The effects of intermittent fasting on liver physiology and metabolism in mice

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Research

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Abstract

Background: Intermittent fasting, as an emerging diet concept, has been widely practiced in the global population. A broad spectrum of health benefits has been reported in animal models and in humans for intermittent fasting. However, the underlying mechanisms remain largely elusive. In this study, we aimed to explore the effects and potential mode-of-action of intermittent fasting in mouse models, with focus on the liver.

Methods: C57BL/6 mice were divided into five groups of 8-14 each, including 30-days *ad libitum* group, 30-days intermittent fasting group, 60-days ad libitum group, 60-days intermittent fasting group and refeeding group (30-days intermittent fasting followed by 30-days *ad libitum*). The food intake, body weight, liver weight and the blood biochemical parameters were detected. Targeted metabolic profiling of liver was performed.

Results: We found that daily 12-hour intermittent fasting for one or two months significantly reduced the cumulative food intake, compared with the mice fed *ad libitum*. Fasting resulted in significantly reduced liver weight, with minimal effect on body weight. This effect on the liver by one month fasting could not be reversed by following one-month *ad libitum* feeding. Among the measured blood biochemical parameters, glucose level was decreased, while alkaline phosphatase was increased in the fasting mice. Surprisingly, targeted metabolic profiling revealed the global elevation of metabolites in the livers of fasting mice. These metabolic molecules include ATP, NADP, NADPH and succinate that are essentially involved in the citric acid cycle and oxidative phosphorylation.

Conclusion: Daily 12-hour intermittent fasting for one to two months significantly reduced liver weight in mice, which is associated with enhanced liver metabolism.

Introduction

Calorie restriction has been shown to mitigate age-associated declines in most pathophysiological parameters and to extend maximum lifespan in various animal species [1–3]. Fasting, as one type of calorie restriction, has been widely practiced for medical applications or religious rituals. It is defined as abstinence or reduction of food, drink, or both for a period lasting typically between 12hours (hrs) and 3 weeks, in short-term, long-term or an intermittent pattern [4, 5]. Intermittent fasting is an umbrella term for various diets that cycle between a period of fasting and non-fasting [6]. It is gaining momentum as a strategy to improve human health. In fact, intermittent fasting has been practiced by the Muslim population in the month of Ramadan for over a thousand years, which typically involves restricting eating and drinking for 12–16 hrs per day for one month.

The health benefits of intermittent fasting have been extensively demonstrated in animal models [7, 8], and in several observational studies associated with decreased cancer risk and metabolic disease phenotype [9, 10]. The mechanisms how fasting promotes health remain largely elusive, but regulation of metabolism is conceivably essential. As a state of negative energy balance, even a single fasting interval

in humans (e.g., overnight) can reduce basal concentrations of metabolic biomarkers that are associated with chronic diseases, such as insulin and glucose [11]. Prolonged food restriction decreases metabolic rate and bodyweight [12, 13], while others reported that fasting improves metabolic state due to less loss of lean body mass and greater fat burning [14, 15]. In the context of Ramadan fasting, changes can range from a reduction to arise in body weight [16–18].

Liver is a central organ for metabolic homeostasis [19, 20]. It executes uptakes, such as glucose, synthesizes glycogen and triglycerides following food intake, releases glucose produced by glycogenolysis or gluconeogenesis, and triggers ketogenesis during fasting [21]. High glucagon is produced during fasting, which stimulates glucose release from liver to provide a continuous fuel for peripheral tissues [22]. Therefore, observing the alterations of small molecules by metabolic profiling in liver allows comprehensive analysis of changes in several metabolic and signaling pathways and their interactions [23, 24].

To largely mimic the fasting patterns that are widely practiced in human population, this study aimed to evaluate the effects of daily 12hrs intermittent fasting for one to two months in mouse models. We mainly focus on the effects and mode-of-action on liver physiology and metabolism.

Materials And Methods

Search strategy and study selection

One author systematically searched the PubMed/Medline, Web of Science, Cochrane Library, and Scopus databases to retrieve articles published by October 2019 without publication period, language, and patient's age restrictions. The search terms or keywords were ("lactotransferrin" or "lactoferrin" or "LTF" or "enamelin" or "ENAM" or "amelogenin X" or "AMELX") and ("dental caries" or "caries" or "decay") and ("gene" or polymorphism* or variant* or genetic*). In addition, the references of the retrieved articles related to the topic including original and review articles were searched to make sure that no study was missed. After article retrieval, another author assessed the titles and abstracts of the articles related to the topic; subsequently, the full-texts of the articles that met our eligibility criteria were downloaded and screened. After screening, the exclusion reason was recorded for any study removed, and the disagreements between the authors were resolved by another author.

