted to the gluconeogenesis pathway to produce glucose and because of that, Krebs cycle is not able to oxidize all of the Acetyl CoA produced, and it is converted into acetoacetate and D-3-hydroxybutyrate (ketone bodies), as represented in Figure 9. Ketone bodies can be used as an energy source by skeletal muscle, heart and brain (Finn & Dice, 2006). A study from Zauner et al. (2000) reported an increase in β-hydroxybutyrate in humans from 182.7 ± 262.9 µmol/L to 1949.9 ± 1458.7 µmol/L after 12 and 36 hours of fasting, respectively.

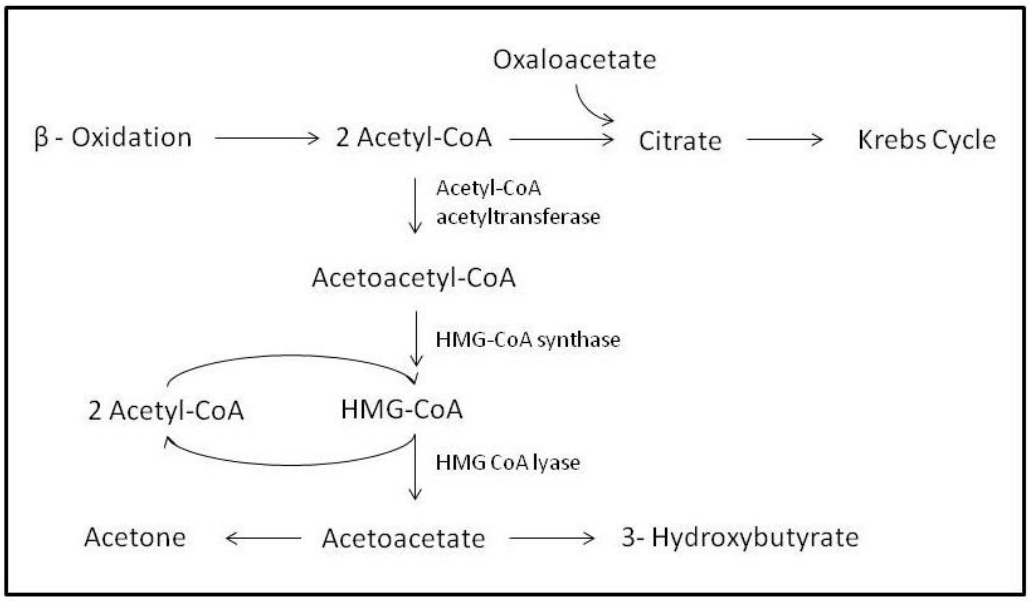

Figure 9: Ketone bodies formation

Due to low insulin levels, skeletal muscle stops almost entirely using glucose as a fuel and starts to use fatty acids (Berg, et al., 2002; Frayn, 2010), which further contributes to the spare of lean tissue (Frayn, 2010) and for the availability of ketone bodies to be used by the brain (Roach & Benyon, 2003).

In summary, it appears that IF regimes promote fat loss due to the metabolic adaptations that occur in the human body as a response to the absence of food supply.

Intermittent Fasting Studies

Overview

The majority of the research conducted in humans in relation to IF was done with the purpose of exploring the health benefits associated with it. Although the fasting practice is something that humans have done during the evolution period, whether due to food shortage or for religious reasons, the truth is that this is a very recent topic and therefore with many gaps to fill when it comes to scientific evidence. For this reason, uncertainties relating to the health benefits of this practice are still abundant but the most frequently reported are summarized in Figure 10.

Despite all these health benefits, they are not going to be discussed in this literature review as the purpose of this paper is to report changes in body composition due to IF interventions, as well as observed modifications in resting energy expenditure (REE) and eating patterns.

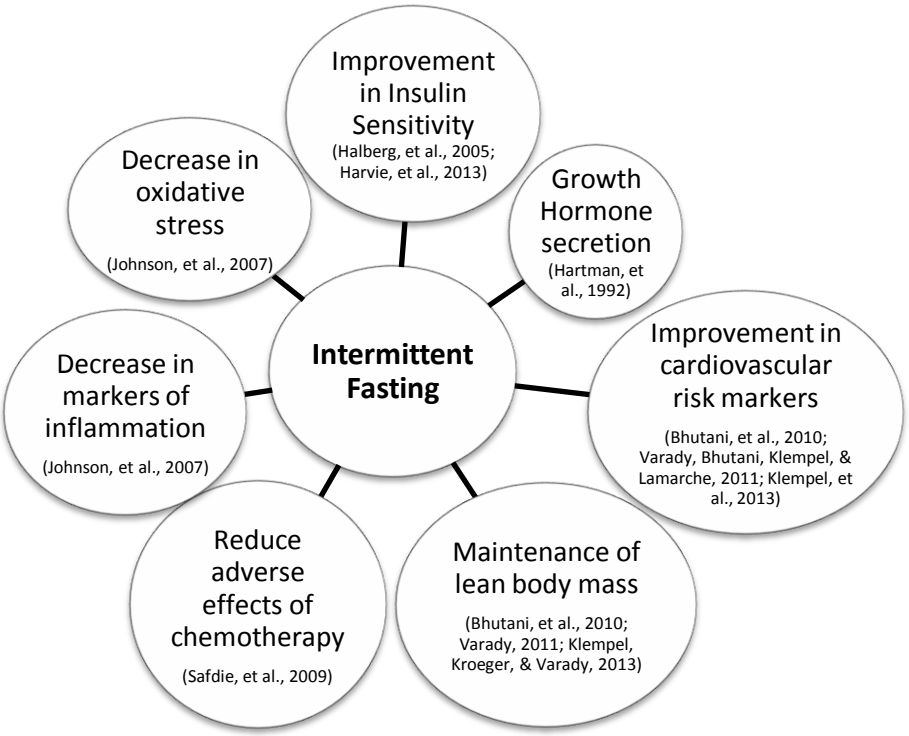

Figure 10: Intermittent Fasting health benefits

Intermittent Fasting and Body Composition

The observed effects of intermittent fasting approaches regarding changes in body weight and body composition are summarized in Table 1.

Body weight and body fat

In despite of being a recent topic and hence, poorly studied, scientific evidence has been consistent with respect to the effectiveness of IF regimes in reducing body weight and fat mass.

In the study of Heilbronn, Smith, Martin, Anton and Ravussin (2005), 16 (8 men and 8 women) healthy non-obese individuals lost an average of 2.5 ± 0.5% of their baseline body weight ($p < 0.001$), from which 0.8 kg were fat mass (4 ± 1 %; $p < 0.001$) after 22 days of alternate day fasting (ADF). The study suggests that IF is feasible for short time periods in non-obese individuals and that the decrease in body weight reveals that participants were not consuming enough calories on the feasting days to maintain their body weight.

Similar results regarding body weight loss were also reported in a short period IF intervention applied by Halberg et al (2005) to eight healthy young non-obese men. After two weeks of doing 20 hours ADF, participants lost an average of 0.9 kg in body weight ($p>0.05$), however they increased 0.3% in body fat ($p>0.05$). Nevertheless, it is necessary to point out that this study was not designed with the purpose of observing changes in body weight and/or body composition, which may explain the small sample size and the short duration of the intervention. Furthermore, participants were instructed to eat enough food to maintain body weight.

A longer IF intervention was applied by Johnson et al (2007) to nine obese participants, who followed an ADF diet protocol characterized by almost 36 hours of very low caloric intake (320 calories for women and 380 calories for men provided by a commercial meal replacement shake) and 12 hours of feeding *ad libitum*. Results revealed a significant body weight loss ($p\leq0.05$) of 8.5 ± 1.7 kg (8.0 ± 1.4%) after 8 weeks of study.

An intervention with the same duration as the above study, which achieved similar results despite having a different IF protocol, was developed by Eshghinia and Mohammadzadeh (2013). In this study 15 obese participants, aged between 20 and 45 years, took part in an eight weeks study divided into two phases: two weeks of control period and 6 weeks of ADF during which participants should consume 25 to 30% of energy needs on three fasting days, eat a 1700-1800 kcal/d diet during

another 3 days of the week and eat *ad libitum* on the seventh day a week. After the six weeks of intervention, body weight significantly decreased ($p<0.001$) by 6 ± 1.2 kg, which represented a mean body weight loss of 7.1%. Additionally, a significant reduction ($p<0.001$) in fat mass (45.82 ± 4.16 kg to 42.98 ±4.01 kg) and in waist circumference (87.87 ± 9.74 to 82.86 ± 9.68 cm) was reported. However, this study presented some limitations, such as, the absence of a control group to compare the results with, a small sample size and a short intervention period.

A similar decrease in body weight was experienced in the study by Varady et al. (2009) after 12 weeks of ADF regime implemented in three phases: two weeks of pre-loss control; four weeks of alternate day fasting with controlled food intake (provision of a meal on fasting days that represented 25% of baseline energy needs) and four weeks of alternate day fasting with self-selected food intake (no food provided but participants could still consume 25% of their baseline needs). Results shown that during the alternate day fasting phases, participants lost a mean of 5.6 ± 1.0 kg (5.8 ± 1.1%) from baseline ($p<0.01$) (0.67 ± 0.1 kg/week during ADF controlled food intake phase and 0.68 ± 0.1 kg/week during ADF self-selected food intake phase). A significant decrease ($p<0.01$) in body fat mass of 5.4 kg (from 45.0 ± 1.6% to 42.1 ± 2.0%) was also experienced by participants.

A study by Eshghinia and Gapparov (2011) tested 26 obese females during a four week study of controlled food intake during ADF diet. Participants consumed a very low caloric diet (VLCD) (25 to 40% of their energy needs) for three days a week and underwent a moderate caloric restriction diet in the remaining four days. After four weeks of study, participants significantly decreased ($p<0.0001$) body weight by 4.7% (from 96.87 ± 21.34 to 92.16 ± 19.85 kg).

Recently, Klempel, Kroeger and Varady (2013) recruited 32 obese females aged between 25 and 65 years and randomized them either to a ADF high fat (ADF-HF) diet (45% fat) or to a ADF low fat (ADF-LF) diet (25% fat) during ten weeks, divided into two phases: two weeks of a weight maintenance period followed by eight weeks of ADF intervention, during which they consumed 25% of their energy needs on the fasting day and 125% of their energy needs on the feed day, either on a high fat or low fat diet. During the 2 weeks of weight loss control, both groups significantly lost body weight ($p<0.001$) despite no caloric restrictions prescribed. During the intervention period, body weight and fat mass significantly decreased from baseline values ($p<0.0001$) by 4.8 ± 1.1% and 5.4 ± 1.5 kg in ADF-HF group and by 4.2 ± 0.8% (4.3 ± 1.0kg) and 4.2 ± 0.6 % (3.7 ± 0.7kg) in the ADF-LF diet, respectively. No significant differences between groups for body weight loss and fat mass were reported at any point, which indicates that an ADF-HF diet is equally as effective as an ADF-LF.

