LITERATURE REVIEW: THE EFFECTS OF INTERMITTENT FASTING ON

METABOLISM AT THE LIVER

By

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Abstract

In recent years, chronic age-related diseases have increased in correspondence with the rising U.S. life expectancy. Metabolic disorders, such as Type II Diabetes, are among such diseases that have increased in prevalence in the aging population. Intermittent fasting has gained traction as preventative and therapeutic measure to prevent metabolic disease. This literature review summarizes the current literature examining the effects of intermittent fasting on lipid metabolism, glycemic control, and insulin sensitivity at the liver. Intermittent fasting improves lipid metabolism and ameliorates hepatic steatosis. Comparatively, the effects of intermittent fasting on glycemic control and insulin sensitivity have conflicting results. These inconsistencies require further examination to reach a consensus on the effects of intermittent fasting on glycemic control and insulin sensitivity.

Introduction

Advancements in healthcare have extended life expectancy in developed nations. In the United States, the number of Americans over 65 years of age is expected to surpass the number of Americans under the age of 18 by 2034 (1); furthermore, the number of Americans in advanced age (over 85) may reach roughly 9 million in 2030, and 18 million by 2050 (1). This rise in life expectancy corresponds with a surge in chronic age-related diseases, which can severely impact an individual's quality of life and strain the healthcare system. Among these diseases, metabolic disorders have risen dramatically in developed nations. Obesity and Diabetes Mellitus (DM), are among the devastating age-related diseases that can lead to a higher risk of morbidity (i.e., cardiovascular disease, amputation), and mortality (2). This has led to a growing body of research dedicated to extending "healthspan" or the period when no major diseases or

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illnesses are affecting the individual (3-6). A longer healthspan will delay the onset of age-related health problems, allowing patients to remain in "good health" despite their advanced age.

Intermittent fasting (IF) has gathered traction as a potential dietary strategy to help extend one's healthspan, by improving metabolic health. This dietary regimen is broadly described as restricting calorie intake (complete or partial restriction) for a set period (16-24 hours). In between fasting periods, there are periods of ad libitum food intake. This regimen can be divided into subtypes with small variations in the level of calorie restriction and the days/time of fasting. Some popular regimens of intermittent fasting are 5:2 and Early Time-Restricted Fasting. 5:2 Intermittent Fasting refers to eating ad libitum for five days of the week and restricting calories the remaining two days of the week. This type of intermittent fasting corresponds to distinct days for the fasting and feeding cycle. Comparatively, the Early Time-Restricted fasting is more concerned with the time of day; all meals/snacks are restricted between 8 am and 2 pm. Outside this set period, the individual will fast. These subtypes of intermittent fasting allow individuals to choose a schedule that fits their preferences.

Numerous studies have shown that intermittent fasting improves metabolic health in both animal models (7-9) and clinical trials (10-12). Other groups have demonstrated the benefits of intermittent fasting are independent of exercise/weight loss (13). Sutton et al. noted early time-restricted fasting improved insulin sensitivity, and decreased appetite in pre-diabetic men when weight loss was controlled (14). Also, under close medical evaluation, patients using hypoglycemic medications can intermittently fast with a low-calorie diet for two days of the week; this creates a robust combination therapy for improving metabolic health (15). These promising results in the clinical setting have encouraged more comparative research studies, investigating the effects of different intermittent fasting regimens on the body.

This literature review aims to synthesize the current evidence of intermittent fasting on metabolic health, specifically hepatic lipid metabolism, glycemic control, and insulin sensitivity. This review will also discuss future research areas and the prospect of intermittent fasting becoming an effective therapy for age-related diseases compared to more traditional dietary patterns (continuous calorie restriction). Studies were excluded if fasting was coupled with exercise, or pertained to religious practices.

