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Review article

Effects of intermittent fasting on metabolism in men☆

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ABSTRACT

This review analyzes the available literature on the impact of intermittent fasting (IF), a nutritional intervention, on different aspects of metabolism. The epidemic of metabolic disturbances, such as obesity, metabolic syndrome (MS), and diabetes mellitus type 2 has led to an increase in the prevalence of cardiovascular diseases, and affected patients might significantly benefit from modifications in nutritional habits. Recent experimental studies have elucidated some of the metabolic mechanisms involved with IF. Animal models have shown positive changes in glucose (lower plasma glucose and insulin levels) and in lipid metabolism (reduced visceral fat tissue and increased plasma adiponectin level), and an increased resistance to stress. Despite the limited number of samples studied, positive results have been reported on the impact of IF for human health. IF is reported to improve the lipid profile; to decrease inflammatory responses, reflected by changes in serum adipokine levels; and to change the expression of genes related to inflammatory response and other factors. Studies on obese individuals have shown that patient compliance was greater for IF than other traditional nutritional approaches (calorie restriction), and IF was found to be associated with low oxidative stress. Recent reports suggest that IF exerts a positive impact on the metabolic derangements commonly associated with cardiovascular diseases, and that it may be a viable and accessible intervention for most individuals. Therefore, further clinical studies are essential to test the effectiveness of IF in preventing and controlling metabolic and cardiovascular diseases.

Efeitos do jejum intermitente no metabolismo humano

Palavras-chave:
Jejum
Doença cardiovascular
Obesidade
Síndrome metabólica
Restrição calórica
Dislipidemia

RESUMO

Esta revisão traz uma análise de dados disponíveis na literatura sobre o impacto do jejum intermitente, uma modalidade de intervenção nutricional, em diferentes aspectos do metabolismo. A epidemia de anormalidades metabólicas, como obesidade, síndrome metabólica e diabetes mellitus tipo 2, tem ocasionado um aumento na prevalência de doenças cardiovasculares, condições em que os indivíduos afetados apresentam importantes melhorias advindas de modificação nos hábitos alimentares. Estudos experimentais recentes têm elucidado a modulação do metabolismo por jejum intermitente. Testes com

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animais têm mostrado alterações positivas no metabolismo glicídico (valores menores de glicemia e insulinemia) e lipídico (redução no volume de gordura visceral e aumento nos valores de adiponectina plasmática), além de uma maior resistência ao estresse. Apesar dos estudos disponíveis apresentarem populações muito reduzidas, observaram-se resultados positivos com esta intervenção também na saúde humana. Os resultados indicam melhorias no perfil lipídico, redução de respostas inflamatórias, com redução na liberação de adipocinas inflamatórias e alterações na expressão de genes relacionados com a resposta inflamatória e de outros fatores. Em indivíduos obesos observou-se uma melhor adesão ao jejum intermitente em relação a intervenções tradicionais (restrição calórica), além da redução no estresse oxidativo desta população. Dessa maneira, por se tratar de uma intervenção viável e acessível para a maioria dos indivíduos, novos estudos clínicos são necessários para testar a eficácia desta intervenção na prevenção e no controle de doenças metabólicas e cardiovasculares.

Introduction

The human genotype is believed to have evolved from 600,000 BC to 25,000 BC, when humans were hunter-gatherers.[1] During this period, major energy oscillations appear to have selected genes that regulate metabolism for efficient nutrient usage and increased fat storage, which represents an evolutionary benefit consistent with the thrifty genotype theory proposed by James V. Neel.[2] While the environment changed drastically with urbanization and easy availability of food, the genotype remains largely unaltered. This imbalance has resulted in an epidemic of conditions characterized by metabolic disturbances, such as obesity, metabolic syndrome (MS), and diabetes mellitus type 2 (DM2).[1,3,4]

While obesity is easily defined by body composition (body mass index [BMI] > 30 kg/m^2), and DM2 by elevated blood glucose levels, MS is recognized by a cluster of metabolic markers whose importance and contribution have greatly changed over time. Despite this difference, the three conditions have common pathophysiological backgrounds. Firstly, all these conditions involve insulin resistance, elevated levels of plasma lipids, and increased levels of chronic inflammatory mediators. Secondly, the consequent metabolic profile considerably increases cardiovascular risk. Finally, individuals with any of these conditions can benefit from significant lifestyle changes.[5–7] Notably, modification of nutritional habits is now considered extremely important for reducing cardiovascular risk.[8,9]