Another recent study from Bhutani, Klempel, Kroeger, Trepanowski and Varady (2013) combined an ADF diet with exercise to report which intervention induces superior changes in body composition compared to each intervention alone. With this purpose, they studied 64 obese participants for 12 weeks that were randomly allocated in one of the following groups: combination of ADF plus endurance exercise; only ADF; only exercise; or control group. After 12 weeks of study, a significant decrease in body weight ($p<0.05$) in the combination, ADF and exercise groups (6 ± 4 kg, 3 ± 1 kg, and 1 ± 0 kg, respectively) were reported. Also, significant reductions were described in fat mass and waist circumference in the combination group. Evidence from this paper suggests that ADF approaches in combination with endurance exercise produces superior changes in body weight and body composition when compared to individual treatments.

Despite all these studies proving the effectiveness of IF approaches, a crossover design study developed by Soeters et al. (2009) found no significant changes in body weight and in body fat from baseline values after two weeks of 20 hours of fasting every second day.

No significant changes ($p>0.05$) in body composition were also reported in a randomized crossover study conducted by Stote et al. (2007) with the purpose to compare meal frequency. Participants followed either a protocol of 3 meals/day (breakfast, lunch and dinner) or 1 meal/day consumed within a four hour period (which means that they were fasting for 20 hours every day), during 8 weeks. Despite no significant differences reported, the consumption of 1 meal/day reduced body weight and body fat mass by 1.4 kg and 2.1 kg, respectively, while no changes were observed after the 3 meals/day diet. According to the authors, the changes in body composition observed after the 1 meal/day diet may be due to a deficit of 65 kcal in daily energy intake and/or to the effect that eating patterns could have on metabolic activity.

Lean tissue

It is documented that short-term fasting increases proteolysis (Fryburg, Barrett, Louard, & Gelfand, 1990; Tsalikian, Howard, Gerich, & Haymond, 1984), and therefore it is expected to observe losses in lean tissue due to IF regimes. However, one of the postulated advantages of IF approaches is that it helps to spare lean tissue (Varady, 2011).

In the study of Heilbronn, Smith, Martin, Anton and Ravussin (2005), a significant loss ($p \leq 0.05$) of 0.6 kg from fat free mass ($p < 0.001$) was observed after 22 days of alternate day fasting (ADF). Similar results were reported by Halberg et al. (2005), when after two weeks in a regime of IF, participants lost body weight but increased body fat, which means that the body weight lost was from lean tissue.

On the other hand, in the study of Varady et al. (2009) no significant changes were experienced by participants in lean tissue from baseline to the end of the study (-0.1 ± 0.1 kg). Furthermore, no significant changes in lean tissue were described in the study of Stote et al.(2007) after the consumption of 1 meal/day or 3 meals/day.

In the study from Soeters et al. (2009), despite a slight increase in lean tissue, no significant differences were described after fasting every second day for 20 hours during two weeks. However, the authors stated that the duration of the study might have been insufficient to observe measurable effects on muscle mass.

In another study from Klempel et al. (2013), no significant changes in lean tissue from baseline values were experienced by the participants after a regime of ADF high fat or ADF low fat diet. Despite this, an increase in lean tissue of 1.2 ± 1.3 kg and 0.5 ± 0.7 kg in the ADF-HF or ADF-LF groups, respectively, was observed.

In conclusion, from the limited literature available in humans, it appears that during IF schemes, losses in lean tissue are minimal or none and, therefore, there is evidence to claim the spare in lean tissue as one of the major advantages of IF regimes.

Intermittent Fasting and resting energy expenditure

During fasting periods, due to the low levels of insulin in the bloodstream, the secretion of epinephrine is stimulated, which in turn was reported to stimulate metabolic rate (Staten, Matthews, Cryer, & Bier, 1987). Beyond the existing lack of literature regarding the effects of IF approaches on REE due to the novelty of the topic, the available research is also discordant and inconclusive. Among this issue, Heilbronn et al. (2005) reported no significant differences in resting metabolic rate (RMR) from participants between baseline (6675 ± 283 kJ/d) and day 22 (6329 ± 260 kJ/d), while Soeters et al. (2009) reported a significantly lower ($p \leq 0.05$) REE after two weeks of IF in comparison with a standard diet (SD). In accordance with the authors, the reduction in REE would result in a difference of 21 535 kcal per year, approximating the equivalent of 3 kg of body fat, which suggests that IF can lead to body weight gain if energy intake were not adjusted.

On the other hand and despite not being a study about intermittent fasting, Zauner et al. (2000) tested the effects of short term starvation on metabolic rate during 84 hours of fasting (measurements were made after the first overnight fast, after 36 hours, 60 hours and 80 hours). Results showed that REE increased from day 1 to day 2 and from day 2 to day 3 and slightly decreased at day 4 (3.97 ± 0.9 kJ/min; 4.37±0.9 kJ/min; 4.53 ± 0.9 kJ/min; 4.43 ± 0.9 kJ/min, respectively). The authors' hypothesized that the increase in metabolic rate could be due to a progressive increase in norepinephrine concentration.

Intermittent Fasting and Energy Intake

Anson et al. (2003) described that mice placed in IF regimes ate almost twice as much as mice fed *ad libitum*, which means that the mice subjected to IF diets were compensating for the fasting periods by increasing their food intake. Once that IF regimes are characterized by fasting periods followed by an *ad libitum* feeding window, it is hypothesized that participants will compensate during feeding periods for the shortage in energy intake promoted by the fasting period, as it was reported in mice.

Regarding this issue, a human trial conducted by Johnstone et al. (2002) submitted 24 normal weight (BMI 20–25 kg/m^2) participants (12 men and 12 women) to a 2-day control period (maintenance) and a separate 2-day test period (fast). During the day one of control period, participants were fed 1.6 x times their RMR and during day two, were given *ad libitum* access to food. During the test period, participants consumed a maintenance diet (1.6 x RMR) and then underwent a 36 hour fasting period. During day two, *ad libitum* access to food was given. Results from this short intervention revealed that participants consumed 10.2 *vs* 12.2 MJ/day ($p = 0.049$) during the post-maintenance and post-fast periods, respectively. On the post-fast period, energy intake was 20% greater than control values, while *ad libitum* intake in control treatment was approximately the required energy to maintain participant's body weight (1.6 x RMR). The intake of fat was significantly higher in the post-fast period (5.1 MJ/day) than in the post-maintenance period (3.7 MJ/day) while no significant differences were reported in carbohydrate and fat intake. Despite these results, participants consumed much less energy than required to compensate for the energy reduction induced by the fast.

On the other hand, Södersten, Bergh and Zandian (2006) reported that after a period of fasting (skipping dinner on the day before and breakfast on the day of testing), women ate about 20% less while men ate about 30% more after food deprivation, in relation to the absence of a fasting period before the testing meal. However, this was a single fasting period experience and therefore, no further conclusions can be extrapolated from it.

Klempel, Bhutani, Fitzgibbon, Freels and Varady (2010) reported that average energy intake on the fast days (501 ± 28 kcal) and on feed days (1801 ± 226 kcal) (26 ± 3 % of baseline needs and 95 ± 6% of baseline energy needs, respectively) was similar to the average energy intake during the control phase (1937 ± 180 kcal). These results reveal that participants did not increase their energy intake on the feed day as a way of compensating for the energy restriction on the fast day, as hypothesized by the authors. This paper suggests that participants are able to consistently limit their

energy intake to 25% of their energy needs on fast days and not over intake on feed days. However, the authors recognized that there is a gap between the caloric restriction calculated by the food records (37% energy restriction every day) and the body weight lost by the participants. If this was the actual percentage of caloric restriction, participants would have lost 1.2 kg/week instead of the 0.7 kg/week as reported, which suggests underreporting of energy intake (Klempel, et al., 2010).

Table 1: Summary of the effects of intermittent fasting on body weight and body composition on human studies. *Significant differences from baseline values; BMI - body mass index; IF - Intermittent fasting; RMR- resting metabolic rate; SD - standard diet; ADF - alternate day fasting; ADF-HF - alternate day fasting high fat diet; ADF-LF - alternate day fasting low fat diet;

Reference	Participants	Trial length	IF Protocol	Findings
Heilbronn et al. (2005)	*n*=16 8 women and 8 men Healthy non-obese BMI 20.0 – 30.0 Age 23 – 53 y	3 weeks	Fast day: 24-h fast Feast day: *ad libitum* feeding (food not provided)	↓ 2.5 ± 0.5% body weight* ↓ 4 ±1% body fat (0.8 kg)* ↓ 0.6 kg fat free mass * = RMR
Halberg et al. (2005)	*n*= 8 Men Age 25±0.1 yr BMI 25.7±0.4 kg/m^2	2 week The aim was not to observe changes in weight and/or body composition	Fast day: 20-h fast (2200–1800) Feed day: *ad libitum* feeding Participants were instructed to eat enough food to maintain body weight	↓ 0.9 kg body weight ↑ 0.3% body fat
Johnson et al. (2007)	*n*= 9 7 women and 2 men Sedentary BMI >30, asthma	8 weeks The aim of this study was not to decrease weight or change body composition	Fast day: 36-h very low caloric diet (320 kcal to women, 380 to men) Feed day: 12-h *ad libitum* feeding (food not provided)	↓ 8% body weight*

Stote et al. (2007)	*n*= 15 10 women and 5 men Age 40–50 y 18 ≥BMI≤25	Randomized crossover design 2 x 8 weeks 11 weeks washout period between treatments	Control: 3 meals/d (breakfast, lunch and dinner) Experimental: 1 meal/d 20-h of fast everyday (2100-1700) (calories for body weight maintenance; same macronutrients distribution; all food provided)	EXPERIMENTAL: ↓ 1.4 kg body weight ↓ 2.1 kg body fat mass CONTROL: no reported changes
Varady et al. (2009)	*n*=16 30 ≥BMI≤39.9 Age 35 – 65 y	10 weeks Phase 1: 2 weeks preloss control Phase 2: 4 weeks ADF controlled food intake Phase 3: 4 weeks ADF self-selected food intake	Fast day: 24-h very low caloric diet (≤25% of energy needs) Feed day: <30% fat (food provided on fast day during ADF controlled food intake)	↓ 5.8 ± 1.1% body weight* ↓ 5.4 kg fat mass* ↓ 0.1 ± 0.1 kg Lean tissue
Soeters et al. (2009)	*n = 8* 20 ≥BMI≤25 Age 18 – 35 y	2 weeks Cross over design 4 weeks washout period between treatments	IF: Fast day: 20-h fast (2200–1800) Feast day: *ad libitum* feeding SD: no skipping meals Caloric intake and macronutrient consumption were equal	No significant differences in body weight, fat mass and lean tissue between IF and SD IF decreased REE*

Bhutani et al. (2013)	*n=64* Obese	12 weeks 4 groups: 1) Combination (ADF plus exercise) 2) ADF 3) Exercise 4) Control	No data about ADF protocol	↓ Body weight 1) 7.1±1.0%, 2) 5.8±1.0% 3) 1.4±0.5% ↓ Fat mass 1) 6.3±1.5%
Eshghinia and Mohammadzadeh (2013)	*n= 15* *Obese* Age 20–45 y	8 weeks Phase 1: 2 weeks of control period Phase 2: 6 weeks of ADF	Control period: maintain body weight ADF phase: Fast day: 3 non consecutive days (25 to 30% energy needs) Feed day: 3 non consecutive days of 1700-1800 kcal/d diet and 1 days of *ad libitum* per week.	↓ 7.1% of weight loss* ↓ Fat mass* ↓ Waist circunference*
Eshghinia and Gapparov (2011)	*n = 26* *Obese females*	4 weeks	ADF diet: VLCD during 3 days/week (25 to 40% energy needs) Caloric restriction during 4 days/week	↓ 4.7% body weight ↓ Waist circunference
Klempel et al. (2013)	*n = 32* *Obese females* *Age 25 – 65 y*	10 weeks Phase 1: 2 weeks weight maintenance Phase 2: 8 weeks ADF either in a high fat diet (45% fat) or low fat diet (25%)	Control period: maintain body weight ADF phase: Fast day: 25% of energy needs Feed day: 125% of energy needs on a high or low fat diet	ADF-HF: ↓ 4.8 ± 1.1% body weight* ↓ 5.4 ± 1.5 kg fat mass* ADF-LF: ↓ 4.2 ± 0.8% body weight* ↓ 4.2 ± 0.6 kg fat mass*

Conclusion

A summary of the effects of IF intervention on body weight, body composition and REE in human trials was presented in this review paper. The use of IF approaches as a method to decrease body weight and change body composition is an emergent topic and therefore, there is a limited and a lack of research on the theme. However, the existent evidence in humans suggests that IF approaches are an effective method to decrease body weight and body mass with no change, or minimal changes in lean tissue. During IF regimes, alterations in body composition can be observed even in short term interventions (3-8 weeks), during which reductions in body weight of 3-8% from baseline can be experienced.