Effects of Intermittent Fasting on Hepatic Lipid Metabolism

One of the main root causes of metabolic dysfunction is poor diet. Increased consumption of lipids, fructose, sucrose, and glucose is associated with an increased accumulation of triglycerides stored in the liver. This can cause hepatic steatosis or non-alcoholic fatty liver disease (NAFLD) (16). Lipid consumption also inhibits insulin production, increasing the risk of insulin insensitivity, obesity, and Type II diabetes (16). With age, basal metabolic rate slows, causing further complication. Intermittent fasting has been shown to improve liver function by decreasing lipogenesis and hepatic steatosis. Marinho et al. (17) studied the effects of intermittent fasting in 20-week-old male mice that were either fed a high-fat (50% kcal lipids) or high-fructose (50% kcal of carb from fructose) diet. Each treatment group (high-fat, high-fructose, and normal chow [control]) were fed ad-libitum for 8 weeks before half of the group started the intermittent fasting routines (fed and fasting cycle alternating every 24 hours). Each of the mice in the high fat/fructose diet exhibited severe hepatic steatosis and liver damage, as demonstrated by elevated levels of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT). AST is an enzyme found in multiple different organs including the liver, while ALT is localized in the liver. High serum levels of either enzyme are indicative of severe liver damage (among other organ damage). Both the high fructose and the high fat diet led

to insulin resistance, as assessed by HOMA-IR (the homeostasis model assessment for insulin resistance), which measures beta cell function and insulin resistance using fasting glucose and insulin levels (or C-peptide concentrations) (18). After two weeks of intermittent fasting, ALT levels and inflammatory signals at the liver (tumor necrosis factor [TNF-α], Nuclear factor-κB [NF-κβ], interleukin - 6 [IL6], interleukin – β [IL$_1$β]) decreased in mice fed a high fat/fructose diet. This was matched with a decrease in hepatic triglyceride levels and gene/protein expression of hepatic de novo lipogenesis. Similarly, expression of genes and associated proteins of fatty acid beta-oxidation increased in the intermittently fasted high-fructose diet group but not in the high fat diet group. Hepatic gene expression and immunostaining of Perilipin 2 (PLIN2) also decreased due to intermittent fasting, regardless of the diet. PLIN2 is a lipid droplet protein that promotes hepatic lipid storage, inflammation and fibrosis in the pathophysiology of NAFLD (19). The HOMA-IR results also improved indicating intermittent fasting helped remedy the insulin resistance the mice developed from their high fat and fructose treatments. These results also suggest intermittent fasting helps drive metabolic adaptations, leading to more efficient utilization of stored fats as an energy source (17).

Baumeier et al. (20) conducted a similar study to test the effects of intermittent fasting when mice were fed a high-fat diet. The authors compared their results to mice on a continuous fasting regimen. The study consisted of three mouse models: high fat-ad libitum diet (AL mice), 90% calorie restricted - ad libitum (CR mice) and intermittently fasted mice (90% calorie intake every 24 hours; IF mice). Body mass in the CR mice and IF mice were both reduced. Comparatively, body mass and blood glucose levels increased in the AL group. The prevalence of diabetes in the AL mice reached nearly 50% when the mice reached 14 weeks old - none of the CR or IF mice developed diabetes. IF and CR mice exhibited greater metabolic flexibility

based on data from the mice respiratory quotient (RQ). During the dark phase, when mice are active and consume the majority of their food (active), RQ increased slightly in CR mice; this indicates a higher reliance on carbohydrate metabolism. During the light phase, when food intake and activity is typically decreased, the RQ decreased slightly (indicating a greater reliance on metabolism of lipids). Comparatively, the IF mice were more efficient at lipid oxidation during the fasting stage and carbohydrate oxidation during the feeding stage. Between the two treatments, IF mice showed the most improvement with a distinct and rapid movement between both fuel sources during the fasting and feeding phases. In comparison, the respiratory quotation of the AL mice rested above 0.90, regardless of the time of day; this indicates the use of carbohydrates as the predominant fuel source. The researchers then fasted the AL mice for 24 hours to determine if the difference in respiratory quotients across treatments were based on an adaptive response from the intermittent fasting routine. The AL mice did switch to lipid oxidation during this fasting period; however, the switch in fuel sources -carbohydrates to lipids- was not as robust as in the IF mice and CR mice. These findings confirm that IF does initiate an adaptive response to increase metabolic flexibility. Baumeier et al. (20) also analyzed hepatic triglyceride and diacylglycerol levels in the liver. Both levels were significantly reduced in the IF mice, with only a moderate reduction in the CR mice. PLIN2 expression also decreased in CR and IF mice. Interestingly, the researchers noted the composition of 18 hepatic lipid droplets was altered in the CR and IF mice. These hepatic lipid droplets are associated with managing lipid storage and structure. Of the 18 identified hepatic lipid droplet proteins, 14 were significantly elevated while 4 deceased in expression based on lipid proteomics. The change in expression of these proteins on liver function are not well known. However, given the beneficial effects of calorie restriction on liver health, the researchers predicted such proteins must contribute to the