Intermittent fasting (IF) is an interventional strategy wherein individuals are subjected to varying periods of fasting. IF has recently attracted attention because experimental studies have highlighted its potential for correcting metabolic abnormalities.[10] This regimen has also shown better adherence than other methods.[11]

This review analyzes existing data on the impact of IF on different aspects of metabolism.

Methodology

Experimental studies and clinical trials on IF available in the PubMed database at the time of manuscript preparation were reviewed. Animal and human studies were searched for by using the key words "intermittent fasting," "alternate day fasting," and "starvation" either alone or combined with "cardiovascular risk," "obesity," and "metabolic syndrome."

The first search retrieved over 22,000 results, mostly by using the keyword "starvation" alone (21,735). A detailed search yielded 26 articles on the impact of fasting or IF on metabolic parameters related to cardiovascular risk.

Pathophysiological basis of obesity and MS

Obesity and MS were the main outcomes analyzed in the studies on IF.

Adipose tissue is now known to function as an endocrine organ involved in regulating metabolism, rather than a passive reservoir for energy storage.[12] Adipocytes, mesenchymal cells, and infiltrating macrophages together produce cytokines and adipokines that have important regulatory effects on inflammation, insulin sensitivity, coagulation, vascular homeostasis, appetite, energy expenditure, etc. When this production is deregulated, e.g., by excessive adipose tissue, the organism appears to develop low-grade chronic inflammation, leading to insulin resistance and cardiovascular disease.[13] Adipocytes produce important proinflammatory adipokines, such as leptin, tumor necrosis factor alpha (TNF-α), resistin, angiotensinogen, interleukin-6 (IL-6), and plasminogen activator inhibitor-1 (PAI-1), as well as nonesterified fatty acids and C-reactive protein (CRP), which are atherogenic.[14,15]

Certain adipokines have cardioprotective action, such as adiponectin, which is abundant in human circulation. Adiponectin was primarily investigated for its ability to promote insulin sensitivity by suppressing gluconeogenesis and increasing fatty acid oxidation, which in turn reduce triglyceride accumulation in the liver.[13,16] Moreover, adiponectin regulates endothelial function by increasing the production of endothelial nitric oxide; by inhibiting endothelial cell activation and endothelium–leukocyte interaction; by enhancing phagocytosis; and by suppressing macrophage activation, macrophage-to-foam cell transformation, and platelet aggregation.[17–19]

Adipokines might represent the evolutionary pathway that led excessively nourished humans to obesity, insulin resistance, MS, and cardiovascular disease. Indeed, obese patients

with excess visceral fat show increased adipokine expression. However, weight loss is related to reduced plasma levels of metabolically active substances.[20]

Effects of intermittent fasting on animal models

Metabolic mechanisms triggered by IF were well elucidated by recent experimental studies. Anson et al.[10] investigated glucose metabolism and enhanced neuronal resistance to stress in C57BL/6 mice subjected to *ad libitum* diet, IF, or limited daily food intake for 22 weeks. Neuronal stress was induced at the end of the 22nd week through an injection of kainate, a seizure- and neuronal damage-inducing exotoxin. The IF group showed significantly lesser histopathological brain changes, and lower plasma glucose and insulin levels than the others. These findings were reproduced in male Wistar rats.[21]

Similarly, a study on the lipid metabolism of male C57BL/6J mice subjected to alternate-day fasting (ADF) or alternate-day 50% calorie restriction (ADCR 50%) showed a significant reduction in the size of adipocytes of both visceral and subcutaneous fat. The authors also found that IF causes oscillation of triglyceride metabolism between anabolism (gluconeogenesis and *de novo* lipogenesis) and catabolism (lipolysis).[22] The same group later reported that female C57BL/6J mice subjected to four-week IF showed significantly reduced visceral fat percentage, increased subcutaneous fat percentage, increased plasma adiponectin levels, and unchanged amount of fat tissue.[11]

Mager et al. investigated heart-rate variability in 12 Sprague-Dawley rats during 16 weeks of nutritional intervention by using an implanted telemetric transistor.[23] After 16 weeks, rats in the IF group showed reduced sympathetic activity associated with increased vagal tone. This reduction might have immunomodulatory effects, which would attenuate the release of proinflammatory substances, such as TNF-α, IL-6, and IL-18.

