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Intermittent fasting's impact on autophagy, insulin sensitivity and cortisol in a clinical setting

A Systematic Review

Metabolism

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Abstract

Background

Intermittent fasting (IF) is a method of voluntarily abstaining from food and fluid containing nutrients, during a set period of time. IF has in various forms been shown to have implications on human physiological processes, as certain conditions - nutrient deprivation turns the body into catabolic metabolism which shifts the body to use its own resources - are met in the brain and body for a certain amount of time. Many of which can lead to long-term benefits in the sense of fighting neurological, cardiometabolic and liver diseases, but also promoting sleep, mental focus and the general quality of life. But exactly to what extent, and depending on how long of a fast it takes, is uncertain.

Aim

The purpose of this systematic descriptive review was to summarize the current evidence of the impact IF has on the physiological processes: autophagy, insulin sensitivity and cortisol in a clinical setting.

Methods

Articles were searched on PubMed with the following eligibility criteria in mind: participants were human adults (≥18years of age); (ii) were of an experimental or observational design; (iii) included data on the following outcomes: insulin sensitivity, autophagy and cortisol. The review followed the guidelines of PRISMA and used EPHPP as a quality assessment tool. After screening, thirteen articles met the eligibility criteria.

Results

The findings revealed in most cases that IF, depending on its window length of fasting, has increasingly significant effects on markers of autophagy, insulin sensitivity and cortisol.

Conclusion

Because the literature is currently scarce, and some of the methods measuring these processes are but most indirect, more research needs to be conducted on the topic to provide a more descriptive account of this territory.

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Abbreviation List

AUC; Area under the curve

iAUC; incremental area under the curve

CR; Caloric Restriction

EPHPP; Effective public health practice project

HOMA-IR; homeostasis modelling assessment of fasting insulin resistance

HOMA (%S); homeostasis modelling assessment of fasting insulin sensitivity

HOMA (%B); homeostasis modelling assessment of fasting β-cell function

LC3-I; Microtubule-associated protein 1A/1B-light chain 3

LC3-II; LC3-phosphatidylethanolamine conjugate (LC3-PE/LC3II)

TOR; Target of Rapamycin

mTORC1: mammalian Target of Rapamycin Complex 1

mTORC2: mammalian Target of Rapamycin Complex 2

PRISMA; Preferred reporting items for systematic reviews and meta-analysis

TRE; Time Restricted-Eating

TRF; Time Restricted Feeding

eTRF; early Time Restricted Feeding

IF; Intermittent Fasting

1. Introduction

1.1 Fasting: An evolutionary perspective

The concept of "fasting" can be defined as the voluntary process of abstaining from food and drink for specified, recurring, periods of time (Phillips, 2019).

Throughout evolution, the organisms able to adapt and tolerate a deficit of nutrients for extended periods of time, held a survival advantage over those organisms unable to do so. The evolutionary process with its mechanism of natural selection has therefore over time led to choosing organisms inherent in traits that enable survival in low-energy environments, and which further has led to several fasting-induced metabolic mechanisms to have been conserved in humans for millions of years (Brandhorst & Longo, 2016).

Humans in history, before the agricultural revolution, have endured periodic phases of food scarcity (Crittenden & Schnorr, 2017). Humans derive from a hunter-gatherer way of living, that is lasted for almost two million years, and it was only up till 12,000 years ago that a transition to agricultural means occurred and food scarcity has been reduced globally. A relatively short time compared to the previous era, and may not be adequate enough for humans to have adapted to the streamlining of the food supply provided by farming (Harari, 2015).

The commonly established pattern of eating in most modern societies remains to three or more meals per day (World Health Statistics, 2018). As potential health benefits of a periodic scarcity of nutrients have been recognized, the more current uprising niche of fasting can be explained (Phillips, 2019).

1.2 Fed state and fasting state

Fasting- and fed state establish different biological conditions in the body. The Fed state being the phase where food is still being emptied from the gastrointestinal (GI) tract, and nutrients are still being absorbed. This state lasts about 4 hours or more after eating has begun. Fasted state or postabsorptive state, follows the fed state. When the GI tract is empty the body shifts from anabolism to catabolism, breaking down its reserves of nutrients to supply itself with energy. Further, the biosynthesis of smaller constituents that build up fats, glycogen and proteins, are led to an end (Marieb & Hoehn, 2019).

This is primarily in order to maintain stable blood glucose levels within the homeostatic range (70-110mg/100ml), to provide the brain with enough energy, as its primary resource is glucose. Therefore the majority of the processes of the fasted state are either to (i) make adequate glucose circulate in the bloodstream or (ii) shift certain organ's metabolism to using fats instead of glucose, to prioritize glucose-using-organs (Marieb & Hoehn, 2019). What further sets into stage are numerous processes that are more prevalent in the body during a catabolic metabolic state. As fasting works as an intermittent stressor, one will experience activation of autophagy, sirtuins, AMPK, ketosis and many more, many that won't be discussed further in this review. Because fasting works as any stressor, stress hormones such as adrenalin, nor-adrenalin and cortisol will become secreted in the body, making the individual more prone to seek out food. Also, because the body is set in a prolonged altered glucagon-insulin relationship, it has been hypothesized that with less stimulation of the cells to secrete insulin and therefore less blood glucose circulation in the system for a long time, it could have later beneficial effects on the cells insulin sensitivity (Laand, 2018).