In respect to REE, the evidence to date is inconsistent, therefore more trials are necessary in order to clarify the actual effects of IF interventions on metabolic rate. Also, more research is needed to elucidate the consequences of fasting periods on energy intake during the feeding window.

The popularity of IF regimes is exponentially increasing, therefore, it appears essential for the development of research in this area, especially regarding the effectiveness of IF schemes in people with normal weight, since most of the literature on this subject was conducted in overweight and/or obese individuals.

Research Article

Journal of choice to submit publication

It is with intention to submit the following scientific article to the *Nutrition & Metabolism* journal. As it is described by the journal's aims and scope, the publication content focuses on integration of nutrition and clinical investigation, with special interest on obesity studies as well as biochemistry of metabolism. In this sense, this article involves the comprehension of the effects of food deprivation on body composition and resting energy expenditure as a result of metabolic changes due to it.

The evidence provided by this article is original and innovative and it would contribute to a further understanding on the topic and to extend the current limited literature regarding it.

Intermittent Fasting – How long is enough?

Abstract

Introduction: Intermittent fasting (IF) has become a popular method to decrease body weight and body fat mass, however, no one to date has studied the optimal time frame of fasting/feeding and the impact it has on total body weight, body composition, resting energy expenditure and the feeding patterns in response to the reduction of energy intake due to these fasting periods.

Methodology: Participants were divided into four different groups: Control group (8 hours of fasting/16 hours of feeding every day), 16-h (16 hours of fasting/8 hours of feeding in alternate days (AD), 20-h (20 hours of fasting/4 hours of feeding in AD), and 24-h (24 hours of fasting in AD). The intervention was designed to last for 9 weeks and was divided into two phases: first phase- 1 week of control; second phase– 8 weeks of intervention.

Results: A decrease in body weight and in body fat mass were observed in the 16-h (0.6 ± 1.2 % and 0.64 ± 0.50 kg, respectively), in the 20-h (5 ± 2% and 3.53 ± 2.94 kg, respectively) and in the 24-h group (3.1 ± 4.6% and 2.70± 4.88 kg, respectively). Regarding REE, no significant differences were observed at the end of the study in relation to baseline values.

Conclusions: Findings from this paper suggest that IF regimes characterized by fasting lengths of 20 hours appear to be more effective to achieve reductions in body weight and in body fat mass, while sparing lean tissue.

Keywords: Intermittent fasting, body composition, resting energy expenditure, energy intake.

Introduction

Intermittent Fasting (IF) approaches have been studied and proposed as a way to mimic the cycles of high and low energy consumption, characteristic of the Paleolithic era (Halberg, et al., 2005; Neel, 1962), to which our genetic profile are programmed. (Chakravarthy & Booth, 2004; Mattson, 2005; Zimmet & Thomas, 2003). Furthermore, for the last ten years, IF regimes have become popular as a successful method to lose body weight and fat mass, while sparing lean tissue (Bhutani, et al., 2013; Eshghinia & Gapparov, 2011; Eshghinia & Mohammadzadeh, 2013; Klempel, et al., 2013; Varady, et al., 2009). However, one of the concerns with the use of IF as a solution to body weight loss is the energetic overconsumption during feeding periods as observed in mice models (Anson, et al., 2003) that can lead to an unsuccessful body weight loss or even to a body weight gain, depending on the rate of energy compensation.

Despite the recent popularity of IF approaches, no one to date has studied the optimal time frame of fasting/feeding and its impact on total body mass, fat mass, lean tissue and resting energy expenditure. Therefore, there is a need to identify the ideal time period of fasting in order to achieve optimal intervention periods for such protocols.

Thus, the aim of this project is to study the impact that different lengths of fasting/feeding have on: 1) body weight; 2) body composition; 3) resting energy expenditure and 4) eating patterns during the feeding period.

The objective of this study is to do an 8 week intervention that examines the changes in body weight, body composition and in resting energy expenditure between the baseline, week four, and week eight. Furthermore, a nutritional food analysis will be conducted in order to determine if participants ingest greater energy amounts during the feeding period, as a way of compensating for the reduction of energy intake over the fast period.

Methodology

Ethical approval

The study was approved by the ethical committee of the University of Chester and participants gave their written informed consent.

Recruitment and inclusion and exclusion criteria

University students and staff were invited to take part in the study via email, and Chester residents were recruited through flyer distribution in local gyms. Exclusion criteria to select participants were: being pregnant or having been pregnant less than 6 months before the commencement of the study, pacemaker users, having diabetes, being on a diet for weight loss, history of cardiovascular disease and taking lipid or glucose lowering medications.

Study design

This was a controlled interventional study with a matched group design. Participants were allocated to four different groups by Body Mass Index (BMI) matching criteria, as follows:

Table 2: Fasting length and feeding window per group

Groups	Fasting	Feeding	Frequency of fasting
Control	8 hours (overnight)	16 hours	Everyday
16-h	16 hours	8 hours	Alternate days
20-h	20 hours	4 hours	Alternate days
24-h	24 hours	0	Alternate days

A control group was added to the study in order to compare the remaining groups against a normal eight hour overnight fast. The study was designed for nine weeks, from which the first was a control week, where data about eating patterns were collected through a 7 day food diary, with the following weeks serving as an intervention. During the remaining eight weeks of study, participants had to fast the length of which depended on the group they were allocated.

Participants received the participant information sheet (Appendix 1), informed consent (Appendix 2), food diary (Appendix 3) and food portion photographs (Appendix 4) by email. As soon as they started filling in the food diary, the control week began. At the end of the control week, participants attended an exercise physiology research laboratory at the University of Chester and brought with them signed informed consent forms and food diaries. Participants were instructed to fast overnight, to abstain from alcohol and caffeine intake for 24 hours and not to conduct any exercise for 48 hours prior to the visit. During the lab session, stature, total body mass, body composition and resting energy expenditure (REE) were measured. The same procedures were repeated again at the end of week 4 and week 8, as demonstrated in Figure 11.

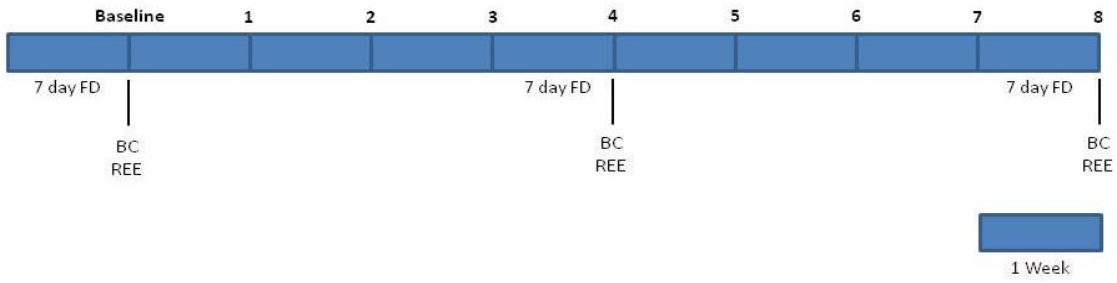

Figure 11: Study Design. FD - Food Diary; BC – Body Composition

After the completion of baseline testing, participants started the fasting periods in accordance to the group where they were allocated. Participants were free to decide at what time they wanted to start fasting but they were told to keep the same starting time during the 8 weeks. An example of a 16 hour fasting scheme is shown in Table 3.

Table 3: Example of a 16 hour fasting scheme starting at 3 pm in alternate days

	Monday	**Tuesday**	**Wednesday**	**Thursday**	**Friday**	**Saturday**	**Sunday**
Week 1		… to 7 am		… to 7 am		… to 7 am	
	From 3 pm…		From 3 pm…		From 3 pm…		From 3 pm…
Week 2	… to 7 am		… to 7 am		… to 7 am		… to 7 am
		From 3 pm…		From 3 pm…		From 3 pm…	

During feeding periods, participants were told to comply with their normal eating habits and on fasting periods, they were allowed to consume up to 25% of their daily energy requirements (DER) (with exception of the control group, who were instructed to not consume food or drinks – only water, coffee and tea without sugar - during their overnight fast).

To calculate DER, REE values were multiplied by the appropriate physical activity level (PAL) (Black, Coward, Cole, & Prentice, 1996), which was reported by the participants during baseline and fourth week assessment. Also, participants received a list of food items with the respective amount of calories supplied by a portions or serving guide (Appendix 5) with the aim of helping them control energy intake during fasting periods .

Stature and body weight

Stature was measured using a wall-mounted Harpenden stadiometer (Holtain, Crymych, Dyfed, UK) with participants in bare feet and the head positioned in the 'Frankfurt plane'. Total body mass was measured using the electronic Tanita®-scale attached to the BodPod® S/T (Life Measurement Inc., California, USA), with participants wearing swimming wear or tight sports clothes.

Body composition

Body composition was assessed with an Air Displacement Plethysmograph (ADP) device, commercially known as the BodPod® S/T (Life Measurement Inc., California, USA), which uses whole body densitometry to determine body composition. Studies regarding validity and reliability of this method have demonstrated a good agreement with that derived from hydrostatic weighing (Biaggi, et al., 1999; Fields, Goran, & McCrory, 2002; Levenhagen, et al., 1999; McCrory, Gomez, Bernauer, & Mole, 1995; Nuñez, et al., 1999), which is considered the gold standard of the densitometric methods (Collins & McCarthy, 2003).

Participants were assessed with swimming wear or tight sports clothes, with a swim cap to provide optimal compression of the scalp, and without jewelry and accessories.