Increased resistance to stress after intermittent fasting

Resistance to stress might be a reasonable basis for increased resistance to cardiovascular and endocrine diseases.

Wan et al. evaluated the response to cardiovascular stress in male Wistar rats undergoing IF by using a telemetric transistor, and found that the rats showed rapid return to basal values of blood pressure and heart rate after induced cardiovascular stress, and presented no alterations in the plasma levels of stress biomarkers, such as adrenocorticotropic hormone and corticosterone, during stress.[21] Further, analysis of heart-rate variability demonstrated a better norepinephrine response to stress, thereby indicating an improved autonomic response in the IF group *versus* the control group.

Wan et al. later reported that a diet supplemented with 2-deoxyglucose (2-DG) - a glycolytic inhibitor and metabolic stressor - on alternate days could mimic the effects of IF in Sprague-Dawley rats.[24] They observed that both IF and 2-DG supplementation were capable of reducing heart rate, blood pressure, and glucose levels to similar extents. The authors suggested that the mechanisms underlying the positive metabolic response to IF may be attributed to the fact that periodic metabolic stress can induce adaptive changes in cardiovascular physiology and glucose metabolism that are associated with a "less atherogenic" profile.

An alternative cardioprotective action of IF was evaluated by Ahmet et al.; they maintained 30 Sprague-Dawley rats under IF for three months and a control group with normal feeding.[25] Thereafter, all animals were submitted to coronary artery ligation to induce myocardial infarction (MI) or to sham surgery. The IF group showed lower left ventricular (LV) mass, lower LV wall thickness, and significantly lesser ventricular remodeling than the control group. Notably, 23 hours after surgery, a significantly reduced degree of apoptosis and neutrophil infiltration was noted in the IF group with MI, possibly contributing to a smaller ventricular size. The authors proposed that IF induces an ischemic preconditioning in the cardiac muscle that protects myocardial cells from ischemic damage. Similar results were recently reported by Wan et al. on male Wistar rats.[26] In this study, plasma adiponectin and IL-6 concentrations were measured, which showed an increase in the former and a decrease in the latter. The authors suggested that the observed benefits of IF might be attributed to increased plasma adiponectin levels.

More recently, Katare et al., performed a study on the impact of IF started after MI induction and maintained for six weeks.[27] The male Wistar rats subjected to IF showed a decrease in cardiomyocyte hypertrophy and fibrosis area, reduced oxidative stress, better cardiac performance, and better survival rates than the control group. The authors also noted an increased expression of the *BDNF* gene, responsible for the enhanced expression of vascular endothelial growth factor (VEGF) in the cardiac muscle; this resulted in increased angiogenesis and decreased apoptosis.

In another experimental study, streptozotocin was used to induce diabetes mellitus type 1 before diet intervention in Sprague-Dawley rats.[28] After eight weeks of IF, the diabetic IF group showed blood pressure levels similar to those of the non-diabetic control group, indicating that glomerular damages promoted by diabetes were somehow prevented. Other findings were normal blood levels of glucose, albumin, HDL-C, and blood urea nitrogen; increased resistance to oxidative stress; and reduced incidence and intensity of degenerative structures in the kidneys. Changes in the expression of some genes involved with cellular survival (*p53*, *p38*, and *Sir 2*) were also demonstrated.