Eligibility criteria

The inclusion criteria were as follows: (I) studies including two independent groups (case group with caries or high caries and caries-free control group or with low/very low caries) without age restriction, (II) studies with any defined Decayed, Missing, and Filled Teeth (DMFT) score for the two groups, (III) studies including one or more polymorphisms of *LTF*, *ENAM*, and *AMELX* genes with a minimum of two relevant

studies for the analysis; for example, four studies assessed *LTF* rs1126478, *ENAM* rs1264848, *ENAM* rs3796704, *ENAM* rs3796703, *AMELX* rs946252, *AMELX* rs17878486, *AMELX* rs6639060, and *AMELX* rs2106416 polymorphisms; and (IV) patients and controls had to have no genetic diseases, chronic illnesses, or other disorders. We excluded irrelevant studies, studies without sufficient data for analysis, studies without a control group, studies including less than 20 individuals in each group, duplicate studies, animal studies, case reports, conference papers, reviews, and systematic reviews.

Data abstraction

Two authors independently abstracted the data of the studies analyzed in the meta-analysis. The data from each study, including first author, publication year, country of residence of the included individuals, ethnicity, mean age of individuals in the two groups, age group of individuals in each study, genotyping method, DMFT score of the two groups, and type of reported polymorphism (s) in each study, were extracted and analyzed.

Statistical analysis

Review Manager 5.3 (RevMan 5.3) software was applied to compute the odds ratios (ORs) and 95% confidence intervals (CIs). To estimate the significance of the pooled OR by the Z test, a *p*-value (two-sided) < 0.05 was considered significant. The I^2 statistic was used to estimate heterogeneity. A p <0.1 or I^2>50% indicated a significant heterogeneity and we used the random-effects model for such cases; if not, the fixed-effects model was used. The publication bias across the studies was assessed using the Egger's and Begg's tests. If p < 0.05 (two-sided) for both tests or one, there was a significant degree of publication bias. In order to evaluate the stability/consistency of the results, the sensitivity analysis with both "the removal of one study" and "cumulative analysis" was performed. The results of these tests were retrieved by Comprehensive Meta-Analysis 2.0 (CMA 2.0) software.

Results

The effects of intermittent fasting on food intake and body weight of mice

During the 30-days experiment, all animals increased their body weight. The body weight had no significant difference between the ad libitum group and the intermittent fasting group at the end of the experiment (AL vs IF, 21.701 ± 1.305 vs 21.610 ± 1.187, mean ± SEM, n = 10), though the cumulative food intake of ad libitum mice was much more than intermittent fasting mice. These results were summarized in Table 1. To confirm this result, we extended the duration of fasting to 60 days. Three groups were included in the 60-days experiment, including ad libitum, intermittent fasting and refeeding group. The refeeding group refers to 30-days intermittent fasting followed by 30-daysad libitum diet (IF&AL). Their body weight and food intake were summarized in Table 1, respectively. The body weight of these three groups had no significant difference by the end of the experiment (AL vs IF vs IF&AL, 26.193 ± 1.680 vs

26.844 ± 1.136 vs 26.457 ± 1.779, mean ± SEM, n = 8–14).Thus,12hrs nighttime intermittent fasting did not affect mouse body weight, although has less cumulative food intake.