Resting Energy Expenditure

REE was evaluated by indirect calorimetry based on respiratory exchange measurement using Oxycon Pro (Viasys Healthcare, Hoechberg, Germany). The device measures oxygen consumption and carbon dioxide production, and therefore it is possible to calculate the total amount of energy produced (Battley, 1995). Oxycon Pro has been shown to be a valid system to measure oxygen consumption and carbon dioxide production, achieving similar results as those obtained with a Douglas bags, the gold standard for the collection and analysis of respiratory gases (Carter & Jeukendrup, 2002).

Device calibration was done before performing each measurement, while participants were resting. A face mask was placed on participants and they were instructed to breathe normally, to lie down on their back and to maintain the same position for 30 minutes, during which time the test ran. Participants were instructed to relax but not to fall asleep during the measurement. REE data were recorded every minute but the first 25 minutes measured were discarded to reduce errors associated with excitement, restlessness and nervousness.

Energy Intake

Energy intake was assessed through food diaries as literature suggests this method is a reliable technique that produces valid results, despite its tendency to underestimate real intakes (De Castro, 1994; Mertz, et al., 1991).

Each participant completed a 7-day food diary on 3 different occasions, during the baseline week and during week 4 and week 8 of the study. Therefore, a total of 21 days of food records were collected for each participant during the 3 different lab assessments. Participants were instructed to record every consumed item (drinks included) with as much detail as possible in the food diary provided, and to include information about the time of the meal and the food preparation. Regarding portion size, participants were told to weigh the food if possible, otherwise, to estimate food quantities with the use of household measures (cups and spoons) or to describe portion sizes as small, medium or large with the aid of food portion photographs provided. Participants were also instructed to attach food labels to the food diary every time a ready-made meal was consumed.

For the analyses of food diaries, all food items were entered into the nutritional software Food Processor SQL*®, version 9.7.0. (ESHA* Research, USA) by the same individual to diminish inter-investigator bias. Food Processor SQL calculates nutritional intake based on food composition tables from the United States Department of Agriculture. Food items that were not available in the software's database were added using the food labels of the foods themselves or through information provided by McCance, Widdowson, Food Standards and Institute of Food (2002). The use of the United States of America food composition table to analyse British food diaries can introduce errors associated with different nutritional composition of foods, particularly fruits and vegetables, and due to different manufacturing processes when it comes to processed foods. Despite this, Food Processor, as determined by McCullough et al. (1999), offers a valid analysis for energy, fat, carbohydrate, protein, cholesterol, calcium, potassium, magnesium, iron and sodium, as the absolute deviations are less than 15%, with the exception of iron, in relation to a chemical analysis.

Statistical Analysis

For statistical analysis, the computer software IBM SPSS version 21 was used. Data were expressed as means ± SD. Tests for normality were included, and if the sample did not violate this assumption, a One-way Independent ANOVA test was performed to identify significant differences between groups and a One-way repeated measures ANOVA test was applied to determine significant differences between baseline, week four and week eight measures (followed by multi paired t-tests). If the sample violated the normality assumptions, a Kruskal Wallis test was conducted to determine significant differences between groups (followed by Mann-Whitney U test) and a Friedman test was applied to determine significant differences between baseline, week four and week eight (followed by Wilcoxon tests).

A significance level was set at $p \leq 0.05$ and effect-size was calculated by Cohen's *d* through the use of an online calculator (Becker, 1999).

Results

Participant's dropout and baseline characteristics

For this study 27 subjects had volunteered. From these, 7 were excluded for not meeting the criteria, 2 dropped out before the beginning of the study (one from the 20-h group and another one from the 24-h group) and 1 dropped out after the fourth week of the study (from the 24-h group). Therefore, 18 people (women=11; men=7) started the study but only 17 completed the 8 weeks of intervention (women=10; men=7).

Baseline characteristics of the participants who completed the study are shown in Table 4. Table 4 also displays data regarding stature and body weight which were used to calculate Body Mass Index (BMI), which was used as matching criteria to allocate participants into groups. A table with the allocated groups by matching BMI criteria is presented in Appendix 6.

Table 4: Participant's reported values regarding stature and body weight and characteristics at baseline from participants who finished the study by group.

	Reported Values (n=17) Mean ± SD	**Baseline Values (n=17)** Mean ± SD			
		Control (n=5)	**16-h (n=5)**	**20-h (n=4)**	**24-h (n=3)**
Age (years)	-	36.8 ± 9.8	33.4 ± 33.1	33.3 ± 15.2	41.0 ± 5.5
Stature (cm)	169.5 ± 8.7	168.4 ± 9.7	168.9 ± 6.7	174.0 ± 8.1	170.1 ± 13.3
Body Weight (kg)	71.5 ± 14.8	67.3 ± 10.4	71.2 ± 18.1	71.7 ± 12.0	76.3 ± 23.3
BMI (kg/m^2)	24.7 ± 3.3	23.7 ± 3.9	24.8 ± 4.6	23.6 ± 2.6	25.8 ± 3.9
Body Fat (kg)	-	17.6 ± 9.8	18.3 ± 5.7	19.8 ± 6.0	23.5 ± 9.7
Lean Tissue (kg)	-	49.7 ± 9.1	52.9 ± 15.8	51.9 ± 6.5	52.7 ± 15.2

Body Weight

Table 5 describes the mean body weight at baseline, week 4 and week 8 in each group. No significant differences regarding body weight were described between groups at baseline.

After 4 weeks of study, the 16-h, 20-h and 24-h groups experienced a decrease in body weight (-0.22± 0.67 kg, p=0.505; -2.48± 1.94 kg, p=0.84; and -2.0 ± 3.50 kg, p=0.433, respectively), while the control group experienced an average increase (p=0.278) of 0.94± 1.67 kg (Figure 12).

Between week 4 and week 8 assessment, a significant change (p=0.000) in body weight (-1.2 ± 0.13 kg) in the 20-h group was observed (Figure 12). At the end of the study, the average body weight change was not significant in the control, 16-h and 24-h groups (0.62 ± 1.2 kg, p=0.314; - 0.24 ± 0.86, p=0.566; - 2.97 ±4.70, p=0.389, respectively) but was significantly different (p=0.29) in the 20-h group (-3.65 ± 1.86 kg) (Figure 12). After 8 weeks of intervention, significant differences (p=0.039) between groups regarding body weight change were observed, namely between the control and the 20-h group (p=0.004) and between the 16-h and 20-h group (p=0.008) (Figure 13).

The magnitude of the effect of fasting on body weight was small in the control (d=-0.06), 16-h (d=0.01) and 24-h (d=0.14) groups, but was considered a medium effect on the 20-h group (d=0.32).

Table 5: Body weight mean values at baseline, week 4 and week 8. P value represents the differences from baseline to week 4 and week 8. Effect size was calculated by difference from baseline to week 8. B – baseline; Wk4 – week 4; Wk8 – week 8.

	Baseline	**Week 4**		**Week 8**		
	Mean ± SD (kg)	**Mean ± SD** (kg)	**p value** (Wk4 - B)	**Mean ± SD** (kg)	**p value** (Wk8 - B)	**d** (Wk8 - B)
Control (n=5)	67.3 ± 10.4	68.2 ± 11.5	0.278	67.9 ± 11.2	0.314	-0.06
16-h (n=5)	71.2 ± 18.1	71.0 ± 18.7	0.505	71.0 ± 18.9	0.566	0.01
20-h (n=4)	71.7 ± 12.0	69.3 ± 10.5	0.084	68.1 ± 10.5	0.029	0.32
24-h (n=3)	76.3 ± 23.3	74.3 ± 20.1	0.433	73.3 ± 19.2	0.389	0.14

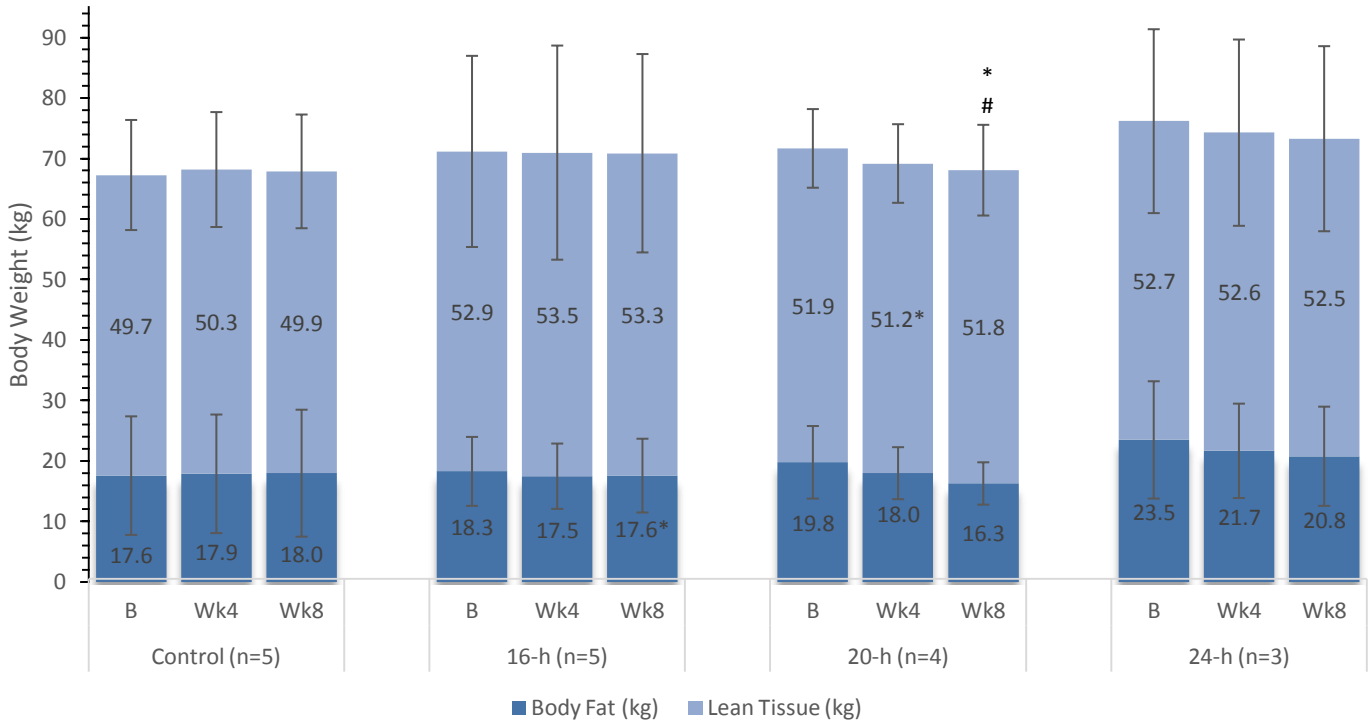

Figure 12: Body weight (and the corresponding values of body fat mass and lean tissue values) at baseline, week 4 and week 8. * Significant difference from baseline, p≤0.05; # Significant difference from week 4, p ≤0.05. B – baseline; Wk4 – week 4; Wk8 – week 8.

Body Fat

Data regarding body fat mass at baseline, week 4 and week 8 is available in Table 6. No significant differences were observed on body fat mass between groups at baseline assessment (p= 0.758).