protective, metabolic effects during fasting states. Among the proteins was autophagy related 3 (ATG3), an enzyme that initiates degradation of lipids through autophagy. The elevated protein expression suggests intermittent fasting increases the rate of lipolysis. The link between intermittent fasting and hepatic lipid droplet composition is an area for future research studies that may elucidate the molecular pathways intermittent fasting activates (20).

Li et al. (21) is among the group of researchers investigating the molecular pathways activated during intermittent fasts. They hypothesized peroxisome proliferator-activated receptor alpha (PPARA) may have a role. PPARA is a nuclear receptor that acts as a transcription factor to promote hepatic fatty acid oxidation, ketogenesis, and hepatic amino acid metabolism during acute fasts (22). Li et al. (21) predicted PPARA may also have a significant role during a long-term regimen such as an intermittent fasting routine. To test their hypothesis, they used two mouse models, PPARA-null mice and wild-type controls, in three experiments. The first experiment established a baseline of values for how an acute fast will impact the control group (wild-type mice- fed ad libitum standard rodent chow). In this first experiment, the control mice exhibited hepatic steatosis after an acute fast. Furthermore, there was an upregulation of PPARA and its target genes: Fibroblast growth factor 21 (Fgf21) and Carnitine Palmitoyltransferase 1A (Cpt1a). Fgf21 and Cpt1a act on the liver to control blood glucose levels and fatty acid oxidation, respectively. Surprisingly, acyl-Coenzyme A dehydrogenase (Acadm) a critical component for fatty acid oxidation was unaffected by the fast. To assess the effect of PPARA on hepatic lipid accumulation, the researchers conducted a second experiment using a PPARA agonist, Wy-14643. Wild-type mice were either given Wy-14643, or a matching diet. The agonist increased expression of PPARA target genes (ACADM and CPT2) and lowered serum triglycerides levels during the fed stage. Contrary to their initial predictions, the agonist did not reduce hepatic

steatosis after an acute fast, and caused severe liver damage. The results indicate that intermittent fasting does not depend on PPARA activation to improve metabolic flexibility. To confirm this finding, Li et al. (21) developed PPARA-null mice and imposed an intermittent fast every 16 hours. Wild type mice were given the same feeding schedule to act as the control. The PPARA-null mice showed severe hepatic steatosis before starting the intermittent fasting routine. The predominant fatty acid present was the long –chain acylcarnitines. These hepatic triglyceride levels in the PPARA-null mice greatly exceeded the levels in the wild-type mice after an acute fast. When the mice were intermittently fasted, the accumulation of long-chain acylcarnitines decreased without the activation of any PPARA target gene pathways. This response confirms intermittent fasting induces a metabolic adaptation to fasting and does not depend on PPARA nor its targeted gene pathways. Future studies must investigate alternative pathways to determine which biological pathways are activated by intermittent fasts to increase metabolic flexibility (21).