1.3 Intermittent fasting/Caloric restriction/Time Restricted Feeding

Intermittent fasting (IF) is a method of voluntarily abstaining from food and fluid containing nutrients, during a set period of time. Caloric restriction (CR) is a method of energy deprivation where one voluntarily undertakes a negative energy balance (Gardner et al., 2018). Many fasting strategies fall under the category of IF. These methods can include or exclude CR in their daily fast. One of these methods being time-restricted feeding or eating (TRF or TRE) which is the term that will be used to describe the overall plan of restricting one's eating window to a particular phase of each 24 hours (usually 12 or less) or in some cases, particular days within a given week (Gardner et al., 2018).

TRF in various forms has been shown to have implications on human physiological processes, as certain conditions - nutrient deprivation turns the body into catabolic metabolism which shifts the body to use its own resources - are met in the brain and body for a certain amount of time. Fasting affects pathways such as autophagy, nutrient-sensing, metabolism of glucose and fatty acids, and many more. Many of which can lead to long-term benefits in the sense of fighting neurological, cardiometabolic and liver diseases, but also promoting sleep, mental focus and the general quality of life (Manoogian et al., 2021).

CR dramatically improve longevity, physiology and metabolic markers of health. Comparatively, study wise CR has a greater amount of evidence speaking to its ability to improve risk profiles. But fasting in its different iterations has benefits of increasing compliance. The more concentrated and shorter bouts of calorie restriction may be preferred as it among other things mitigates the hunger that normally is involved in CR (Horne et al., 2015).

1.4 Autophagy

Autophagy is a process of self-degradation in response to low levels of nutrients and it is important to balance energy. As nutrient deposits are scarce when one has entered a catabolic state, the body shifts into using its own material to resynthesis energy and does its own “housekeeping” by recycling cellular debris. Autophagy translates from the Greek work *Autóphagos* meaning eating of the self or self-digestion. As well as its importance in energy balance it also serves a vital function of degrading misfolded proteins, damaged organelles and the removal of pathogens. The activity of autophagy in the cell is modulated by the nutrient and energy sensing proteins mammalian Target of Rapamycin (mTOR) and adenosine 5’-monophosphate-activated kinase (AMPK). mTOR is activated by growth signals, insulin and nutrients and inhibits autophagy. AMPK senses low levels of energy and inhibits mTOR which leads to an increased activation of autophagy (Glick et al., 2010).

1.4.1 Biomarkers affecting autophagy - ULK1, ATG, LC3, p62

Rapamycin has been found to bind to a certain complex of proteins in the cells, which has been named mTORC1, mTORC1 should be distinguished from mTORC2 (which rapamycin does not bind to). When there is nutrients in the system mTORC1 will bind and phosphorylate ULK1 (Unc-51 like autophagy activating kinase), this will inhibit the formation process of autophagosomes that are the driver of autophagy (Laand, 2018).

Further, during the lysosomal degradation pathway of autophagy in mammalian cells, there are several components that together serve as origin for the development of the autophagosomes that engulfs cytoplasmic components, including cytosolic proteins and organelles. Some of them being autophagy-related genes(ATG) that help in the formation of microtubule-associated protein 1A/1B-light chain 3 (LC3) a precursor to the autophagosome membrane (Tanida et al., 2008; Green & Levine, 2014).

More specifically LC3's cytosolic form Light Chain 3-I (LC3-I), which is later conjugated with the molecule phosphatidylethanolamine, that afterwards forms into LC3-phosphatidylethanolamine conjugate (LC3-PE/LC3II), is attached as a component to the autophagosome membrane. The autophagosome then fuses with either lysosomes or endosomes to degrade organelles, proteins and pathogens. One can therefore point out that LC3-II is the only protein of autophagy that stably associates with the autophagosome in its mature state, and therefore often used as a measure of autophagy (Tanida et al., 2008; Green & Levine, 2014).

p62 is an intracellular protein which is stress-inducible and known to be found in the regulation of various signal transduction pathways of many cellular life/death activities, including autophagy. Autophagy modulates the level of p62 by degrading it in the process, p62 can also suppress autophagy through the activation of mTORC1 and accumulates with the inhibition of autophagy. Therefore would an increase in p62 be an indirect marker for a reduction in autophagy, and a decrease in p62 would indirectly mark an increase in autophagy (Komatsu et al., 2012).

1.5 Insulin sensitivity - resistance

Increases in serum insulin lead to increased glucose-uptake by adipose tissue and skeletal muscle. It also inhibits the release of fatty acids from adipose tissue and inhibits the release and production of glucose from the liver. Insulin sensitivity (IS) means how well the target tissues respond to insulin and therefore insulin's ability to clear out glucose from the blood. The response to decreased IS also known as insulin resistance (IR) is increased production and release of insulin (Shenck et al., 2008). Insulin resistance is a fundamental component of the development of Diabetes type 2 in most cases. Lifestyle changes that increase insulin sensitivity and improve glucose disposal can be implemented to prevent the development of diabetes type 2 (Fujimoto., 2000).

1.5.1 Measurements of Insulin - Homeostatic Model Assessment

Homeostatic Model Assessment (HOMA) is a mathematical method that one can use to assess IS, IR and beta-cell function. This occurs by taking samples of fasting blood glucose and insulin. The fasted state's relationship between insulin and glucose reflects the balance between insulin secretion and hepatic glucose output (from which HOMA is used). This relationship is maintained by a homeostatic feedback loop between the beta-cells and the liver. The HOMA model is a derivate from the mathematical assessment of interactions between IR and beta-cell functions in a standardized model which one then use to compute the steady-state insulin and glucose concentrations. The choice of method that one use to assess beta-cells or IR levels usually depends on the type and size of the study. As clamps (described in later paragraph) are more suitable techniques for physiological studies that are of smaller size, HOMA is a simpler tool which is more suitable for larger epidemiological studies. (Wallace et al., 2004).

