Fasting and Experimental Animals, Detrimental or Positive?

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ARTICLE INFO	**ABSTRACT**
Article type: Review article	While fasting has been practiced for centuries, its beneficial effects were unknown until recently. This review tries to analyze the current literature of how fasting and intermittent fasting (IF) could affect clinical pathological parameters like red blood cell count, hemoglobin, hematocrit and etc. Animal experiments have elucidated fasting and IF could exert positive effects on learning, mood and brain, plus metabolic functions such lowering plasma glucose and insulin level and improvement in lipid metabolism (reduced visceral fat tissue and increased plasma adiponectin level), and an increased resistance to stress. Thus, more clinical studies are necessary to test the effectiveness of fasting and IF in preventing different diseases.

Introduction

Fasting is defined by not ingesting food and caloric beverages for periods ranging from 12 hr to 3 weeks. Fasting is an integral part of many religions. For instance Muslims, fast from dawn until dusk during the month of Ramadan, and Christians, Jews, Buddhists, and Hindus traditionally fast on special days. Fasting is different than caloric restriction (CR), in which the meal frequency is maintained but the daily caloric intake is reduced chronically by 20%–40%. Fasting results in ketogenesis and exerts significant changes in metabolic pathways and cellular processes. Investigations on experimental animals and new findings from human studies indicate that fasting may provide effective strategies for weight loss, delay aging, and optimize health. Here we review the fascinating and potent effects of fasting, and intermittent fasting (IF) on experimental animals.

Effects of Fasting on Clinical Pathological Parameters

In a study by Kale VP et al., a major decrease of body weight within 4 hours of fasting in all rats, and a noticeable increase of hemoglobin concentration after 16 hours of fasting in males were seen. In this research, significant decrease in triglyceride concentrations and serum glucose in both sexes and cholesterol and high-density lipoprotein-C (HDL -C) concentrations in females at 16 hours of fasting were reported. In addition, in females, there was an increase in creatinine concentration following 16 hours of fasting. These findings showed that 8 hours of fasting in male rats and 16 hours of fasting in females result in a considerable decrease in the activity of serum alkaline phosphatase and alanine aminotransferase. Maximum

modifications of clinical-pathological parameters were detected within 16 hours of fasting, with negligible later changes (1). Zeng et al. showed the effects of fasting on hematologic and clinical chemical values in cynomolgus monkeys. They compared the hematologic values based on the sexuality. Consequently, they found the BW (body weight) significantly decreased after 24 hours of fasting. Major decreases in red blood cell count, hemoglobin, hematocrit, and mean corpuscular volume and increases in mean cell hemoglobin and mean cell hemoglobin concentration were seen at 16 hours in males. In females, the increase in the duration of fasting caused a significant time-dependent increase in platelets. After fasting, blood urea nitrogen showed noticeable decreases in both sexes. Alkaline phosphatase increased in females after fasting. After 8 hours of fasting, aspartate transaminase significantly increased both in females and males. In females, also alanine transaminase and lactate dehydrogenase significantly increased. Albumin significantly decreased in males after 24 hours of fasting, but increased in females 16 hours after fasting. Serum glucose and triglyceride were not affected by fasting. In males, serum calcium decreased and inorganic phosphorus increased after fasting. These results suggested that clinical pathology data would vary after fasting. So the decision to fast or feed before blood collection for clinical purposes should be made based on careful consideration (2).

Some studies investigated the impact of fasting on alcohol metabolism. In 1936, Le Breton found that fasting rats that had been administered alcohol, oxidized the alcohol only a little more than half as rapidly as did well fed controls (3). Leloir and Munoz in 1938 showed that *in vitro* liver alcohol oxidation in fasting rats was slower compared to fed rats (4). Vitale et al. in 1953 suggested that fasting could possibly lead to the loss of essential liver enzymes responsible for alcohol oxidation; therefore it causes a decrease in oxidation rate. These investigators have also demonstrated that diphosphopyridine nucleotide (DPN) could limit the oxidation rate (5). This inhibition has been shown not to be due to a deficiency in alcohol dehydrogenase, diphosphopyridine nucleotide (DPN) level, nor to a metabolic block in the oxidation of the intermediaries acetaldehyde or acetate.