Studies evaluating the potential of IF in the recovery from spinal cord injury have also shown intriguing results. Plunet et al. assessed the effect of ADF in a group of male Sprague–Dawley rats after cervical spinal cord injury.[29] The intervention proved to be neuroprotective, with a 50% reduction in lesion volume and increased sprouting of corticospinal axons. The intervention also promoted plasticity; improved behavioral recovery, evident by improved gait-pattern and forelimb function during ladder-crossing; and enhanced vertical exploration. Jeong et al. investigated the effect of the same dietetic intervention, started before or after a different spinal cord lesion (thoracic contusion) in Sprague–Dawley rats.[30] Both groups subjected to the intervention (before or after the lesion), showed positive results, with a better functional

recovery, along with improvement of several parameters of their walking pattern. The prophylactic group (IF started before the lesion) performed slightly better than the therapeutic group (IF started after the lesion). The results were also superior in benefits when compared with a group of rats consuming the same amount of calories as the ADF group (25% calorie restriction) everyday. Davis et al. also found positive results assessing not only spinal cord lesion but also traumatic brain lesion (TBL) induced in Sprague–Dawley rats.[31] They found that fasting for 24 hours after moderate TBL confers neuroprotection, maintains cognitive function, and improves mitochondrial function. The results confirm the beneficial role of this kind of calorie restriction in other organisms.

Metabolic changes during fasting in humans

Metabolic changes due to fasting in humans were first investigated in the beginning of the century for treating obesity and other conditions, such as seizure disorders.[32–34]

Kerndt et al. investigated the metabolic effects of long-term fasting in human subjects who underwent a 36-day complete fasting regimen for religious reasons.[35] They noted a significant decrease in blood pressure, reaching significance on the 33rd day, accompanied by negative sodium balance. Changes in metabolic fuel were observed soon after the fasting period started. Plasma glucose levels dropped immediately at the beginning of the studies and remained low throughout the fasting period. Lipolysis and ketogenesis increased, gluconeogenesis remained higher than baseline levels, and glycogenolysis was reduced to undetectable levels.

Carlson et al. subjected healthy human volunteers to 60 hours of fasting and collected blood samples at 12 and 60 hours.[36] They observed a decrease in plasma glucose by 30% and in insulin by 50%; a significant increase in the extent of lipolysis and fat oxidation; and moderate increase in the extent proteolysis and protein oxidation. This increase of fat oxidation provides the substrate for gluconeogenesis and compensates for the decline in carbohydrate oxidation and glycogenolysis, thus confirming a switch of metabolic fuels. ADF is considered a variation of complete fasting that is easier to maintain for longer periods and has remarkable metabolic benefits. Studies on healthy subjects demonstrated a similar adaptation of energetic substrates. This action was also confirmed by changes in the muscular expression of genes, such as *GSK-3*, which is responsible for the regulation of glycogen synthesis pathways, favoring glycogen reposition.[37] IF also down-regulates *mTOR* expression in muscle tissue,