Further, there are different HOMA models, one being HOMA-IR which assess insulin resistance. There are also HOMA that assess insulin sensitivity (HOMA %S) and β-cell function (HOMA %B). Where both HOMA-IR together with HOMA%S reflect hepatic rather than peripheral insulin sensitivity. Thus improvements of insulin sensititivty in the liver corresponds to decreased concentrations of insulin in fasted state and an increase of the process gluconeogenesis from amino acids and glycerol (Wallace et al., 2004).

1.5.2 Measurements of Insulin - Hyperinsulinemic-euglycemic clamp

As mentioned, measurements of insulin can also be derived from another method called an insulin clamp (hyperinsulinemic-euglycemic clamp). This is known as the “gold standard” method in the scientific community for the assessment of insulin in vivo and may be more suitable for studies of smaller size. The method is performed by acutely raising plasma insulin concentration to 100microU/ml by a continuous infusion of insulin and then maintaining it. At the same time, the plasma glucose concentration is maintained at a constant basal level of a variable of glucose infusion. As a steady-state is achieved, the rate of glucose infusion is equivalent to the glucose uptake in all the tissues in the body and hence a measure of tissue insulin sensitivity (Ayala et al., 2011).

1.5.3 Measurements of Insulin - Glucose tolerance test and area under the curve

Oral glucose tolerance test (OGTT) is a method of measuring blood glucose response. The area under the curve (AUC) of the OGTT is used to diagnose impaired glucose tolerance. AUC doesn´t take differences in baseline glucose into consideration. Incremental Area under the curve (iAUC) is a variation of AUC that subtracts individual baseline glucose values (Cheng et al., 2015).

1.6 Cortisol

Cortisol is considered a stress hormone. The secretion of cortisol is to an extent controlled by the circadian rhythm. Cortisol is elevated in the morning during the waking process and is the lowest before we go to sleep. It acts immunosuppressive and regulates metabolism in favor of releasing energy from storage and the synthesis of glucose from lipids and amino acids. Long term exposure to elevated cortisol can lead to effects like increased subcutaneous and visceral fat, increased insulin resistance, hypertension and muscle atrophy.
Cortisol can be measured through blood, urine and saliva (Manenschijn et al., 2011).
Dysregulation of cortisol secretion may have detrimental effects to sleep quality. Where a larger difference between cortisol levels during sleep onset and duing awakening showed better subjective sleep quality (Pulopulos et al., 2020).
Rodent studies have shown a delay of 2 hours for the peak secretion of corticosterone, the rodent hormone closely similar to human cortisol. While human studies show longer fasts to shift the peak secretion from morning to afternoon with higher mean values (Kim et al., 2021).

1.7 Modern times

In a modern time of rising healthcare costs and an increasing prevalence of neurological disorders and metabolic diseases, it would serve the individual to have a cost-free, effective therapeutic option for a range of metabolic and neurologic disorders, as an addition to a way of treatment for physicians and healthcare professionals (Phillips, 2019). Deficient autophagy has been seen to be related to a wide variety of diseases, for example neurodegenerative disorders such as a Alzheimer's and Parkinson's diseases (Marieb & Hoehn, 2019). One of the biggest metabolic diseases is diabetes mellitus (DM) which results from either a hyposcretion or hypoactivity of insulin. In the absence of insulin the body results in a type 1 diabetes, and if insulin is secreting normaly but doesn’t have adequate effects enough, it results in type 2 diabetes. But in both cases blood glucose remain high because it is unable to enter the cells. With fasting working as a potential tool for altering glucagon-insulin balance and later on, cells insulin sensitivity this could potentially be working as a preventive intervention for developing diabetes amongst other metabolic diseases. Even though fasting might have increasing beneficial effects on the body, it is still a stressor and therefore one have to take into account the elevated concentrations of stress hormones that are secreted in this process. Especially if individuals already have other buffering stressors affecting them from other areas of their everyday life as well, an intervention such as this might not be suitable. An understanding of the cortisol secretion in relation to fasting could be as important as understanding the concentrations of the beneficial markers, as too much stress can have deletarious effects on the system as well (Marieb & Hoehn, 2019). But for an adequate application of these strategies, a proper collection and presentation of the current results regarding IF and the health beneficial effects it could have in humans needs to be displayed, something which this study will seek to provide.

1.8 Aim

The aim of this study is to summarize the current evidence for the effect of different forms of fasting on autophagy, insulin sensitivity and cortisol in adult humans in a clinical setting.

- Does a prolonged length of abstaining from food, effect the concentrations of markers for autophagy, insulin sensitivity and cortisol in adult humans in a clinical setting?

2. Methods

This systematic descriptive review was conducted according to the international PRISMA statement - The PRISMA 2020 statement: an updated guideline for reporting systematic reviews (Prisma Statement, 2021).

2.1 Eligibility criteria

The articles that were included are all clinical trial nature, investigating the relationship between intermittent-fasting, and their following physiological outcomes. The studies were included (i) if the study participants were human adults (≥18years of age); (ii) were of an experimental or observational design; (iii) included data on the following outcomes: insulin sensitivity, autophagy and cortisol. Also any studies that were of the meta-analysis and literature review design were excluded.