Moreover, they found that DPNH re-oxidizing agents such as ferricyanide and methylene blue raise the alcohol metabolism rate *in vitro*, while this increase does not occur in fed rats. According to Vitale et al. results, DPN: DPNH ratio lowers by alcohol metabolism in the intact animal. This decrease was noticeable in fasting. Alanine and pyruvate simultaneously boosted the DPN: DPNH ratio and the rate of alcohol metabolism. The greatest increase was observed in fasting state. Based on these findings, DPN: DPNH ratio could lower the rate of alcohol metabolism in fasting animals and to some degree in fed animals (6).

Fasting, Learning, Memory, and the Mood

Some researches were conducted by Fontan-Lozano et al. and Singh et al., to reveal the effect of fasting on learning, memory, and the mood of the experimental animals. In rodents, alternating days of normal feeding and fasting can enhance brain function, as indicated by improvements in performance on behavioral tests of sensory and motor function and learning and memory (7, 8). Li et al. showed chronic intermittent fasting improves cognitive functions and brain structures in mice. Their results suggested that intermittent fasting improves brain functions (9).

Intermittent Fasting

Intermediate or intermittent fasting (IF) refers to alternate periods of AL (ab libitum) intake with complete or partial restriction of calories. IF does not mean severe nutrient deprivation/starvation and it gets adequate vitamin and mineral intake (10). IF has been widely accepted, for the advent of the "5, 2" diet, in which there are two days of complete or partial CR (caloric restriction) during a weekly period (11). Overall caloric consumption and body weight (BW) do not necessarily decrease in IF regimens since subjects may compensate for the reduced intake throughout the restriction period by overeating on the AL phase, although a 20–30% reduction of caloric consumption following IF regimen has been reported (12, 13).

It has been proved that IF regimens could efficiently impact age-related diseases in

animals, such as the attenuation or prevention of diabetes, and also phenotypes and cardiovascular diseases as well as increasing maximal life span (12).

At the molecular level, IF is thought to engage adaptive cellular stress response pathways and appears to engage many of the pathways described for CR (14, 15).

The Effects of IF on Brain Plasticity

IF particularly develops brain plasticity and at cellular and molecular level with concomitant improvements in behavior (16). Additionally, hippocampal neurons displayed more resistance to chemically induced degeneration in rats on maintained IF regimen (17, 18) and in experimental models of stroke (19). Moreover, neuroperformance such as memory and learning would be preserved by the neuroprotective effect of IF (19, 20). Moreover, the effects of IF following excitotoxic challenge associated with lower levels of corticosterone, leading not only to decreased hippocampal cell death, but also to increased levels of hippocampal brain-derived neurotrophic factor (BDNF) and phosphorylated cAMP responsive-element binding (pCREB) and reversal of learning deficits (18).

IF fed mice showed the development of neurogenesis and glycogenesis and further survival of newly born cells in the dentate gyrus (DG) (21). This growth led to learning progress regarding the improvement of LTP long-term potentiation, the expression of long-lasting activity-dependent synaptic modifications in response to high frequency stimuli and expression of the N-methyl-D-aspartate receptor subunit NR2B (22). The resilience of hippocampal neurons to excitotoxic stress IF but not CR for 20 weeks increases, suggesting distinct neuroprotective effects of IF (23).

IF and Mood/Anxiety

Since neither epidemiological nor interventional study of the effects of IF on mood/anxiety in the human beings has taken place; this section will focus on the promising findings of IF in animal studies.

While the majority of studies on the effects of fasting have focused on chronic procedures, a few have also examined the results of acute interventions. In this regards, a recent study has shown significant antidepressant effects of 9 h fasting in mice (24).

Plus, administration of antidepressant drug (imipramine) in conjunction with fasting has showed synergic effects. According to the authors, these findings could be used as a new strategy to boost antidepressant effects in clinical practice. Furthermore, acute fasting boosted the p-CREB/CREB ratio (p-CREB: phosphorylated CREB), as a biological effect in accordance with some caloric restriction (CR) studies' findings (25).