At week 4, body fat mass decreased in the 16-h, 20-h and 24-h groups (0.74 ± 1.44 kg, p=0.316; 1.8± 1.86 kg, p=0.148; and 1.8± 3.64 kg, p=0.482: respectively) and increased (p=0.505) by 0.28 ± 0.86 kg in the control group (Figure 13). No significant changes were observed on body fat from week 4 to week 8.

After 8 weeks, a significant decrease (p=0.047) in body fat mass was experienced by the 16-h group (from 18.3 ± 5.7 kg to 17.6 ± 6.1 kg; -0.64 ± 0.50 kg). In the remaining groups, namely the control, 20-h and 24-h group, body fat change was the following: 0.34 ± 1.6 kg, p=0.656; - 3.53 ± 2.94 kg, p= 0.096; and -2.70± 4.88 kg, p=0.439 respectively. No significant differences (p=0.889) in body fat were seen between groups at the end of the study.

Regarding Cohen's effect size, *d* value suggested a small practical significance in the control (d=-0.04) and 16-h group (d= 0.12), while a medium magnitude was observed in the 20-h (d=0.71) and 24-h group (d=0.30).

Table 6: Body fat mass mean values at baseline, week 4 and week 8. P value represents the difference from baseline to week 4 and week 8. Effect size was calculated by difference from baseline to week 8. B – baseline; Wk4 – week 4; Wk8 – week 8.

	Baseline	**Week 4**		**Week 8**		
	Mean ± SD (kg)	**Mean ± SD** (kg)	**p value** (Wk4 - B)	**Mean ± SD** (kg)	**p value** (Wk8 - B)	**d** (Wk8 - B)
Control (n=5)	17.6 ± 9.8	17.9 ± 9.8	0.498	18.0 ± 10.5	0.715	- 0.04
16-h (n=5)	18.3 ± 5.7	17.5 ± 5.4	0.345	17.6 ± 6.1	0.047	0.12
20-h (n=4)	19.8 ± 6.0	18.0 ± 4.3	0.148	16.3 ± 3.5	0.096	0.71
24-h (n=3)	23.5 ± 9.7	21.7 ± 7.8	0.482	20.8 ± 8.2	0.439	0.30

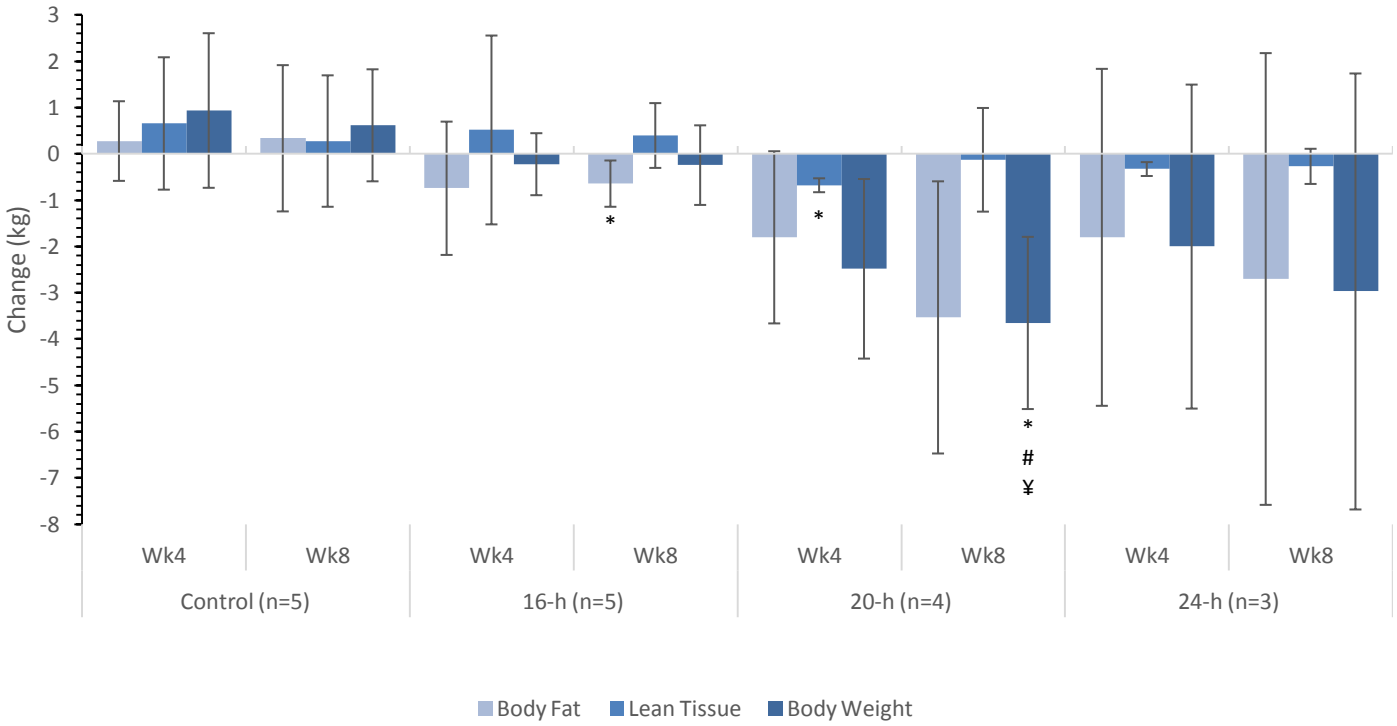

Figure 13: Changes in body weight, body fat mass and lean tissue at week 4 and week 8 by fasting group. * Significant difference from baseline, p≤0.05; # Significant difference from week 4, p ≤0.05; ¥ Significant difference from control and 16-h group, p ≤0.05. Wk4 – week 4; Wk8 – week 8.

Lean Tissue

Lean tissue values at baseline, week 4 and week 8 are shown in Table 7. At baseline, no significant differences in lean tissue were observed between groups.

After 4 weeks of intervention, the control and 16-h group saw an increase of 0.66 ± 1.4 kg (p=0.361), and 0.52 ± 2.04 kg (p=0.680), respectively. On the other hand, the 20-h group experienced a significant decrease (p=0.003) of 0.68 ± 0.15 kg and the 24-h group a reduction (p=0.199) of 0.17± 0.15 kg (Figure 13). During the last 4 weeks of study, no significant changes in lean tissue were experienced by any of the groups.

At the end of the study, no significant changes in lean tissue were experienced between groups (p=0.978). From baseline to week 8, lean tissue increased by 0.28 ± 1.4 kg in the control (p=0.680) and by 0.40 ± 0.70 kg in the 16-h group (p=0.268), and decreased by 0.13 ± 1.12 kg in the 20-h (p=0.837) and by 0.27 ± 0.38 kg in the 24-h (p=0.347) (Figure 13). A low *d* value was observed in all the groups which means that fasting had a low effect on changing lean tissue (control: d=-0.02; 16-h: d=-0.02; 20-h: d=0.01; 24-h: d=0.01).

Table 7: Lean tissue mean values at baseline, week 4 and week 8. P value represents the differences from baseline to week 4 and week 8. Effect size represents the difference from baseline to week 8. B – baseline; Wk4 – week 4; Wk8 – week 8.

	Baseline	**Week 4**		**Week 8**		
	Mean ±SD (kg)	**Mean ±SD** (kg)	**p value** (Wk4 - B)	**Mean ±SD** (kg)	**p value** (Wk8 - B)	**d** (Wk8 - B)
Control (n=5)	49.7 ± 9.1	50.3 ± 9.5	0.361	49.9 ± 9.4	0.680	-0.02
16-h (n=5)	52.9 ± 15.8	53.5 ± 17.7	0.680	53.3 ± 16.4	0.268	-0.02
20-h (n=4)	51.9 ± 6.5	51.2 ± 6.5	0.003	51.8 ± 7.5	0.837	0.01
24-h (n=3)	52.7 ± 15.2	52.6 ± 15.4	0.199	52.5 ± 15.3	0.347	0.01

Resting Energy Expenditure

Data concerning REE at baseline, week 4 and week 8 is presented in Table 8. No significant differences were observed between groups at any time. In appendix 7 is shown data about REE in kilojoules.

After 4 weeks, an increase in REE was observed in the control, 16-h and 24-h groups (79 ± 293 kcal, p=0.579; 118 ± 470 kcal, p=0.605; and 137 ± 141 kcal, p=0.233, respectively), while a decrease (p=0.74) of 331 ± 245 kcal was reported in the 20-h group (Figure 14).

At the end of the study, an increase of 164 ± 230 kcal and of 163 ± 340 kcal in the control (p=0.186) and the 16-h group (p=0.344), respectively, was observed. On the other hand, a decrease of 225 ± 258 kcal, and of 7 ± 243 kcal in the 20-h (p=0.179) and in the 24-h group (p=0.964), respectively, was reported (Figure 14). Despite no significant differences, a moderate effect size was reported regarding the effect of fasting on REE in the control (d=-0.41), 16-h (d=-0.23) and 20-h group (d=0.53).

Table 8: Resting energy expenditure mean values at baseline, week 4 and week 8. P value represents the differences from baseline to week 4 and week 8. Effect size represents the difference from baseline to week 8. B – baseline; Wk4 – week 4; Wk8 – week 8.

	Baseline	**Week 4**		**Week 8**		
	Mean ± SD (kcal)	**Mean ± SD** (kcal)	**p value** (Wk4 - B)	**Mean ± SD** (kcal)	**p value** (Wk8 - B)	**d** (Wk8 - B)
Control (n=5)	1631 ± 322	1710 ± 524	0.345	1795 ± 465	0.186	-0.41
16-h (n=5)	1588 ± 759	1706 ± 958	0.605	1751 ± 625	0.344	-0.23
20-h (n=4)	1479 ± 338	1148 ± 454	0.074	1253 ± 502	0.179	0.53
24-h (n=3)	1515 ± 683	1652 ± 543	0.233	1508 ± 675	0.964	0.01

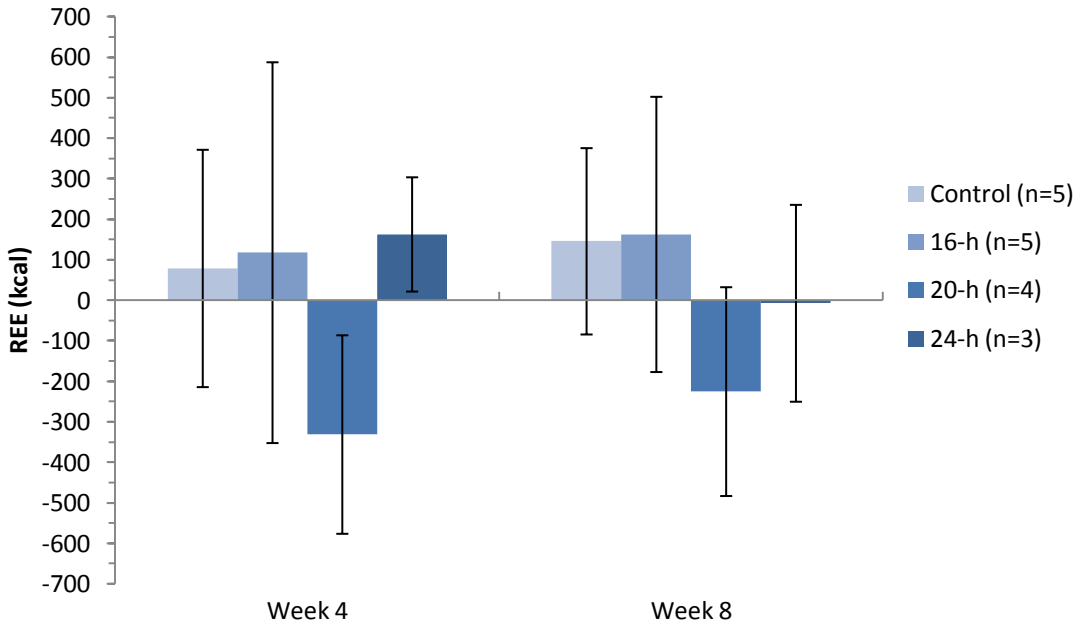

Figure 14: Changes in resting energy expenditure at week 4 and week 8 by fasting group. No significant differences within and between groups. REE – resting energy expenditure.