Table 1

Body weights and food intakes of the mice

Groups	30-days experiment		60-days experiment		
	AL	IF	AL	IF	IF&AL
Body weight (g) Beginning of experiment	**15.578 ± 1.172**	**14.890 ± 0.853**	**21.764 ± 1.381**	**20.900 ± 1.166**	**20.613 ± 1.629**
End of experiment	21.701 ± 1.305	21.610 ± 1.187	26.193 ± 1.680	26.844 ± 1.136	26.457 ± 1.779
Gain of weight	39.305%	45.132%	20.350%	28.440%	28.351%
Food intake (g) Beginning of experiment	**3.667 ± 0.343**	**3.380 ± 0.305***	**3.314 ± 0.422**	**4.000 ± 0.444****	**3.745 ± 0.511***
Middle of experiment	3.770 ± 0.393	3.219 ± 0.238**	3.221 ± 0.691	2.889 ± 0.389	2.714 ± 0.613*
End of experiment	3.765 ± 0.477	2.721 ± 0.393***	3.536 ± 0.371	2.433 ± 0.424***	2.976 ± 0.612**

AL: ad libitum group; IF: intermittent fasting group; IF&AL: intermittent fasting followed by ad libitum group

* P < 0.05, ** P < 0.01, *** P < 0.001

Reduced liver weight by intermittent fasting

Livers were isolated, observed and weighted after sacrificing the mice. The livers of intermittent fasting mice appear smaller than those of ad libitum mice (Fig. 1D and G). Subsequently, the wet livers were weighted and liver weight/total body weight (LW/TBW) was determined. Our results showed that the liver mass of intermittent fasting mice was significant less than that of ad libitum mice (Fig. 1E and H), and LW/TBW ratios of was much lower in intermittent fasting compared to ad libitum mice (Fig. 1F and I). Ad libitum refeeding for 30 days failed to recover fasting-induced loss of liver weight.

Subsequently, the morphological changes of mouse liver were examined. Histology assessment of the liver tissues by H&E staining showed that the hepatocyte plates were well developed and the sinusoids were clearly visible in all the livers (Fig. 2A and B).

Intermittent fasting alters liver-related biochemical markers in blood of mice

We next profiled the blood biochemical markers that are associated with liver physiology or functions, including glucose, total protein (TP), albumin (ALB), globulin (GLO), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenate (LDH) and γ-glutamyl transferase (γ-GT). We found that blood glucose level was significantly decreased in intermittent fasting compared with ad libitum mice (IF vs AL, 1.391 ± 0.914 vs 5.726 ± 0.648, mean ± SEM, n = 7, $P < 0.01$, Fig. 3A). The level of alkaline phosphatase was significantly increased in intermittent fasting group (IF vs AL, 171.571 ± 13.465 vs 152.829 ± 17.130, mean ± SEM, n = 7, $P < 0.05$, Fig. 3B). No significant change was observed among other tested parameters (Fig. 3C-K). These results suggest that intermittent fasting appears not to affect liver function, but likely regulates metabolism.

Intermittent fasting rewires liver metabolism

To finally assess the effects on liver metabolism by intermittent fasting, we performed targeted metabolomics by LC-MS/MS analysis. Principle component analysis showed clear clustering based on the diet patterns of the mouse groups (Fig. 4A). The distribution of the most significant metabolites that separated the mice was visualized in the heatmap (Fig. 4B). Twelve metabolic molecules were increased more than 1.5-foldin intermittent fasting mice when compared with ad libitum mice (Fig. 5A-L), including nicotinamide adenine dinucleotide phosphate (NADP), adenosine triphosphate (ATP), succinate, reduced nicotinamide adenine dinucleotide phosphate (NADPH). Most of these metabolites are essentially involved in the citric acid cycle and oxidative phosphorylation. We postulate that intermittent fasting enhances liver metabolism, and this may explain the reduced liver mass in these mice.

Discussion

Fasting has been suggested as a promising approach for health benefits in animal models and humans [2, 25]. Intermittent fasting in obese people showed that reducing calorie intake one to six days per week over at least 12 weeks was effective for reducing body weight [26]. Especially, the 5:2 diet has become a popular intermittent fasting regimen and has a potential to be considered for medical interventions.

Our study showed that daily intermittent fasting of 12hrs at night for one to two months reduced blood glucose level and enhanced liver metabolism, although did not affect body weight in mice. Given the feeding habits in our model that mice are nocturnal in biological opposition to human circadian rhythms, our study resembles Ramadan fasting to some degree, which is a lunar month and lasts 29–30 days. Ramadan fasting requires Muslims to fast daily from dawn to dusk, and food can be taken without any restriction in the night [4]. Studies reported a modest weight loss or gain by the end of Ramadan, and the mean weight loss was regained a few weeks after Ramadan [17, 18].