2.2 Information sources and Search strategy

All of the data was extracted from the database of Pubmed. No restrictions were set in terms of publication dates of the articles that were searched, this was due to the scarcity of literature on the topic. Therefore all literature history was searched up to the current year (2022). The following keywords were searched: “Time-restricted Feeding” OR “Time-Restricted Eating”

OR “Intermittent Fasting” OR “Fasting” AND “Autophagy”OR “Insulin Sensitivity” OR “Cortisol”

2.3 Screening and Data selection process

Two authors independently collected and screened all abstracts and titles of the studies that were found during the literature search of the potential eligibility criterias. Any duplicates that were passed by, were removed. Each relevant article was extracted for authors and year of publication, participants, study design, characteristics of their intervention, and the outcomes of interest. Any uncertainties were resolved through consensus.

2.4 Quality assessment

The Quality Assessment Tool for Quantitative Studies developed by the Effective Public Health Practice Project (EPHPP) was used to assess study quality (Thomas et al., 2004), which evaluates six key methodological areas: selection bias, study design, confounders, blinding, data collection method and withdrawals. For each study, the areas were rated using a scale of weak, moderate and strong. Based on the rating of each of the areas a global score was given reflecting the overall quality of the methodology of the study.

2.5 Data Synthesis

For each study, results were summarized by: (i) intervention characteristics (intervention methods, exercise or test protocols and length of each trial); (ii) changes in autophagy; (iii) changes in insulin sensitivity; and (iv) changes in cortisol.

2.6 Ethics

As the researchers assessed the articles, the following articles were also screened for their ethical eligibility, looking at if they were conducted by the Declaration of Helsinki by ethical permit number and were approved by any established research center.

2.7 Sustainability

In a time of increased consumption of food, energy and resources there is a certain simplicity to the idea of a health promoting lifestyle intervention that derives its benefits from the act of non-consumption. It might therefore have beneficial effects on human and environmental health by highlighting the effects of not over-consuming. It´s also an intervention that is really cost-efficient as it has no required starting cost but could rather be a potential reduction in expenses for those who practice it.

3. Results

3.1 Study Selection

The search of articles on the database of Pubmed yielded 378 studies. After duplicates were removed (n=1), articles were screened for eligibility whereas 344 of the articles were excluded based on their title, abstract and method. This left out a total of 33 articles that were screened more precisely for their relevance. From this 13 articles came out meeting the eligibility criteria for this review and further, went through a quality assessment presented later in this review (Figure 1).

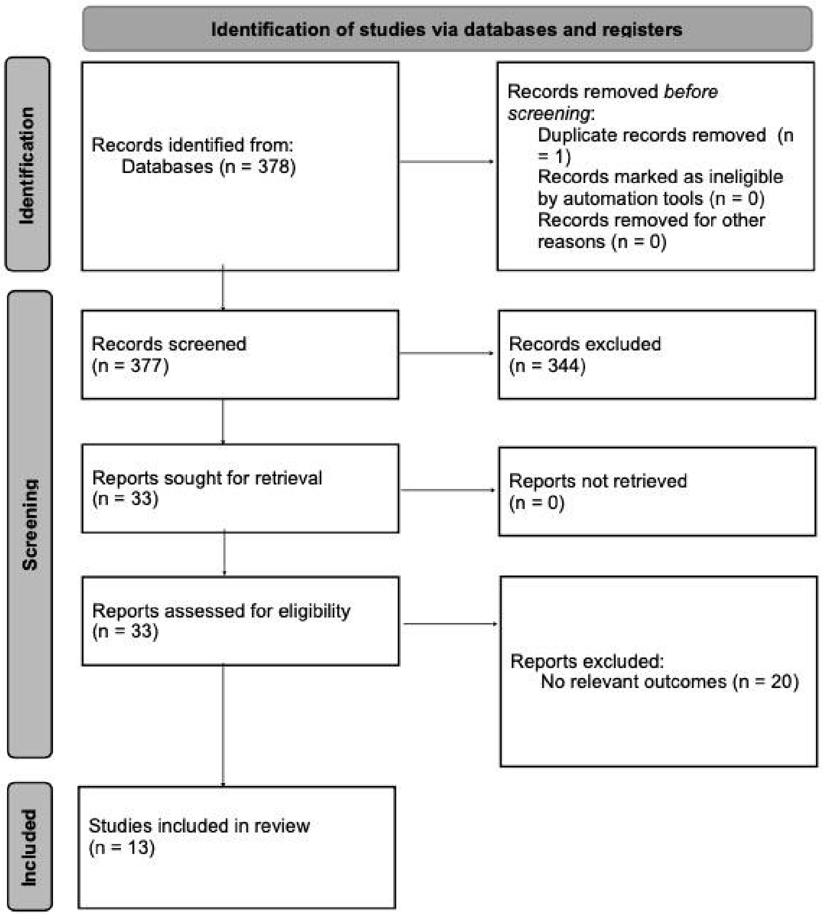

Figure 1. *Flow chart of the methodology for the search results (Prisma Statement, 2021).*

3.2 Quality Assessment Results

Table 2. depicts a detailed categorization of each quality domain together with overall methodological quality for the included studies in this review.