Energy expenditure and energy intake

Energy expenditure

Participants were provided with information concerning the energy value that they were allowed to consume during fasting periods, which represented 25% of DER (Table 9). Table 9 is presented in the appendix 8 in kilojoules.

Table 9: Daily energy requirements calculated at baseline and week 4 assessment. Daily energy requirements were calculated by multiplying resting energy expenditure by physical activity levels. Data to the control group was not calculated because they were instructed to not consume food or caloric drinks during the 8 hour of overnight fast. DER - daily energy requirements

	Baseline		**Week 4**	
	DER (kcal)	**25% of DER** (kcal)	**DER** (kcal)	**25% of DER** (kcal)
Control (n=5)	-	-	-	-
16-h (n=5)	2684 ± 935	671 ± 234	2626 ± 1484	656 ± 371
20-h (n=4)	2224 ± 519	556 ± 130	1857 ± 660	464 ± 165
24-h (n=3)	2403 ± 992	601 ± 248	2474 ± 894	619 ± 223

Energy Intake

From the 17 participants that completed the study, 3 of them did not complete food diaries during week 4 and week 8, which means that only 82% of the sample gave food diaries back.

The average of the weekly energy intake during baseline, week 4 and week 8 (including fasting and feeding periods) are described in Table 10, as well as the percentage of energy intake at week 4 and week 8 from baseline values. In the appendix 9 is shown the weekly mean of energy intake in kilojoules.

All groups, either at week 4 or week 8 were consuming less energy in relation to baseline values, showing a significant restriction in the 20-h group at week 4 (p=0.023) and week 8 (p=0.047) and in the 24-h group at week 8 (p=0.033). The energy restrictions are derived from the fasting periods and for a better understanding, see Figure 15 and 16. Moreover, a large effect size was observed in the control (d=0.83), 20-h (d=1.38) and 24-h groups (d=1.89), while a moderate effect size was reported in the 16-h group (d=0.74).

Figure 15 and 16 show the mean energy intake during 3 complete periods of fasting and 3 complete periods of feeding for each group at week 4 and week 8, respectively. From these figures it is notable that participants were able to restrict food intake during fasting periods to 25% of the daily energy requirements (Table 9).

Table 10: Weekly mean of energy intake at baseline, week 4 and week 8. P value represents the differences from baseline to week 4 and week 8. Effect size represents the difference from baseline to week 8. B – baseline; Wk4 – week 4; Wk8 – week 8.

	Baseline	**Week 4**			**Week 8**			
	Mean ± SD (kcal)	**Mean ± SD** (kcal)	**% Baseline**	**p value** (Wk4 - B)	**Mean ± SD** (kcal)	**% Baseline**	**p value** (Wk8 - B)	**d** (Wk8 - B)
Control (n=4)	18907 ± 3193	14016 ± 1094	74	0.064	16044 ± 3695	84	0.098	0.83
16-h (n=3)	18596 ±5786	14753 ± 2281	79	0.200	14962 ± 3765	80	0.192	0.74
20-h (n=4)	17219 ± 3748	11182±2238	65	0.023	12142 ± 3618	71	0.047	1.38
24-h (n=3)	14052 ± 2701	10359 ± 2171	74	0.16	9210 ± 2424	66	0.033	1.89

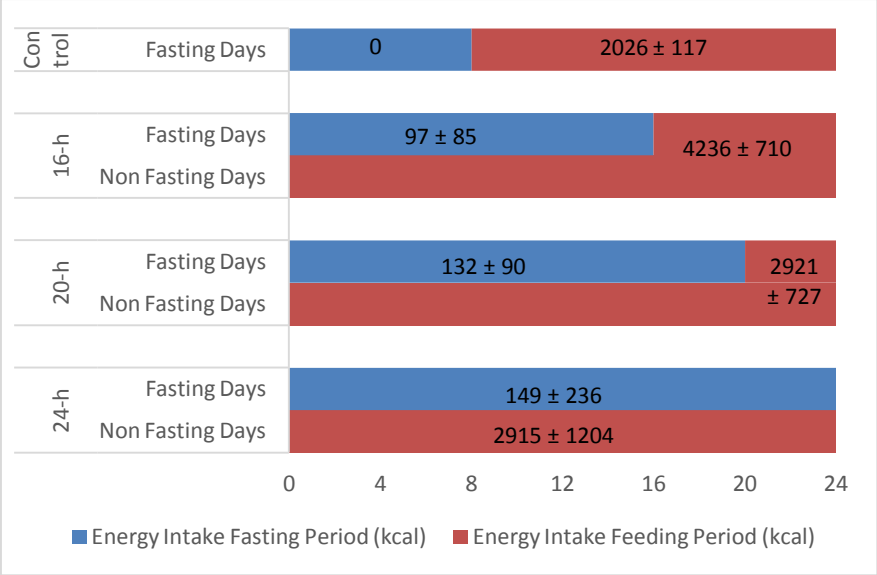

Figure 15: Average of energy intake on fasting and feeding periods at week 4 by group

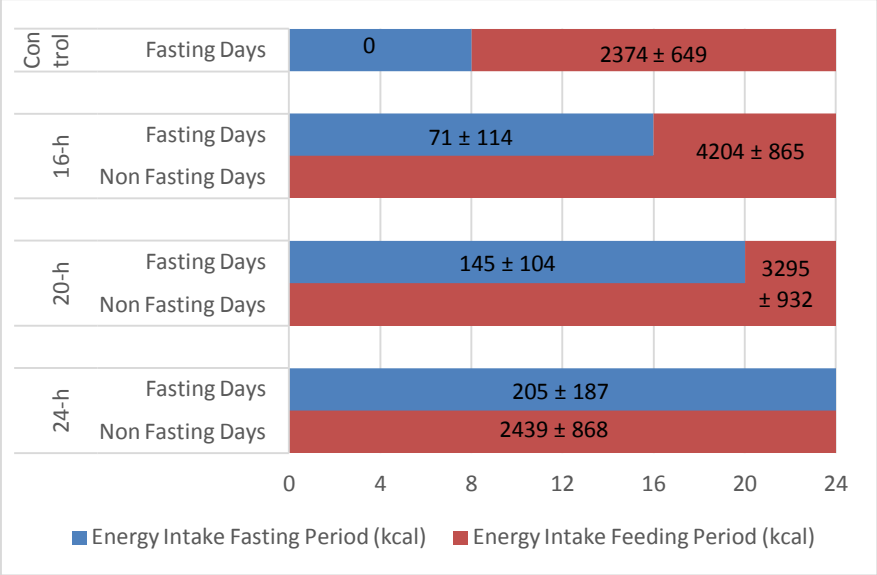

Figure 16: Average of energy intake on fasting and feeding periods at week 8 by group

Discussion

The present study evaluated the effect of different lengths of fasting on body weight, body composition, REE and eating patterns. From the presented data it can be concluded that IF regimes that include fasting lengths of 20 hours or longer are suitable for body fat loss as was previously demonstrated (Eshghinia & Gapparov, 2011; Eshghinia & Mohammadzadeh, 2013; Heilbronn, et al., 2005; Klempel, Kroeger, Bhutani, Trepanowski, & Varady, 2012; Klempel, et al., 2013; Stote, et al., 2007; Varady, et al., 2009). However, it appears that the length of fasting has influence over the amount of body fat mass lost, with higher reductions being experienced by the 20-h and 24-h groups (- 3.53 ± 2.94 kg and -2.70 ± 4.88 kg from baseline values, respectively) in relation to the control (+0.34 ± 1.58 kg) and the 16-h group (-0.64 ± 0.5 kg). In accordance with Klein, Sakurai, Romijn and Carroll (1993), a notable shift in substrate oxidation occurs between 18 and 24 hours of fasting, with a marked increase in the rate of fat oxidation and a decrease in glucose oxidation, which might explain the greater decrease in body fat by the 20-h and 24-h group. Interestingly, the 20-h group lost more body weight and body fat (3.65 ± 1.86 kg and 3.53 ± 2.94 kg, respectively) than the 24-h group (2.97 ±4.70, and -2.70± 4.88 kg, respectively). From food diary analyses it is possible to observe that the 20-h group was consuming less energy in relation to baseline values both in week 4 and week 8 (energy restriction of 35 and 29%, respectively) than the 24-h group (energy restriction of 26 and 34%, respectively) which might explain the differences between the 20-h and 24-h group regarding body weight and body fat losses. However, it is unclear whether there are other reasons that may explain these differences, and due to the absence of blood samples collection and analysis, it is not possible to clarify whether these differences were due to metabolic adaptations or hormonal alterations that might occur in the human body between 20 and 24 hours of fasting. Furthermore, the lack of literature regarding the tracking of hormones every two to three hours during the first 24 hours of fasting is notable, making these results impossible for further clarification.

The decrease in body weight indicates that participants were not compensating during the feeding period for the reduced energy intake during fasting periods, as observed by the food diary analyses. As a matter of fact, all the groups were experiencing some level of energy restriction, probably due to the knowledge of being a participant in a weight loss study. These results are opposite to the ones described in mice but in agreement with the ones stated by Klempel, Bhutani, Fitzgibbon, Freels and Varady (2010) in humans.

The mean energy restriction in week 4 and week 8 reveals a mean of 21% in the control, 21% in the 16-h, 32% in the 20-h and 30% in the 24-h group in relation to baseline values. Theoretically, if participants had kept the same energy restriction during the entire study, body weight would have decreased by 4.5 kg in the control, 4.5 kg in the 16-h, 6.3 kg in the 20-h and 4.8 kg in the 24-h group, which reveals a gap between the actual weight lost and the energy restriction reported, which have also been identified by Klempel et al. (2010).

However, the equation which dictates that energy potential of body mass remains constant when caloric intake equals caloric expenditure (Change in energy stores = energy intake - energy expenditure) is only truth in closed systems and not in open systems, like the human body (McArdle, Katch, & Katch, 2009). Furthermore, different macronutrient distribution on a diet lead to different biochemical pathways and consequently to different rates of weight loss (Feinman & Fine, 2003). Therefore, the divergence observed between the actual weight loss and the energy restriction reported are more than just an underreporting case.