Much of the research has focused on what to eat to prevent diseases. Recently, a novel theorem has emerged that when to eat also matters, with research showing that the timing of food intake affects metabolic health and cancer development [27–29]. A large prospective cohort of 2413 patients with breast cancer reported that short nightly fasting duration (< 13 hours per night) was associated with 36% increased risk for cancer recurrence [30]. Thus, erratic feeding behaviors can have detrimental health

consequences [31]. Circadian disruption has recently been identified as a common risk factor for obesity, metabolic disorders, non-alcoholic fat liver diseases as well as liver cancer [32–34]. At least 10% of the liver transcriptome demonstrates rhythmic expression, implying that the circadian clock regulates a large set of hepatic genes [35]. It has been demonstrated that chronic circadian disruption promotes weight gain and hepatic lipid storage in mice [36, 37]. This may be the reason for weight loss of liver and slight weight gain of body in our mouse model. Certainly, better-quality animal and more studies are needed to provide mechanistic data on time-restricted feeding in this field.

In normal condition, liver serves as the main organ for supplying energy to body, while 12 to 24 hrs fasting results in depletion of the hepatic glycogen, accompanied by a switch to a metabolic mode in which nonhepatic glucose, fat-derived ketone bodies, and free fatty acids are used as energy sources [25]. It has been suggested that intermittent energy deprivation is adequate for improving metabolic health, however, identifying how these energy restrictions initiate the processes are still major challenges. Our results identified that the majority targeted metabolites were significantly increased in the livers of intermittent fasting mice. These metabolites are essential components of the citric acid cycle, oxidative phosphorylation and glycolysis cascades, indicating the enhancement of liver metabolism. Although the metabolic consequences of intermittent fasting are complex and the mechanisms by which intermittent fasting benefits the metabolic regulation are unknown, studies of these molecular changes in liver provide potential for offering promising nonpharmacological approaches to improving health at the population level with multiple public health benefits.

In conclusion, intermittent fasting reduces liver weight and rewires liver metabolism in mice. This may partially contribute to the health benefit of fasting, and bear implications for intervention of chronic diseases, such as non-alcoholic fatty liver disease. Nevertheless, the mechanisms of intermittent fasting regulating liver metabolism remain to be investigated.

Abbreviations

AL: *Ad libitum*; IF: Intermittent fasting; IF&AL: Intermittent fasting followed by *ad libitum*; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; AST/ALT: Aspartate aminotransferase/alanine aminotransferase; TP: Total protein; ALB: Albumin; GLO: Globulin; A/G: Albumin/globulin; LDH: Lactate dehydrogenase; γ-GT: γ-glutamyl transpeptidase; NADP: Nicotinamide adenine dinucleotide phosphate; ATP: Adenosine triphosphate; PEP: Phosphoenolpyruvate; NADPH: Reduced nicotinamide adenine dinucleotide phosphate; GDP: Guanosine diphosphate; AMP: Adenosine monophosphate; FMN: Flavin mononucleotide; TPP: Thiamine pyrophosphate; NAD: Nicotinamide adenine dinucleotide

Declarations

Ethics approval and consent to participate: The animal studies were performed with the approval of the Experimental Animal Committee of Lanzhou Veterinary Research Institute, Lanzhou, China.

Consent for publication Not applicable.

Availability of data and materials: All data generated or analyses during this study are included in this published article.

Competing interests: The authors declare that they have no competing interests.

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Author contributions: J.M, Y.W, L.L and Q.S performed the experiments, J.M and Y. C analyzed the data and wrote the manuscript; W.A, G.L, Z.M, Z.Q, Q.P and K.C performed project discussion and analyzed the data, Q.P and K.C supervised the research and edited the manuscript.

The authors declare no conflict of interest.