Impacts of IF on Aging

Singh et al. aimed to test whether short-term late-onset exposure to an IF regimen could improve age-related declines in cognitive and motor functions, in association with possible changes in the expression of plasticity markers (9). Memory function improved in old IF rats. Besides, these authors demonstrated the impacts of short-term IF on Ca2+ signaling, the main regulator of synaptic plasticity. They particularly evaluated the expression of serine/threonine protein phosphatase calcineurin (CaN), as the mediator of the effects of Ca2+ signaling on synaptic plasticity, cell survival, and ultimately cognition (26), as well as the Ca2+-dependent protein kinase (CaM kinase), known as a functional protein in synapse formation and neurotransmitter release, thus affecting neuroplasticity, learning, and memory performance (27, 28). Short-term IF partially restored the expression of CaN and synaptophysin, decreasing with age in the region III of hippocampus proper (CA3) and DG subregions of the hippocampus. This evidence proposes the recovery of synapse density loss and parallel increases in neurotransmission. The probable expression of synaptic proteins regulating calcium homeostasis known as the advantageous effect of IF regimen regarding the decreased levels of CaN and decreased expression of CaM in the hippocampus of aged rats (9). Another research performed by Mladenovic Djordjevic et al. revealed the induction of synaptophysin expression in the DG and CA3 as the consequence of IF regimen (29). Similar effects of CR in moderating age-related declines in synaptophysin levels approve the above mentioned findings (30, 31). They all indicate that hippocampus stress reduction,

synaptic plasticity enhancement, and neurogenesis growth underlie the preservation of synaptic functionality linked to CR and IF regimens. IF has also been shown to reverse the age-related impairments in the neuronal plasticity marker, neural cell adhesion molecule (NCAM) (32).

Effects of IF on Metabolism

Recent investigational studies have fine clarified the IF-activated metabolic mechanisms. Glucose metabolism and boosted neuronal resistance to stress in C57BL/6 mice subjected to ad libitum diet, IF, or limited daily food intake for 22 weeks has been revealed by Anson et al. (33). An injection of kainate, a seizure- and neuronal damage-inducing exotoxin raised neuronal stress at the end of the 22nd week. The IF group showed less significant histopathological brain changes and lower plasma glucose and insulin levels compared with the control group. Analyses in male Wistar rats achieved the same results (34). A remarkable decrease in the size of adipocytes of both visceral and subcutaneous fat was also noticed in 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%). The authors also realized that IF causes oscillation of triglyceride metabolism between anabolism (gluconeogenesis and de novo lipogenesis) and catabolism (lipolysis) (35). A noticeable decrease in visceral fat percentage, amplified subcutaneous fat percentage, increased plasma adiponectin levels, and unaffected amount of fat tissue female C57BL/6J mice subjected to four-week IF were all observed by the same research team (36). Moreover, using an implanted telemetric transistor, Mager et al. studied heart-rate variability in 12 Sprague-Dawley rats within 16 weeks of nutritional intervention. Based on their findings after 16 weeks, the sympathetic activity related with increased vagal tone declined in IF group rats. The immunomodulatory effects of this reduction would lessen the release of proinflammatory substances, such as TNF-α, IL-6, and IL-18 (37).

Intermittent Fasting (IF) and Increased Resistance to Stress

Wan et al. assessed the response to cardiovascular stress in male Wistar rats undergoing IF regimen using a telemetric transistor. Interestingly, they noticed a rapid return to basal values of blood pressure and heart-rate after induced cardiovascular stress, and no alterations in the plasma levels of stress biomarkers, such as adrenocorticotropic hormone and corticosterone. A better norepinephrine response to stress was found in further analysis of heart-rate variability, thus indicating an improved autonomic response in the IF group versus the control group (34).

In addition, the same authors demonstrated that both rat groups receiving a diet supplemented with 2-deoxyglucose (2-DG) - a glycolytic inhibitor and metabolic stress or - on alternate days and receiving IF in Sprague-Dawley rats could be affected in the same way. They observed that heart rate, blood pressure, and glucose levels could be decreased to similar extents by both IF and 2-DG supplementation. The authors also 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 (38).