Reference	Selection Bias	Study Design	Confounders	Blinding	Data Collection Method	Withdrawals & Drop-Outs	Global
[Jamshed et al., 2019]	3	1	1	2	1	2	Moderate
[Vendelbo et al., 2014]	3	2	3	3	1	3	Weak
[Sutton et al., 2018]	1	1	1	3	1	2	Moderate
[Moro et al., 2021]	2	1	2	2	1	2	Strong
[Liu et al., 2021]	1	1	3	2	1	1	Moderate
[Atkinson et al., 2020]	1	2	3	2	1	1	Moderate
[Allison et al., 2021]	3	1	2	2	1	2	Moderate
[McAllister et al., 2020]	3	1	3	3	1	1	Weak
[Ruppert et al., 2020]	3	2	3	3	1	1	Weak
[Bogdan et al., 2001]	3	2	3	2	1	3	Weak
[Bergendahl et al., 1996]	2	1	2	2	1	3	Moderate
[Elimam et al., 2002]	3	1	3	2	1	2	Weak
[Bergendahl et al., 2000]	3	2	3	2	1	2	Weak

Table 1. Note. 1 - Strong; 2 - Moderate; 3 - Weak. The categories are described below in chronological order.

Selection bias, stands to explain if the researchers have chosen their participants randomly out from a target population or not. *Study Design*, reflects the type of allocation process which was used in the study, this is commonly a well used indicator of the extent of bias. *Confounders are* different variables that can have causal effect on the outcome of the intervention because they are closely associated with it. *Blinding*, speaks for whether the participants or researchers (or both) are aware of the research question or aware of which participants were disposed to which groups respectively. *Data Collection Method,* asks whether the methods used were reliable and valid for their primary interest of outcome. *Withdrawals & Drop-Outs*, reflects how many of the initial percentage of participants completed the study intervention. *Global,* represented the summated rating from the previous results, if an article had two or more Weak ratings this was viewed as a global Weak, if an article contained one Weak rating it was viewed as Moderate. Lastly, if an article consisted of no Weak ratings it was viewed as Strong (Thomas et al., 2004).

3.3 Study Characteristics

Table 1. Summary of studies evaluating the effects of intermittent fasting on autophagy, insulin sensitivity and cortisol

Reference	***Parameters***	***Participants***	***Age***	***Trial length & Intervention***	***Autophagy***	***Insulin Sensitivity***	***Cortisol***
[Jamshed et al., 2019]	*LC3A; MTOR; ATG12; Serum Cortisol*	*11 overweight adults(4 men & 7 women); 7 African-American, 3 Caucasian, 1 Other)*	*32 ± 7 years*	*4-day eTRF schedule: a 6-hour eating*	*LC3A ↑ MTOR↓ No significant changes in ATG12*	-	*Morning cortisol↑ Evening cortisol↓*
[Vendelbo et al., 2014]	*LC3B-II; p62; MTOR; Serum Cortisol*	*8 healthy men, with no family history of diabetes*	*26 ± 4 years*	*3-day RCT; control schedule: 10-hour fasting period; IF schedule: 72-hour fasting period, crossover to other arm occured after >1 month.*	*LC3B-II ↑ P62 ↑ MTOR↓*	-	*Cortisol↑*
[Sutton et al., 2018]	*Fasting insulin; Fasting glucose; Insulin sensitivity; Insulin Resistance; β-cell function*	*8 overweight men with prediabetes; 6 Caucasian, 1 African-American, 1 South Asian*	*56 ± 9 years*	*5-week isocaloric & eucaloric controlled feeding trial testing eTRF; eTRF schedule: 6-hour eating period*	-	*Fasting insulin ↓ Postprandial insulin ↓ β-cell responsiveness ↑ Insulin resistance ↓*	*No changes in cortisol*

Reference	***Parameters***	***Participants***	***Age***	***Trial length & Intervention***	***Autophagy***	***Insulin Sensitivity***	***Cortisol***
[Moro et al., 2021]	*Fasting glucose; Fasting Insulin; Insulin Resistance*	*20 Healthy Adults*	*29.21 ± 3.8 years*	*12 Month TRE and ND Protocol; TRE schedule: 8-hour eating period; ND: Normal diet no calorie/time restrictions.*	-	*Fasting Glucose ↓ Fasting Insulin↓ HOMA-IR↓ Fasting Glucose↓ Fasting Insulin↓*	-
[Liu et al., 2021]	*Insulin Sensitivity Clamp*	*76 women overweight*	*51 ± 3 years*	*8 week, RCT; CR70%, IF70%, IF100%*	-	*8 week(pre 24h fast): No statistical difference between groups 8 week(post 24h fast): Insulin Sensitivity IF70↓ Insulin Sensitivity IF100↓*	-
[Atkinson et al., 2020]	*Plasma glucose; Insulin; Insulin sensitivity; Insulin Resistance; β-cell function*	*24 lean, young, healthy adults (12 men, 12 women); 11 European–Caucasian & 13 Southeast Asian*	*23 ± 2.6 years*	*Nine separate testing sessions in which the fasting period lasted for 12, 14, or 16 h. Each fasting duration was tested three times (3 × 3) by each participant.*	-	*Fasting plasma glucose ↓ Insulin concentrations ↓ In females: HOMA-%S↑ HOMA-IR↓ HOMA %B - no changes In males: No significant changes in HOMA-%S/ IR/ %B*	-
[Allison et al., 2021]	*cortisol*	*12 Adults (7men, 5 women)*	*26,3 ± 3,4 years*	*8 week daytime 08-19, 8 week delayed 12-23a*	-	-	*No changes in cortisol.*