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Figures

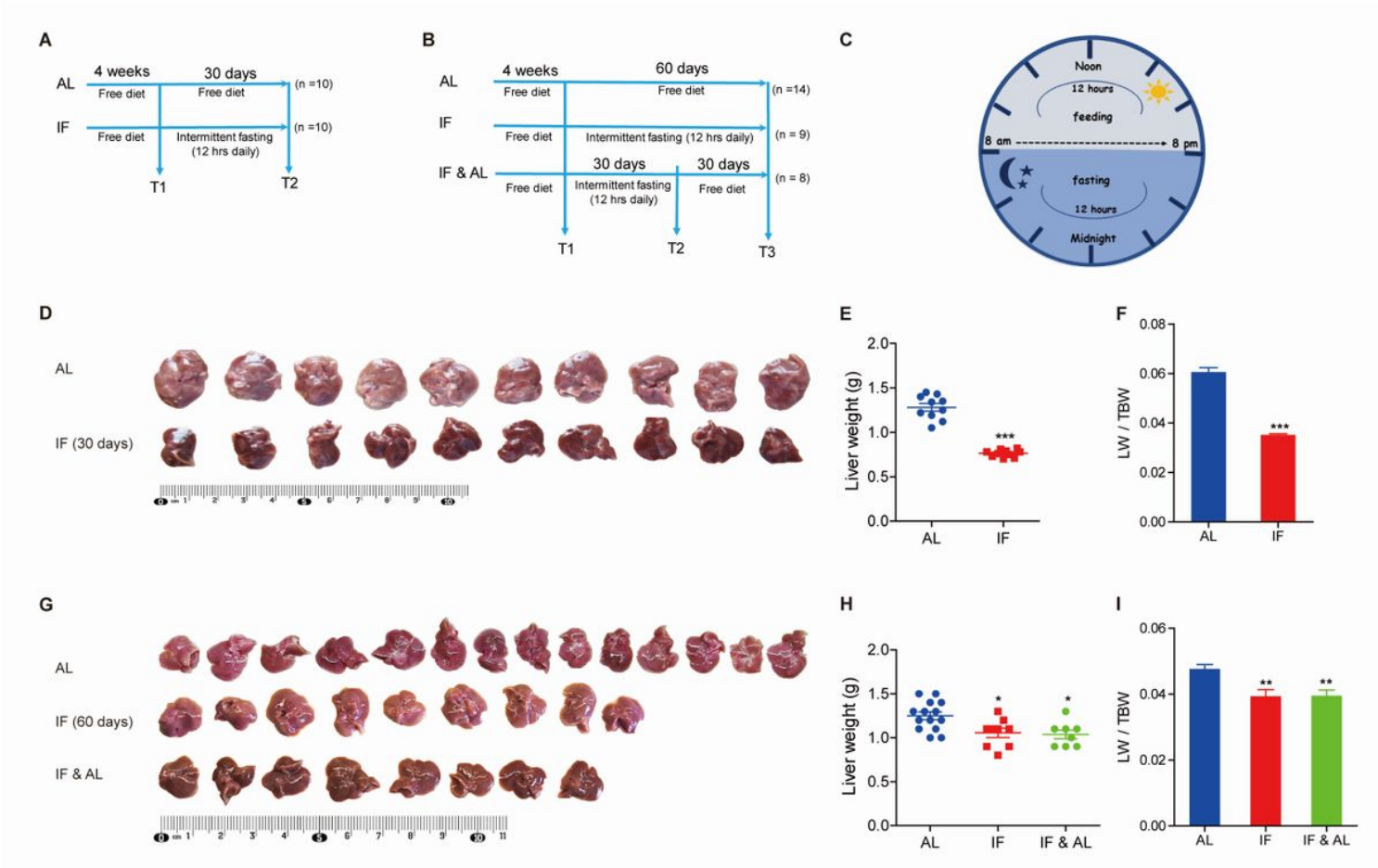

Figure 1

The effects of intermittent fasting on mouse liver. (A) Experimental design for the ad libitum (AL) group (n=10) and intermittent fasting (IF) group (n=10). In AL group, mice had free access to food and water, while in IF group, food and water were taken away for 12hrs during nighttime per day; (B) Experimental design for AL group (n=14), IF group (n=9) and IF followed by AL (IF&AL) group (n=8). In AL group, mice had free access to food and water. In IF group, food and water were taken away for 12hrs during nighttime per day. In IF&AL group, the mice had free access to food and water after one month of IF; (C) Experimental design for time of feeding and fasting; (D, G) The corresponding mouse livers were photographed; (E, H) Quantification of the liver weight; (F, I) Quantification of liver weight/total body weight (LW/TBW). (mean ± SEM, *P < 0.05, **P < 0.01, ***P < 0.001).