Ahmet et al. assessed an alternative cardioprotective action of IF; they maintained 30 Sprague-Dawley rats under IF for three months and a control group with normal feeding (39). Then, all experimental animals were transferred to coronary artery ligation to induce myocardial infarction (MI). Left ventricular (LV) mass, LV wall thickness and ventricular remodeling IF group were slighter in comparison with control group. Besides, 23 hours following surgery, apoptosis and neutrophil infiltration remarkably dropped in IF group with MI, possibly contributing to a smaller ventricular size. The authors proposed that IF regimen leads to an ischemic preconditioning in the cardiac muscle that protects myocardial cells from ischemic damage. Wan et al. have recently achieved the similar results in male Wistar rats (40). In this study, the measurement of plasma adiponectin and IL-6 concentrations indicated an increase in the former and a decrease in the latter. The authors proposed that the observed benefits of IF might arise from the augmented plasma adiponectin levels.

More recently, Katare et al. conducted a study on the impact of IF following MI induction and continued for six weeks (41). 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 (42). 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 alternate-day fasting (ADF) in a group of male Sprague–Dawley rats after cervical spinal cord injury (43). 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 (44). 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 alternate-day fasting (ADF) group (25% calorie restriction) every day. Davis et al. also found positive results assessing not only spinal cord lesion but also traumatic brain lesion (TBL) induced in Sprague–Dawley rats (45). 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 (46). Another study showed some factors including WBC parameters, glucose, corticosterone skewed high, low, high respectively after short-term fasting in animals (47). Daniel E. Naya et al. investigated the effect of short- and long-term fasting on digestive and metabolic flexibility in the Andean toad (*Bufo spinulosus)*. They investigated the effect of short-term fasting and hibernation on the hydrolytic activity of digestive enzymes, histology of the small intestine, gross morphology of digestive and other internal organs and standard metabolic rate. There was no difference in body mass or snout–vent length (SVL) among groups. Except for the large intestine, which did not differ among groups, digestive organ length and mass were both greater in feeding than in fasting toads and in fasting compared with hibernating animals. Liver dry mass also changed gradually between feeding, fasting and hibernating animals but in this case differences between fasting animals and the other two groups did not reach statistical significance. Abdominal fat bodies were heavier in both summer groups than in hibernating toads, whereas kidneys were heavier in feeding animals than in both fasting groups (48). Another study focused on carnitine metabolism in the fasting rats. The results showed the concentration of carnitine began to fall early during the fast, being significantly lower 12 hours after fasting. After reaching a nadir after 24 h of starvation, the concentration began to increase slowly, returning to pre-fast levels. In contrast, the concentration of acid-soluble acylcarnitines rose slowly throughout the fast. Total carnitine, reflecting the sharp drop in carnitine, was significantly depressed early in the fast. The plasma concentrations of free fatty acids and P-

hydroxybutyrate. Both were significantly elevated after 12 h of starvation. After reaching a peak between 24 and 72 h of starvation, concentrations of the compounds decreased in the late stages of the fast. These observations serve to characterize the fast with respect to the shift to fatty acids as a fuel source and the development of ketosis (49).

Many of the metabolic changes in starvation, including the production of ketone bodies, occur in the liver. Following a decrease during the first 12 h of starvation, levels of carnitine increased in the liver and plateaued after 72 h. In contrast, acid-soluble acylcarnitines began to increase immediately and remained stable after 24 h of starvation. Acid-insoluble acylcarnitines also began to increase immediately, reaching maximum 18 h into the fast and decreasing slowly after 36 h. As a result of this increase in acylcarnitines, total liver carnitine per g of liver was elevated after 18 h of starvation and plateaued after 24 h of starvation (50).

Conclusion

Based on the existing evidence from animal studies, we conclude that fasting and IF are two key factors that may affect clinical pathological parameters like red blood cell count, hemoglobin, hematocrit and etc. in toxicological and pharmacological studies. In animals, fasting results in major decrease in red blood cell count, hemoglobin and hematocrit. It is also responsible for the decreased rate of oxidation. Fasting can also enhance brain function in experimental animals, as indicated by improvements in performance on behavioral tests of sensory and motor function and learning and memory. IF particularly exerts positive effects on brain plasticity at cellular and molecular level with concomitant improvements in behavior. Intermittent fasting (IF) is thought to engage adaptive cellular stress response pathways and appears to involve many of the pathways that have been described in caloric restriction. Moreover, IF has been associated with significantly lower histopathological brain changes. Animal studies have showed strong and replicable effects of IF on health indicators such as improvement in insulin sensitivity and decrease in body fat, blood pressure, IGF-I, insulin, glucose, atherogenic lipids, and inflammation.

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