Reference	*Parameters*	*Participants*	*Age*	*Trial length & Intervention*	*Autophagy*	*Insulin Sensitivity*	*Cortisol*
[McAllister et al., 2020]	*Serum Cortisol*	*22 subjects*	*22 ± 2.5 years*	*28 days, 16 hour fast 8 hour feeding window, 2 groups one eating isocaloric and one ad libitum*	-		*Cortisol Isocaloric↑* *Cortisol Ad Libitum ↓*
[Ruppert et al., 2020]	*Serum Cortisol*	*24 healthy subjects*	*40-70 years*	*Standardized meal and 26 hour fast once.*	-	-	*Cortisol↑*
[Bogdan et al., 2001]	*Serum Cortisol*	*10 healthy male*	*34 ± 3.7 years*	*23 days, ramadan fasting, measurements taken before ramadan and last day of*	-	-	*Cortisol onset in the morning delayed* *Cortisol in the afternoon↑*
[Bergendahl et al., 1996]	*Serum Cortisol*	*8 healthy male*	*29 ± 1,6 years*	*5 day fast, measures taken every 5 min for the last 24hour of the fast and before.*	-	-	*Cortisol↑*
[Elimam et al., 2002]	*Serum cortisol*	*13 healthy subjects*	-	*one group overnight fast, one group 48h fast*	-	-	*No changes in Cortisol*
[Bergendahl et al., 2000]	*Serum Cortisol*	*8 young healthy women*	*21-28 years*	*2,5 day fast*	-	-	*Cortisol↑*

Definitions: ND; normal diet, eTRF; early time-restricted fasting(8am-2pm), eucaloric; number of calories consumed is equal to calories expended, Isocaloric; a diet of equal caloric value to their normal diet, Ad Libitum; eating as much as desired, CR70%; diet consisting of 70% of the estimated caloric expenditure, IF70%; a schedule of intermittent fasting paired with diet of 70% of the estimated caloric expenditure, IF100%; a schedule of intermittent fasting paired with eating 100% of the estimated caloric expenditure.

3.4 Changes in biomarkers

What follows is a literary presentation of the results gathered from the screening process of this review, the following studies each kept a consistency of attaining to a significance level of $p<0.05$ as they presented their results.

3.4.1 Autophagy

Two studies presented data of the association between IF and changes in autophagy levels and both showed increases in autophagy.

Jamshed et al. (2019) presented increased levels of LC3A (22 ± 5%; $p = 0.001$) and ATG12 (5 ± 2%; although not significant after adjustments) following a 4-day eTRF without CR. As presented by Vendelbo et al. (2014) a 72 hour fast significantly reduced phosphorylation of an activation site (Ser) on mTOR (40%; $p = 0.05$), phosphorylation of ULK1 was significantly decreased ($P = 0.05$) and increased LC3B-II protein content with (ca. 30%) in comparison to LC3B-I. Lastly, an increase of p62 of ca.10% was also found (Vendelbo et al., 2014).

3.4.2 Insulin Sensitivity

There were four studies presenting data on different fasting regimens and insulin sensitivity.

Sutton et al. (2018) presented a 5-week eTRF without weight loss and CR. Fasting glucose did not seem to be affected ($D = 2 \pm 2$ mg/dL; $p = 0.49$), therefore mean glucose remained unchanged ($D = 5 \pm 5$ mg/dL; $p = 0.40$). Reduction in fasting insulin was seen 3.4 ± 1.6 mU/L ($p = 0.05$), together with postprandial insulin $t = 60$ min and 90 min post-load ($p = 0.01$). Mean and peak values of insulin were reduced by 26 ± 9 mU/L ($p = 0.01$) and 35 ± 13 mU/L ($p = 0.01$) respectively. Further, beta-cell responsiveness showed an increase in responsiveness by 14 ± 7 U/mg ($p = 0.05$). Finally, insulin resistance was seen to decrease by the measurement of 3-hr incremental AUC ratio with 36 ± 10 U/mg ($p = 0.005$) (Sutton et al., 2018). As presented by Moro et al. (2021) fasting glucose and fasting insulin levels had decreased (glucose −9.26%, $P < 0.0001$; insulin −28.26%, $P < 0.0001$) after 2 months of TRF without CR. After 12 months of the same intervention fasting glucose and fasting insulin decreased by (glucose −9.92%, $P < 0.0001$, $d = −0.95$; insulin −31.66%, $P < 0.0001$, $d = −1.06$). They also saw a reduction in HOMA-IR by 34.80% ($P < 0.0001$) after 2 months and

37.83% ($P < 0.0001$, $d = -1.32$) at 12 months (Moro et al., 2021). Liu et al. (2021) showed no statistical difference in changes of insulin sensitivity following an intermittent fasting regimen with determining caloric intake. Whereas another study saw a reduction in insulin sensitivity in both IF groups after the 24h of fasting (all $P < .05$) (Liu et al., 2021). Atkinson et al. (2020) presented data on 12h, 14h and 16h fasts and showed that fasting glucose and fasting insulin were significantly reduced ($p = 0.002$ and 0.045, respectively) after a 16h fast. There was an increase in IS (HOMA%S +42, $p = 0.016$, marginally significant) after 16h fast in females only. IR was shown to reduce in the window of 16h compared to 12h by (Log of HOMA-IR −12, $p = 0.024$). Beta-cell response was not affected in either of the sexes. Men had a non-significant increase in IS by (HOMA%S +56,9, $p = 0.107$) after a 16h fast compared to 12h. IR was shown to decrease in the window of 16h compared to 12h by (Log of HOMA-IR −7, $p = 0.107$) (Atkinson et al., 2020).