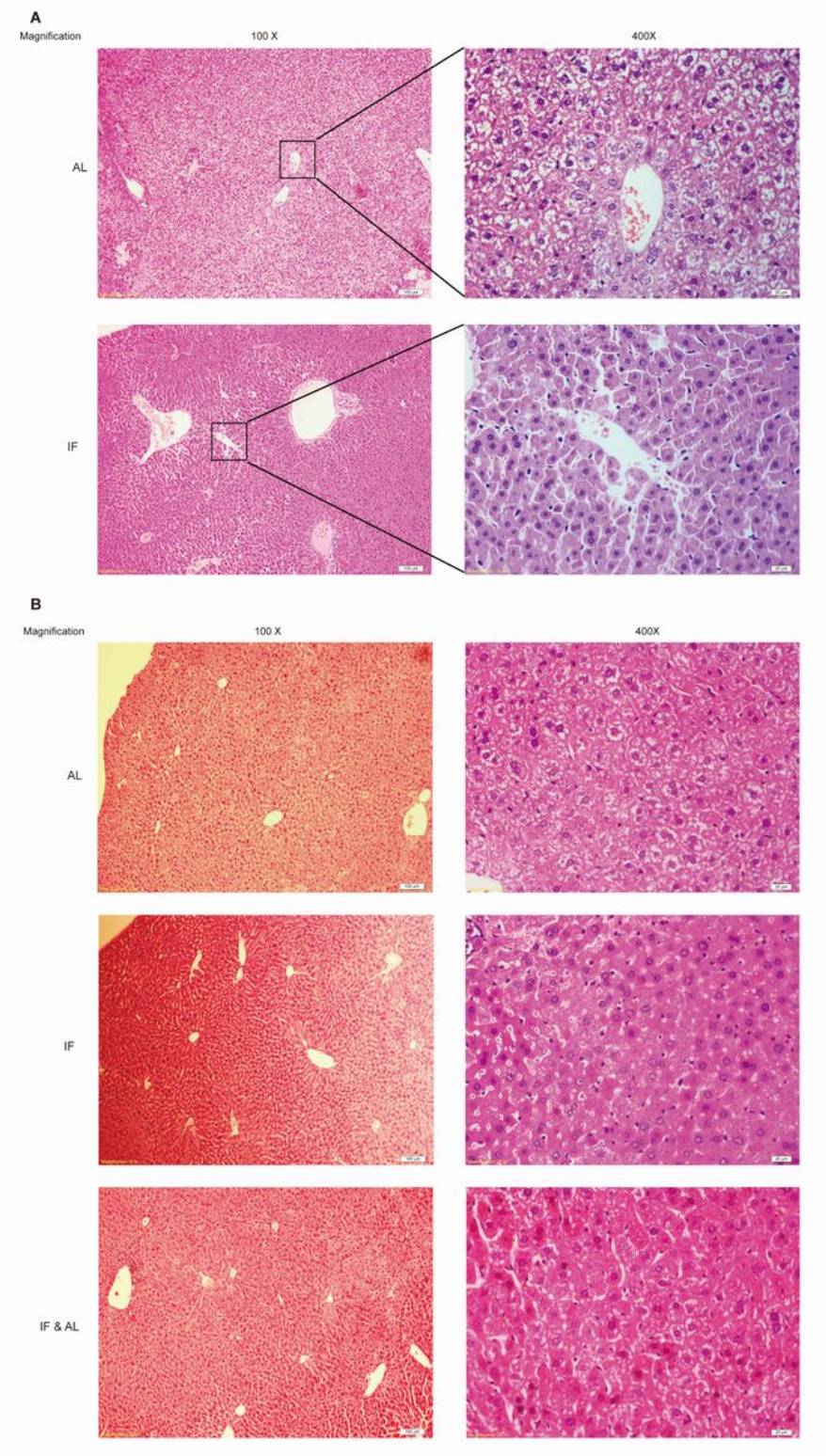

Figure 2

The histologic morphology of livers with H&E staining. (A) 30-days ad libitum or intermittent fasting was performed in 2 groups of mice, respectively; (B) 60-days ad libitum or intermittent fasting, or 30-days intermittent fasting followed by 30-days ad libitum was performed in 3 groups of mice, respectively.