Effects of Intermittent Fasting on Glycemic Control and Insulin Sensitivity

Unlike the effects of IF on hepatic lipid metabolism, researchers have found it difficult to reach a consensus on the effects of intermittent fasting on glycemic and insulin control. Over the years, various studies have documented a range of possible outcomes, spanning from beneficial (or neutral) to being harmful and damaging. Wei et al. (23) are among the researchers who support intermittent fasting for glycemic control. The study utilized a mouse model of obesity-induced Type II diabetes, db/db (mice deficient in leptin signaling) and a model of streptozotocin-induced Type I Diabetes (STZ injection intraperitoneally to ablate pancreatic beta cells). Additionally, two intermittent fasting routines were tested: 2:5 and 5:9 regimen (ie: 2 or 5 days of ad libitum and 5 or 9 days of partial calorie restriction). On fasting days, the 2:5 and 5:9

regimen employed a partial restriction of calories (81% and 76% of total calories, respectively). These two treatments were compared to a continuous calorie restriction group with equal calorie intake levels (81% and 76% of total) as the two intermittent fasting routines and a control group with normal rodent chow. Due to deletion of the leptin receptor, the db/db mice were obese, insulin resistant, and hyperglycemic. The researchers discovered that db/db mice exhibited a lower fasting blood glucose level when fasted intermittently (2:5 diet pattern) or continuously. The glucose tolerance test (GTT) and the insulin tolerance test (ITT) also indicated marked improvements in glucose tolerance and insulin sensitivity in the mice fasted intermittently. Continuous calorie restriction in db/db mice moderately improved insulin sensitivity, and there was no change in glucose tolerance. The study was repeated using db/db mice with a 5:9 intermittent fasting routine compared to continuous calorie restriction. This experiment yielded similar results with intermittent fasting improving insulin sensitivity and fasting blood glucose levels more than a continuous fasting regimen. However, both diet plans proved to be equally successful in improving glucose tolerance. The researchers transitioned to a Type 1 Diabetes mouse (T1DM) model, using the same study design described for the db/db mice. Intermittent fasting (2:5 regimen) and continuous calorie restriction significantly ameliorated the high fasting blood glucose levels. Other groups have also shown that intermittent fasting improves insulin sensitivity in mice, with no change in glucose tolerance (20). The 5:9 intermittent fasting plan and continuous calorie restriction proved to be slightly more successful than the 2:5 routine. Both plans reduced fasting blood glucose levels and improved insulin sensitivity. Intermittent fasting also increased glucose tolerance significantly, but there was little change to blood insulin level in either treatment plan. The overall assessment of the data revealed that intermittent fasting for the Type 2 diabetic mouse fared better in improving glycemic control and insulin

sensitivity than continuous calorie restriction. Comparatively, in the Type I Diabetic mouse model, the two dietary plans (intermittent versus continuous fasting) were more evenly matched in efficacy (23).

Studies performed by Carter et al. (24) yielded similar results, whereby intermittent fasting proved to be an effective treatment for glycemic control in Type 2 diabetic patients. Their study design included 137 adult participants with Type II diabetes. The group was divided into two treatments - intermittent fasting (2:5- partial calories on fasting days with 75% restriction) and continuous energy restriction – to compare the efficacy of the dietary plans. The patients were closely monitored by an endocrinologist for hypoglycemic events during the 1-year duration of the experiment. Patients' blood glucose levels were assessed via hemoglobin A1c (HbA1c), a measure of the amount of glucose attached to hemoglobin (Hb) in erythrocytes; this serves as an indication of an average of the patient's blood glucose levels over the course of roughly three months (25). Patients' HbA1c fluctuated during the course of the experiment but had an overall downward trend for both treatment groups. As anticipated, the patients with the highest incoming HbA1C values had the greatest change. There were corresponding decreases in fasting blood glucose levels in both treatment groups. As a result, the patients did have to decrease their oral hypoglycemic medication dosage. In addition, there was a significant decrease in the insulin dosage for patients in the intermittent fasting treatment group compared to the continuous calorie restriction group. Based on these results, it appears that both treatments are evenly matched in their effectiveness at controlling Type II Diabetics blood sugar levels; however, intermittent fasting appears to be slightly better at controlling blood sugars levels for patients who are taking insulin (24).