3.4.3 Cortisol

There were 10 studies looking at cortisol in relation to some kind of fasting protocol. Four studies showed no significant changes in cortisol. Five studies showed an increase in cortisol. One study showed an increase in the morning and a decrease in the afternoon. Jamshed et al. (2019) showed an increase of 1.5 ± 0.9 μg/dl ($p=0.10$)in the morning and a decrease of 1.4 ± 0.6 μg/dl ($p = 0.03$) in the afternoon following an 18/6 eTRF schedule.

McAllister et al. (2020) having two groups following a 16/8 TRF schedule showed increase cortisol from 31.8 ± 4.7 to 35.2 ± 4.3 12 in the group eating Isocaloric. The group following an Ad Libitum diet showed a decrease from 39.8 ± 12.6 to 37.3 ± 10.0 μg/dL. Ruppert et al. (2020) presented results before and after a 26 hour fast that showed a significant increase in cortisol after 24 hours of fasting. As presented by Bogdan et al. (2001) there was a delayed onset of cortisol in the morning but also increased levels in the afternoon following 23 days of Ramadan fasting. These findings were not significant. Bergendahl et al. (1996) presented statistics for 24 hours before as well as the last 24 hours of a 5-day fast. The 24-h mean serum cortisol concentrations increased 2.2-fold as a result of the 5-day fast ($P < 0.001$). Bergendahl et al. (2000) presented results from a 2,5-day fast where they showed significantly increased cortisol secretion per pulse and significantly increased 24-h mean serum cortisol concentrations.

4. Discussion

The aim of this systematic descriptive review was to summarize the current evidence for the effects of different forms of fasting on insulin sensitivity, autophagy and cortisol markers in adult humans. Thirteen studies ended up passing the quality assessment with results suggesting that IF affects insulin sensitivity, autophagy and cortisol on various levels.

4.1 Discussion - Results

During different time intervals of IF, autophagy was indirectly shown to be affected by a significant increase in either LC3A or LC3B-II, a precursor and component of the autophagosome membrane, respectively. Where the first marker was found to be increased after an intervention of a lower time-span (6/18h) Jamshed et al. (2019) and the second one being of more length (72h) (Vendelbo et al., 2014). This suggests an increase in autophagosome appearance and therefore correlates with elevations in the autophagy process. (Mizushima & Yoshimori, 2007). This is in congruence with the findings of simultaneous fasting-induced reduction of mTOR activity, during the periods of fasting in both studies. Interestingly, however, Vendelbo et al. (2014) had findings which spoke for a significant increase in levels of p62 - protein concentrations which increase in the absence of autophagy and which elevated presence, has been seen to inhibit the autophagic process (Bjørkøy et al., 2005) - contradicts the findings of elevated levels in LC3B-II and therefore makes the results ambiguous to interpret from this study. Even though the changes in p62 are not as pronounced as the elevated levels of LC3B-II, Vendelbo et al. (2014) were not able to exclude the probability of inhibition of autophagy rather than induction, also because LC3B-II is at best an indirect measure of the autophagic flux (Mizushima & Yoshimori, 2007). Whilst these findings are controversial, there was however a finding of a decreased mTORC1 activation in both articles, something which has been seen over and over to positively regulate the catabolic process of autophagy that promotes protein and organelle recycling (Deleyto-Seldas & Efeyan, 2021).

IF consisting of various protocols - from single fasts of 12h,14h,16h, protocols of 8/16h over weeks, to longer fasts of 96h - were shown to alter the responses of insulin sensitivity, insulin resistance, beta-cell responsiveness in participants with both significant and non-significant results. This was measured by the methods of HOMA, iAUC or by a clamp. There were even findings of significantly increased measures of IS in participants that underwent protocols where CR was excluded, without fasting caused weight loss, something which previously have not been found in interventions. Most of the studies suggested an increase in IS during the fasted state, although one of the studies, Liu et al. (2021), actually found a reduction in insensitivity. As discussed in the article, this is not unnormal as it is been seen that both more acute, and prolonged fasts can be associated with the development of IR in humans with obesity as described in Nowotny et al., (2013), due to increased lipid deposition in skeletal muscle. IR in conjunction with fasting has been seen to function as a protective mechanism to spare glucose, this process should be distinguished from the IR that develops as a response to obesity, something that is well associated with hyperinsulinemia and hyperglycemia. Why this was not the overarching findings in all of the studies, could be due to Liu et al. (2021), providing a different protocol(24h fast on 3 non-consecutive days per week/8 weeks), participants were all overweight or obese in nature, also the study could have further confounders affecting the outcome. The results from the articles showed one outcome of significantly increased beta-cell responsiveness. As mentioned, acute fasting has been seen to potentially induce insulin resistance, but another interesting finding is that it can also impair beta-cell responsiveness - this is by a mechanism of increased triglycerides and/or free fatty acids that are secreted into the bloodstream through lipolysis. Although none of these studies showed this to be true, it is yet something to take in mind (Sutton et al., 2018).