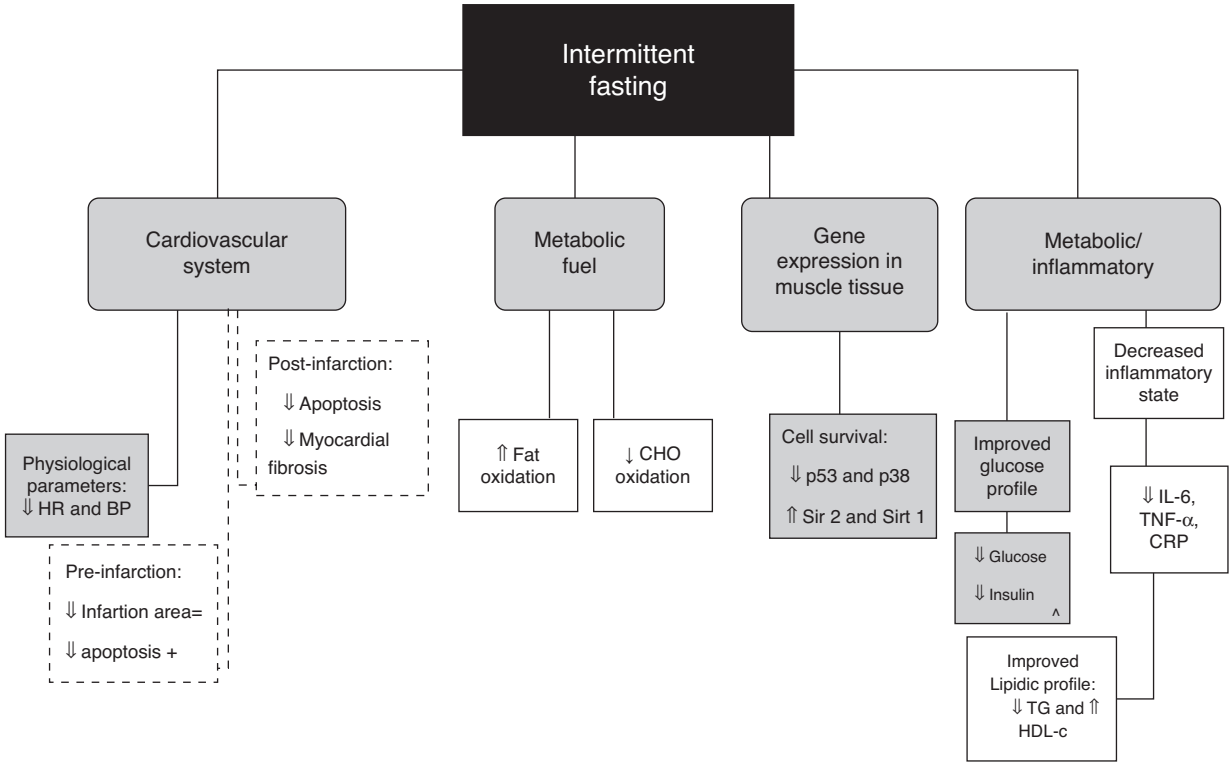

Fig. 1 – Potential targets for interventions using intermittent fasting (IF). IF impact in each different pathway of the organism. This is a summary of the different actions of this intervention (IF) within the metabolism demonstrated in all the studies that were used to compose this review. CHO, carbohydrate; HR, heart rate; BP, blood pressure; TG, triglycerides; HDL-c, high-density lipoprotein cholesterol; CPR, C-reactive protein; IL-6, interleukine 6.
Grey boxes – Verified in human and animal studies
---------- - Verified in animal studies only
_____ - Verified in human studies only
⇑ - Increase
⇓ - Decrease.

another important gene responsible for the modulation of nutrient signaling, and could reflect lesser protein synthesis and increased expression of carnitine acyltransferase 1 (CPT-1) in muscle tissue, as well as an increase in fat oxidation.[38,39]

Results from these and other studies on similar populations have shown improvements in the lipid profile, with higher HDL-cholesterol levels in women and lower triglyceride levels in men, and increased muscular expression of *Sirt 1* (40) –a gene involved with the regulation of food intake, fat metabolism, cell differentiation, apoptosis, and prevention of aging.[35] In these studies, an increase in stress resistance was demonstrated after three weeks of intervention.[40] A summary of the main findings is presented in Fig. 1.

Clinical implications

The general health benefits involved with intermittent fasting in humans still have not been fully explored in the literature. Johnson et al. described this phenomena after studying the article by Vallejo, published in 1956 in a Spanish scientific magazine. In his study of elderly subjects living in a nursing home, Vallejo did not change the overall calorie consumption, only the pattern of eating.[41] The subjects would alternate between *ad libitum* diet for one day, followed by a very restrictive diet on the next day. The results were quite interesting, revealing an important increase in the lifespan, demonstrated by a shorter number of days spent in the infirmary. It is important to emphasize that there was no calorie restriction involved in this intervention, thus the results were a consequence of the meal pattern alterations. Johnson concluded that this was probably just the tip of the iceberg, and that the health improvements derived from IF are probably seen in very different aspects of the organism, as observed in other studies exploring different conditions.[41]

Some latter studies were designed to evaluate the consequences of this intervention in specific health conditions. One interesting model analyzed consisted of individuals following Ramadan.