Results from the food diaries also revealed that participants were able to keep energy intake during the fasting periods below 25% of energy requirements, as was instructed. Despite no significant differences being observed regarding energy intake between groups, a trend towards consuming more energy as the length of fasting increased was observed. Similar percentage of non compliant participants with the food diaries has also been observed by Klempel et al. (2010).

One of the benefits attributed to IF regimes in relation to daily calorie restriction diets is the preservation of lean tissue, as evidenced by the low effect size reported in this study. Other studies have previously demonstrated this sparing effect in lean tissue (Bhutani, Klempel, Berger, & Varady, 2010; Varady, 2011; Varady, et al., 2009).

The decrease in body weight and in fat mass achieved by the 20-h group (5 ± 2% and 3.53 ± 2.94 kg, respectively) does not agree with the values obtained by Soeters et al. (2009) and by Halberg et al. (2005), who did not find any significant differences in body weight and fat mass after two weeks of 20 hours of fasting on alternate days. However, it is important to highlight that these studies were not intended to achieve changes in body composition and that participants were instructed to consume food to maintain body weight. On the other hand, the changes in body weight and body fat mass experienced by the 20-h group were greater than the ones reported in the study of Stote et al. (2007). The results reported by the 24-h group were similar to the ones described by Heilbronn et al. (2005).

No significant differences were observed in REE at any time, as previously shown by Heilbronn et al. (2005), however, a strong effect size regarding the impact of fasting on REE was observed. Nevertheless, it is important to point out that the face masks used to measure REE were all of the same size, being possibly too big for some participants, thus allowing room for air to escape, which could have negatively affected the measurement.

This is the first study to appraise the impact of different lengths of fasting and feeding on body weight and body composition and to report its effects on REE.

A key limitation of this study was the size of the sample which is too small to draw strong conclusions from the observed results. Despite this, some significant differences were observed, particularly in the 20-h group, which means that more investigation should be conducted in this field.

In addition, the study was conducted during summer time, so most of the participants were on holiday, which may have introduced alterations in the normal routine of physical activity and eating patterns.

Also, the alternate day regime was not accomplished by all the participants as evidenced by the food diary analyses, with the tendency to skip periods of fasting on Sundays, possibly because it is a day associated with family-time meals.

Conclusion

It is suggested by the data presented in this paper that IF regimes with a length of fasting of 20 hours in alternate days are more efficient than true intermittent fasting protocols normally used (24 hours of fasting in alternate days). However, more research in this area is needed, specifically a similar design study using a bigger sample and one that involves the collection of blood samples from participants in order to achieve a better understanding of metabolic adaptations in response to different lengths of fasting.

Furthermore, it appears to be extremely important to keep records of food intake during the entire length of the study, or at least, a record of the weekly number of fasting days accomplished.

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Appendices

Appendix 1

Participant information sheet

Participant Information Sheet

Intermittent Fasting: How long is enough?

You are being invited to take part in a research project. Before you decide, it is important that you understand why the research is being done and what it will involve. Please read the following information carefully and ask for clarification, if there is something that is not clear. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

Intermittent fasting is getting a lot of attention lately as a viable option to lose body weight and change body composition. The aim of this study is to determine if there are optimal fasting periods during intermittent fasting approaches.

Do I have to take part?

It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. Even if you decide to take part on the study, you are free to withdraw at any time and without giving an explanation.

What will happen to me if I take part?

You will participate in a study lasting nine weeks, of which the first will be a control week and the following will be intervention weeks. In the control week, your height, body composition (total body weight, fat mass and fat free mass) and resting metabolic rate will be measured. This procedure will be repeated again at week four and week eight. In order for this to be possible, you will have to

come to University of Chester sports laboratory (on three different occasions, at the start, week four and week eight) after an overnight fast.
During the eight weeks of intervention, you will have to do fast periods in alternate days, which length depend on the group where you were allocated:

Group 1 - 8 hours of fast/16 hours of feeding everyday (overnight fast);

Group 2 - 16 hours of fasting /8 hours of feeding in alternate days;

Group 3 - 20 hours of fasting/4 hours of feeding in alternate days;

Group 4 - 24 hours of fasting in alternate days;

Allocation in groups will be made by matching criteria such as gender and body mass index.

What are the possible disadvantages and risks of taking part?

In some cases fasting periods can cause general tiredness, weakness, dizziness, headaches, stomach aches and in extreme events even fainting. In addition, you can suffer from stress and inadaptation due to changes in your lifestyle caused by fasting periods. However, please rest assured that these side effects only occur in a few people and most people do not experience the extreme versions of these. You might need a couple of days to adjust to the fasting period.

What are the possible benefits of taking part?

It is expected that you will change your body composition mainly by decreasing body fat. If this happens, you will benefit from a series of improvements in health, which are characteristic of body fat loss, such as improving in lipid profile, less fatigue, more energy, etc..
You will also receive a detailed body composition analysis, as well as a nutritional analysis of your food intake.

What if something goes wrong?

If you feel concerned over any aspect about the way you have been approached or treated during the course of this study, you can contact Professor Sarah Andrew, Dean of the Faculty of Applied Sciences, University of Chester, Parkgate Road, Chester, CH1 4BJ, 01244 513055.

Will my taking part in the study be kept confidential?

All information collected during the course of the research will be kept strictly confidential so that only the researcher carrying out the research will have access to it.

Assessment in the laboratory is going to be individual and none of the measures will be revealed to anyone except yourself.

In addition, to perform statistical analysis of the data, participants' names are going to be replaced by codes and thus, the researchers will have no knowledge of the connection between the codes and the real person. This step ensures the confidentiality of the data since these can not be associated with a name.

What will happen to the results of the research study?

The results will be written up into a dissertation as a final project from my MSc. Individuals who participate will not be identified in any subsequent report or publication.

Who is organising the research?

The research will be conducted as part of a MSc in Exercise & Nutrition Science within the Department of Clinical Sciences at the University of Chester. The study is organised with supervision from the department, by Andreia Filipa Luís de Castro, an MSc student.

Who may I contact for further information?

If you would like more information about the research before you decide whether or not you would be willing to take part, please contact:

Name: Andreia Castro;

Email: 1219680@chester.ac.uk

Thank you for your interest in this research.

Appendix 2

Informed consent

Intermittent Fasting: How long is enough?

Consent Form

Please initial box

1. I confirm that I have read and understand the information sheet for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason and without my legal rights being affected. ☐

3. I agree to take part in the above study. ☐

Name of Participant	Date	Signature
Researcher	Date	Signature

Appendix 3

Food diary

Intermittent Fasting: How long is enough?

Food Diary Instructions

Write down everything you eat and drink over the day.

Time: Write the time of day you ate the food and which meal it represents for you, i.e. breakfast, morning snack, dinner, etc.

Description of food and/or drink: Write down the type of food and/or drinks you consumed. Give details and be as specific as you can, differentiating between foods of the same type but that are different in content, eg wholemeal vs. white bread or freshly squeezed orange juice vs. orange juice made from concentrate. Include information about the way that food was prepared, for example, fryed, boiled, baked and steamed. Don't forget to write down "extras," such as salad dressing, mayonnaise, butter, sugar and ketchup.

Portion Size: Try to weigh and measure what you eat and drink. If you don't have a scale for this, estimate the quantities using containers (1 cup, 1 mug, a level tea spoon or heaped cereal bowl) or use the food portion photographs that are attached to these instructions in order to describe portion sizes (small, medium or large). If you have consumed a ready made meal, then attach the food label to your food diary for later analysis.

Some basic rules to remember:

- Try to be as honest as possible and **record everything you eat and drink** (remember that this information is confidential).
- It's hard to remember what you've eaten at the end of the day, so try to record things as you go.

Physical Activity: Write down the time and duration of exercise performed

Please use the following example as a guideline to a proper filling of your food diary.

Time	Description of food and/or drink	Portion Size
8 am Breakfast	Rolled oats (microwaved with water) Cinnamon Frozen raspberries Honey Water	40g Teaspoon 100g 1 tablespoon 1 glass
11 am Morning snack	Red Apple Almonds Coffee Sugar	Medium size 10 units 200 ml 2 teaspoons
1.30 pm Lunch	Wholemeal bread Tuna in brine Cottage cheese Tomato Lettuce Water	2 thick slices Small can 2 tablespoons Small size 1 leaf 330 ml bottle
4.30 pm Afternoon snack	Fat free Greek yoghurt Almonds Coffee Sugar	1 cup 10 units 200 ml 2 teaspoons
7 pm Dinner	Basmati rice boiled Chicken breast roasted Broccoli steamed Olive oil	Small portion Medium portion 2 cups 1 tablespoon

Intermittent Fasting: How long is enough?

Food Diary

Week:

Monday

Time	Description of food and/or drink	Portion Size

Physical Activity:

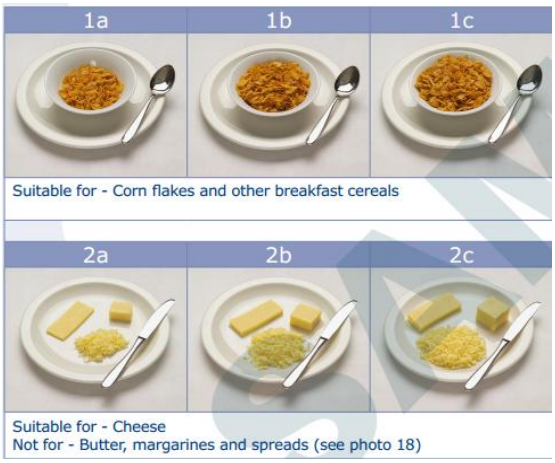
Appendix 4

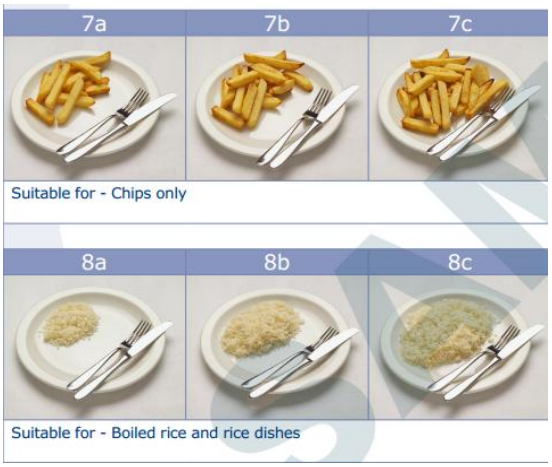
Food portion photographs

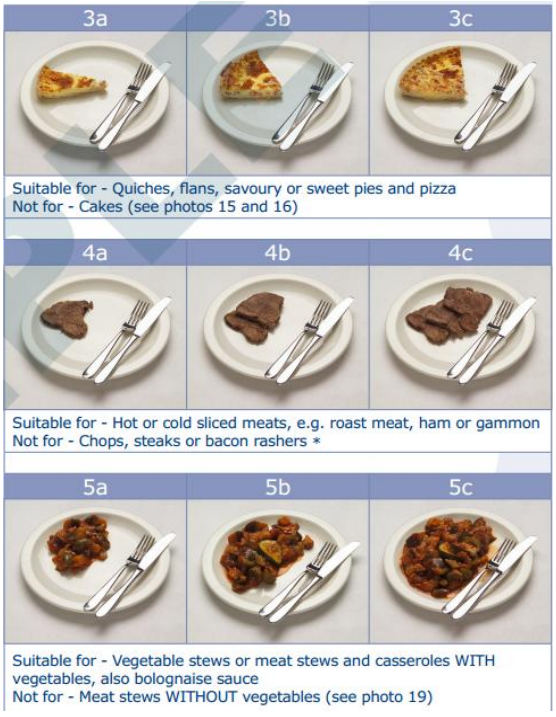
University of Chester

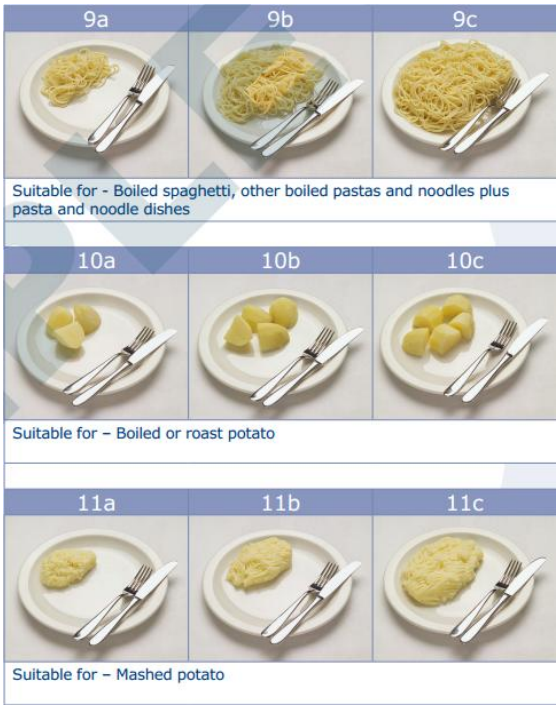
Food Portions Photographs

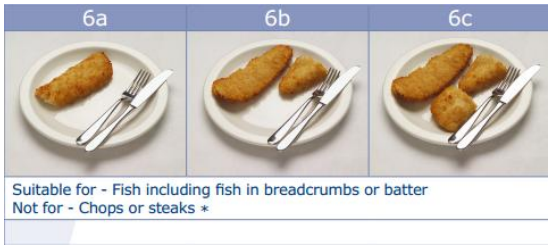
Intermittent Fasting: How long is enough?

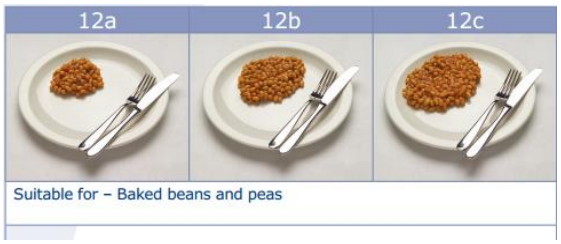


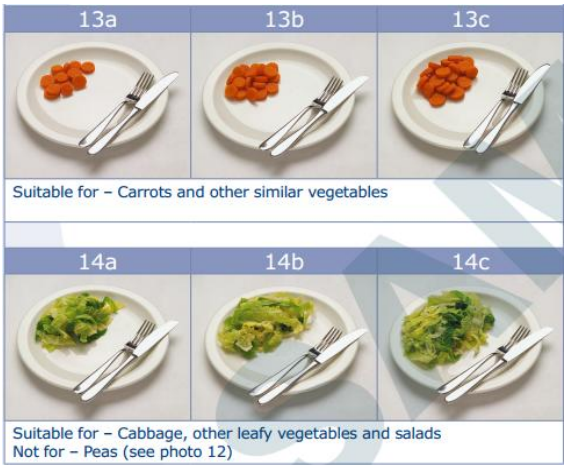


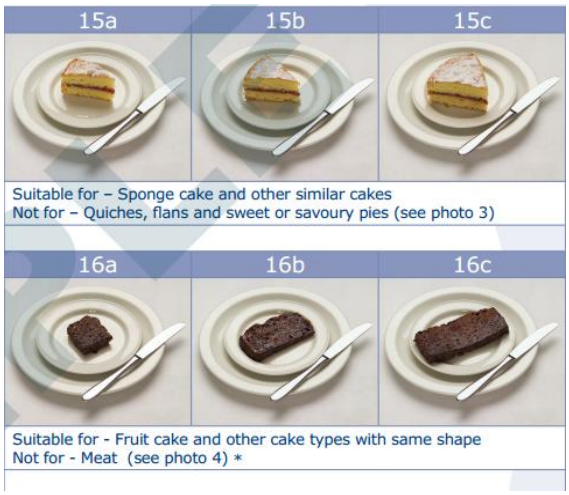


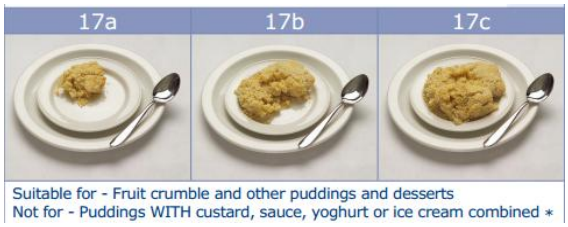


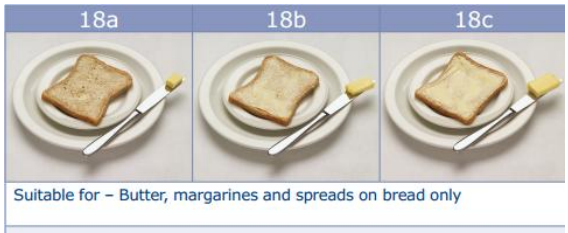


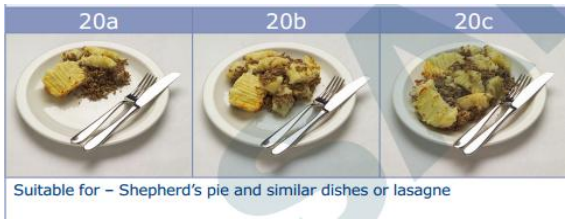












Adapted from: EPIC/CNC study. Food Diary. *Available at*:

http://dapa-toolkit.mrc.ac.uk/documents/en/non/non-study_EPIC_diary.pdf

Appendix 5

Food List

Intermittent Fasting: How long is enough?

During your fasting period, If you feel weak, dizzy, or have headaches then please consume foods in small quantities. These foods may be from the following options.

You will be provided with an individualised total amount of energy that you can consume from this list. This amount will be no more than 25% of your total energy requirements.

Daily Energy Requirements: _____

25% of Daily Energy Requirements: _____

Food List

Food	Total Energy (Kcal)
Fruit	
Apple (medium size)	70
Banana (medium size)	120
Pear (medium size)	60
Avocado (medium size)	230
Strawberries (10 medium units)	40
Raspberries (20 units)	20
Grapefruit (medium size)	100
Tomato (medium size)	25
Vegetable	
Lettuce (medium portion)	10
Spring Onions (medium size)	5
Cucumber (medium size)	24
Celery (1 medium stalk)	6
Beetroot (medium size)	45
Legumes	
Baked beans (small can)	205
Chickpeas (3 tbsp)	60
Peas (3 tbsp)	45
Cereals	
Oats (30g uncooked)	126
Rice (100g cooked)	130
Pasta (75g uncooked)	175

Bread (2 slices)	220
Meat/ Fish/ Egg	
Tuna in Brine (canned)	140
Egg	80
Chicken breast (grilled)	145
Nuts and dry fruits	
Raw/toasted Almonds (10 units)	60
Raw/toasted Cashews (10 units)	55
Raw/toasted Macadamia nuts (10 units)	200
Walnuts (10 units)	260
Pistachio (25 units)	80
Pecan (10 units)	195
Raisins (25 units)	40
Apricots (5 units)	95
Figs (5 units)	100
Prunes (5 units)	210
Cranberries (25 units)	123
Drinks	
Almond milk - unsweetened (250 mL)	35
Soya milk - unsweetened (250 mL)	70
Dairy	
Semi-skimmed milk (250 mL)	150
Skimmed milk (250 mL)	110
Whole milk (250 mL)	200
Yogurt Low Fat (125mL)	65
Cheddar Cheese (1 slice)	110
Cottage cheese – low fat (portion 28g)	24

If the symptoms still persist after the consumption of these foods, then please contact the researcher on the following email address: *1219680@chester.ac.uk* for further instructions.

Appendix 6

Allocation in groups by BMI matching criteria

Allocation in groups by BMI matching criteria

Control		16-h		20-h		24-h	
Gender	**BMI (kg/m^2)**	**Gender**	**BMI (kg/m^2)**	**Gender**	**BMI (kg/m^2)**	**Gender**	**BMI (kg/m^2)**
Female	22.2	Female	22.9	Female	22.7	Female	22.3
Male	24,1	Male	26.6	Male	26.2	Female	26.9
Male	23.1	Female	23.1	Female	22.9	Female	22.8
Female	30.3	Male	31.1	Female	30.5	Male	30.4
Male	22.1	Female	20.9	Female	21.2	Female	21.0

The highlighted cells represent the participants that have dropped out

Appendix 7

Resting energy expenditure mean values at baseline, week 4 and week 8 in kilojoules

Resting energy expenditure mean values at baseline, week 4 and week 8 in kilojoules

	Baseline	**Week 4**	**Week 8**
	Mean ± SD (kJ)	**Mean ± SD** (kJ)	**Mean ± SD** (kJ)
Control (n=5)	6524 ± 1288	6840 ±2096	7180 ± 1860
16-h (n=5)	6352 ± 3036	6824 ± 3832	7004 ± 2500
20-h (n=4)	5916 ± 1352	4592 ± 1816	5012 ± 2008
24-h (n=3)	6060 ± 2732	6608 ± 2172	6032 ± 2700

Appendix 8

Daily energy requirements at baseline, week 4 assessment in kilojoules

Daily energy requirements at baseline, week 4 assessment in kilojoules

	Baseline		Week 4	
	DER (kJ)	**25% of DER** (kJ)	**DER** (kJ)	**25% of DER** (kJ)
Control (n=5)	-	-	-	-
16-h (n=5)	10736 ± 3740	2684 ± 936	10504 ± 5936	2624 ± 1484
20-h (n=4)	8896 ± 2076	2224 ± 520	7428 ±2640	1856 ± 660
24-h (n=3)	9612 ± 3968	2404 ± 992	9896 ±3576	2476 ± 892

Appendix 9

Mean energy intake at baseline, week 4 and week 8 in kilojoules

Mean energy intake at baseline, week 4 and week 8 in kilojoules

	Baseline	Week 4	Week 8
	Mean ± SD (kJ)	**Mean ± SD** (kJ)	**Mean ± SD** (kJ)
Control (n=4)	75628 ± 12772	56064 ± 4376	64176 ± 14780
16-h (n=3)	74384 ± 23144	59012 ± 9124	59848 ± 15060
20-h (n=4)	68876 ± 14992	11182 ± 8952	48568 ± 14472
24-h (n=3)	56208 ± 10804	41436 ± 8684	36840 ± 9696