Higashida et al. (26) also concluded that intermittent fasting has some positive effects on glycemic and insulin control in rodents. Using a rat model, the authors compared three treatment groups: high-fat ad-libitum, high fat intermittent fasting (every 24 hours) and ad libitum standard rodent chow. After six weeks, fasting serum glucose levels did not change significantly in any of the three groups. Rats in the intermittent fasting and ad libitum chow group had decreased overnight fasted serum insulin levels, compared to high fat diet fed rats. The researchers also used the homeostasis model assessment to evaluate insulin resistance (HOMA-IR) at the liver. The researchers found intermittent fasting markedly improved the HOMA-IR results which corresponds to an improvement in hepatic insulin sensitivity (26).

Heilbronn et al. (27), however, identified negative implications of intermittently fasting, especially in women. The study used non-obese human subjects, ranging from 20-55 years old. The participants have not been diagnosed with Type II Diabetes. The subjects were instructed to double their food intake on feeding days in order to maintain body weight levels. The team observed their glucose tolerance and insulin sensitivity (among other factors) after a 24 hour fast every other day. This treatment was implemented for 22 days. The results from the study concluded the rate of glucose clearance was reduced in female subjects after intermittently fasting, whereas male subjects had no difference in their glucose clearance efficiency. Additionally, insulin sensitivity was not affected in female subjects but improved in male subjects. These results suggest that the effects of intermittent fasting on glycemic and insulin control vary greatly. Furthermore, intermittent fasting may have some negative effects on women compared to men (27).

Park et al. (28) study also concluded that intermittent fasting is not an effective dietary plan. This study was similar in design as Higashida's team, which consisted of male rats being

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divided into paired treatment groups: high-protein with ad libitum feeding (HP-AL), high-protein

with intermittent fasting (HP-IMF), high-fat diet with ad libitum feeding, and high-fat diet with

intermittent fasting (HF-IMF). The intermittent fasting consisted of a 3-hour feeding period

during the dark (active cycle) followed by an overnight fast. The final results for the study

showed that after an overnight fast, the HF-IMF and HP-IMF had the highest serum insulin and

glucose levels compared to the other treatment groups. Insulin sensitivity decreased the most

among the intermittent fasting groups (high-fat and high protein). The study found there was

reduction of AKT phosphorylation (or Protein kinase B), which is responsible for assisting with

glucose metabolism. Similar results were found with the GLUT 2 transporter, which serves as a

transmembrane carrier protein of glucose in hepatocytes. A lower expression of this protein

signifies intermittent fasting has disrupted glucose transport and utilization in the liver. The

HOMA-IR results also increased, indicating higher hepatic insulin resistance. Based on these

results, intermittent fasting, on a high-fat or protein diet, would not be a safe choice for young

patients or those suffering with metabolic disorders. It appears intermittent fasting would

augment insulin resistance and glucose intolerance and may be linked to a wide-spread

disruption of glucose and insulin homeostasis (28).

Conclusion

Intermittent fasting is an effective dietary regimen for improving lipid metabolism (17,

20, 21). The aforementioned studies have shown intermittent fasting can ameliorate hepatic

steatosis on a high-fat diet by increasing lipolysis (20). Intermittent fasting has also been linked

to having an adaptive response, switching between carbohydrates and lipids as a fuel source

during a fast, thereby decreasing hepatic lipid accumulation. Intermittent fasting has also shown

to be equally as effective (or more effective, in some cases) than continuous calorie restriction, allowing it to be a great alternative for overweight or obese patients.

Given these results, intermittent fasting appeared to be a successful therapeutic measure to ameliorate metabolic diseases associated with hepatic lipid accumulation. However, there have been mixed results on the effectiveness of intermittent fasting when improving glycemic control and insulin sensitivity. Some researchers have found intermittent fasting is a successful diet plan for glucose metabolism and insulin sensitivity in patients with Diabetes Mellitus (Type I and Type II), and during a high fat diet. Whereas other studies have found intermittent fasting can disrupt glucose homeostasis and increase insulin resistance. Importantly, the effects intermittent fasting may be impacted by the gender of the patient. These conflicting results suggest there needs to be more research on intermittent fasting before it is promoted to the clinical setting. Future studies should focus on deciphering the specific conditions that maximize the beneficial metabolic effects of intermittent fasting.

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