Overall the evidence points to the abstinence from food leads to increases in cortisol during the fast. Some studies showed no changes at all which might point to the variations between individual physiologies. The results seem to paint the picture of cortisol secretion being increasingly elevated as the duration of the fast increases. McAllister et al. (2020) presented results that indicate there might be an effect based on calorie consumption. Where the group who could eat as much as they wanted to, had lower cortisol levels during the fast. Jamshed et al. (2019) presented that cortisol was elevated early and lowered later in the day following an early feeding window. While those who followed Ramadan fasting as presented by Bogdan et al. (2001) showed a slightly delayed cortisol onset in the morning but the concentration remained the same while afternoon concentrations were increased. Bergendahl et al. (1996) presented results from the longest fast out of the selected studies and also presented the highest both mean and per pulse concentrations of cortisol.

TRF and fasting as shown by the studies can inhibit mTORC1 and increase autophagy. The overactivation of mTOR has been implicated in cancer and other diseases. Autophagy has shown promise in preventing the occurrence of neurodegenerative diseases and cancer. Therefore it can be used as a relatively safe and inexpensive lifestyle for the prevention of cancer and neurodegenerative diseases (Gudden et al., 2021).

Diabetes type 2 is a disease of impaired glucose control and insulin resistance can be greatly affected by improving insulin sensitivity. Insulin sensitivity also has a correlation to weight loss. Fasting and TRF have a positive effect on insulin sensitivity and have the potential to be an effective lifestyle intervention to prevent and even reverse Diabetes type 2 (Fujimoto., 2000). As fasting is a form of stressor that puts the body in an energy-deficient state it also can have deleterious effects on the body in the same way other stressors can. Therefore for the sustainability aspect of fasting for general health, it is important to understand the interplay between fasting as a beneficial stressor and as a detrimental stressor. But also finding which eating pattern optimizes the pulses of cortisol so that it is high when you want it to be high and low when you want it to be low. Especially important for those who already live a stressful life and/or are prone to diseases that are affected by stress. TRF can be an inexpensive method of optimizing or adjusting your cortisol concentration for overall health benefits, wakefulness and sleep efficiency.

CR has some of the same benefits as fasting in regards to autophagy activation and improved insulin sensitivity as well as weight loss. Limiting the daily feeding window by the way of TRF has the potential to ease the process of adhering to a CR protocol (Horne et al., 2015).

The present review has some limitations that need to be addressed. First of all the amount of studies that present information on these areas especially autophagy is very scarce. There is significant heterogeneity among the study designs which therefore makes it harder to pinpoint the effects of certain time spans of fasting. We can see that longer windows seem to increase all the markers but it is hard to make out when the benefits start to really show. There is an inherent difficulty in these studies to adhere to the gold standard of double-blinding, simply because it is impossible to keep the participants oblivious to the fact that they´re fasting. The apparent lack of studies examining specific windows of TRF and IF leaves room for future studies to fill. IF having low inherent risks when executed responsibly paired with the potential positive effects on metabolic health and hormonal regulation makes it a prime target to study in the name of general health of the world.

4.2 Discussion - Method

This study was done as a systematic descriptive review, with the purpose of gathering results of outcomes from studies performed in clinical settings. As with any systematic review, one needs to adhere to guidelines that have been assessed by an audience to be valid and reliable. In this review, the researchers conducted the study out on the guidelines of PRISMA(The PRISMA 2020 statement: an updated guideline for reporting systematic reviews). These guidelines were recently updated (2020) and is well-established guidelines for conducting systematic & metanalysis reviews (Prisma Statement, 2021).

In terms of the screening process of articles, the researchers used a narrow search field (based on the eligibility criteria), which allowed for scarce but precise search results of articles viewed on the database of Pubmed. Due to the area of interest - fasting and autophagy/insulin sensitivity/cortisol - being something which is fairly novel in the scientific community to research about, the search history was limited with a total finding of 377 articles found to be potentially relevant for the topic. To note is, that this review's aim was to find primary outcomes in humans, with that said, there are multiple articles done on rodents as they provide leeway in terms of ethical considerations.

This was the researcher's first conduction of a systematic review and therefore various different technicalities around how to create a systematic review had to be worked out prior to starting. As an effect, the search period had to be postponed and for that, because the new time constraint, the researchers decided to make a more shallow search in the different databases and found that, (i) the databases didn't vary largely from a first glimpse into the literature, (ii) Pubmed seemed to have the most search results.

For any review a quality assessment needs to be conducted, the researchers performed this with the help of the Quality Assessment Tool for Quantitative Studies developed by the Effective Public Health Practice Project (EPHPP). Simultaneously, the articles were examined for their ethical considerations of their researchers conduction of intervention. EPHPP is described as a method apt for assessing study methodology for this in the area of medecine (Thomas et al., 2004).

Even though six of the thirteen articles global ratings were acknowledged as ‘Weak’, the researches took into consideration their limitations and decided upon sharing their results transparantly anyway. Also because there was a common theme among authours in articles to not be explicit enough with their methodology of use, and henceforth, challenging to rate accurately.

5. Conclusion

From these findings, the existent research investigating the impact of IF on autophagy, insulin sensitivity and cortisol in a clinical setting has generated mostly coherent, but also a minority, of conflicting data. Our findings reveal that in most cases, IF and depending on its window length of fasting, have increasingly significant effects on markers of autophagy, IS and cortisol. Although, the litterature is yet scarce, and some of the methods measuring these process are but most indirect, more research needs to be conducted on the topic to provide a more descriptive account of this territory.

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