Ramadan is a holy month in the Islamic calendar, in which Muslims must refrain from eating or drinking during daylight hours, thereby resulting in approximately 12 hours of fasting. These features make Ramadan a natural model to study IF in humans. Aksungar assessed cardiovascular health in Muslim individuals during Ramadan, with special emphasis on coagulation.[42] The results showed improvements in the lipid profile, with increased HDL-cholesterol levels and decreased values of HDL risk factor (CT/HDL), during the fast and 20 days after it; decreased levels of D-dimmer and reduced homocysteine, which translates in an improved coagulation profile, were also observed. Three years later, in a similar study design, the same author measured inflammatory markers levels, such as IL-6 and CRP, during Ramadan. The results demonstrated a decrease in the inflammatory response, since plasma levels of IL-6 and CRP were consistently reduced by fasting.[43]

Allard et al. assessed cellular stress in healthy individuals subjected to IF for three weeks.[44] Serum samples obtained before and after the intervention were added to cultures of human hepatoma cell line (HepG2). The effects on growth, stress resistance, and gene expression were observed. HepG2 cells were then subjected to acute stress by treatment with freshly prepared hydrogen peroxide. The results demonstrated cell proliferation and increased stress resistance. This study also confirmed previous reports of an increased expression of the Sirt 1 gene after IF. The results obtained showed that IF had a significantly greater cell-protective effect than calorie restriction.

Another study on healthy subjects appraised changes in adipokine levels after the energetic oscillations caused by IF. After two weeks of intervention, subjects showed an increase in plasma adiponectin levels and increased insulin sensitivity in muscle and fat tissue, which was confirmed by increased lipolysis inhibition.[3]

Finally, Varady et al. presented a revision of the impact of IF on the prevention of chronic diseases in healthy human and animal models.[45] Although most human results were found to be inconclusive, they showed a tendency to improve metabolic conditions (glycemia and blood lipids), which would probably reflect in cardiovascular risk reduction. The lack of significance found in human studies is probably a consequence of the short length of the interventions and of the absence of a control group in most studies, reflecting the need for more studies to precisely determine the impacts of this intervention in humans.[45]

Although there are important differences in the strength of the results found, these findings in humans reflect the benefits observed in animal models, and also show similar underlying mechanisms, such as genetic modulation. Indeed, the described results motivated the investigation of IF and its possible therapeutic effects in diseased individuals.

Protective actions of intermittent fasting in obese individuals

IF for a period longer than a few weeks is difficult for most individuals, since they tend to develop headaches, dizziness, and irritability.[40] Therefore, an alternative approach - alternate-day calorie restriction (ADCR) - has been developed to promote patient compliance. In this approach, patients have *ad libitum* and very-low-calorie diet on alternate days (ranging from 25% to 85% restriction). When tested on obese individuals, this regimen showed higher adherence scores than others.[22]

Johnson et al. investigated the therapeutic properties of ADCR for two months in ten obese individuals with asthma.[46] The study revealed that IF affected improvement in the lipid profile, systemic inflammatory status (reduced serum TNF-α levels), and oxidative stress (decreased levels of reactive oxygen species). The authors concluded that ADCR induces mild stress, thereby causing cells to adapt by activating antioxidant mechanisms; this would imply that IF may be beneficial in other disorders involving inflammation and oxidative stress, such as atherosclerotic heart disease.

The cardio-protective action of IF was examined by Varady et al.[11] 16 obese individuals underwent eight weeks of ADCR 25%, i.e., they consumed 25% of their baseline energy needs, and *ad libitum* food on alternate days. The study results confirmed the significant cardio protective action of IF, such as weight loss; reduction of fat tissue mass, blood pressure, and heart rate; and improvements in lipid profile, with decrease in

total cholesterol and LDL-cholesterol levels, and an increase in HDL-cholesterol levels.

Conclusions

The development of cardiovascular disease, the leading cause of death worldwide, is directly connected to lifestyle factors causing metabolic disorders. Traditional approaches to counter these risk factors have been proven ineffective in most individuals. However, IF has recently been shown to have a positive impact on cardiovascular health.

Few studies conducted hitherto have explored the effectiveness of this intervention in metabolism regulation. The studies discussed in this paper have mostly been conducted in very small populations, on healthy individuals, and for short periods, which limits the strength of the results achieved. Further investigations are required to determine the frequency and/or duration of IF required to exert a positive effect on metabolism and cardiovascular outcomes.

Conflict of interest

All authors declare to have no conflict of interest.

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