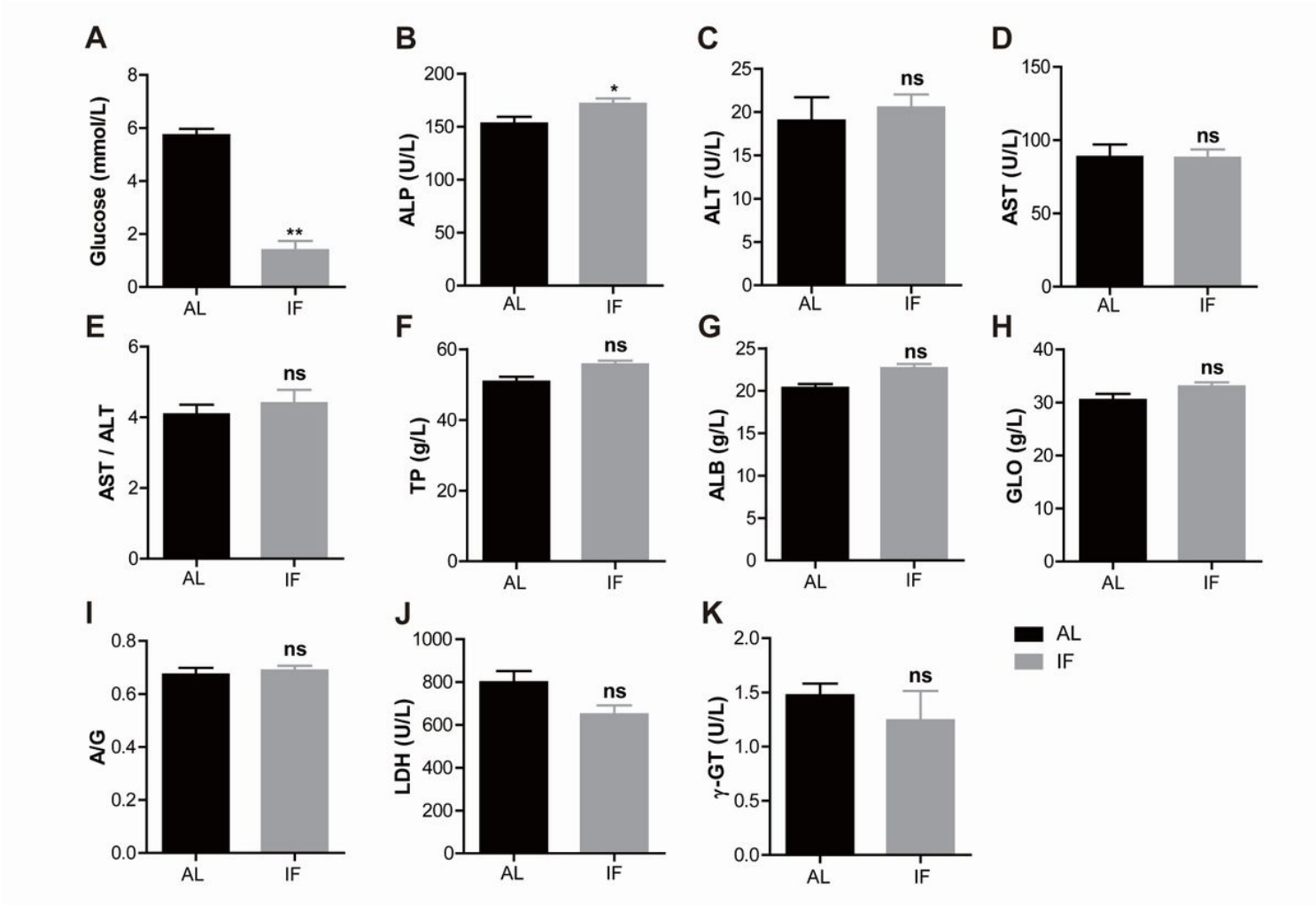

Figure 3

The levels of blood biochemical markers. (A) Blood glucose; (B) ALP: alkaline phosphatase; (C) ALT: alanine aminotransferase; (D) AST: aspartate aminotransferase; (E) AST/ALT: aspartate aminotransferase/alanine aminotransferase; (F) TP: total protein; (G) ALB: albumin; (H) GLO: globulin; (I) A/G: albumin/globulin; (J) LDH: lactate dehydrogenase; (K) γ-GT: γ-glutamyl transpeptidase (mean ± SEM, n = 7, *P < 0.05, **P < 0.01). AL: the ad libitum group; IF: intermittent fasting group.

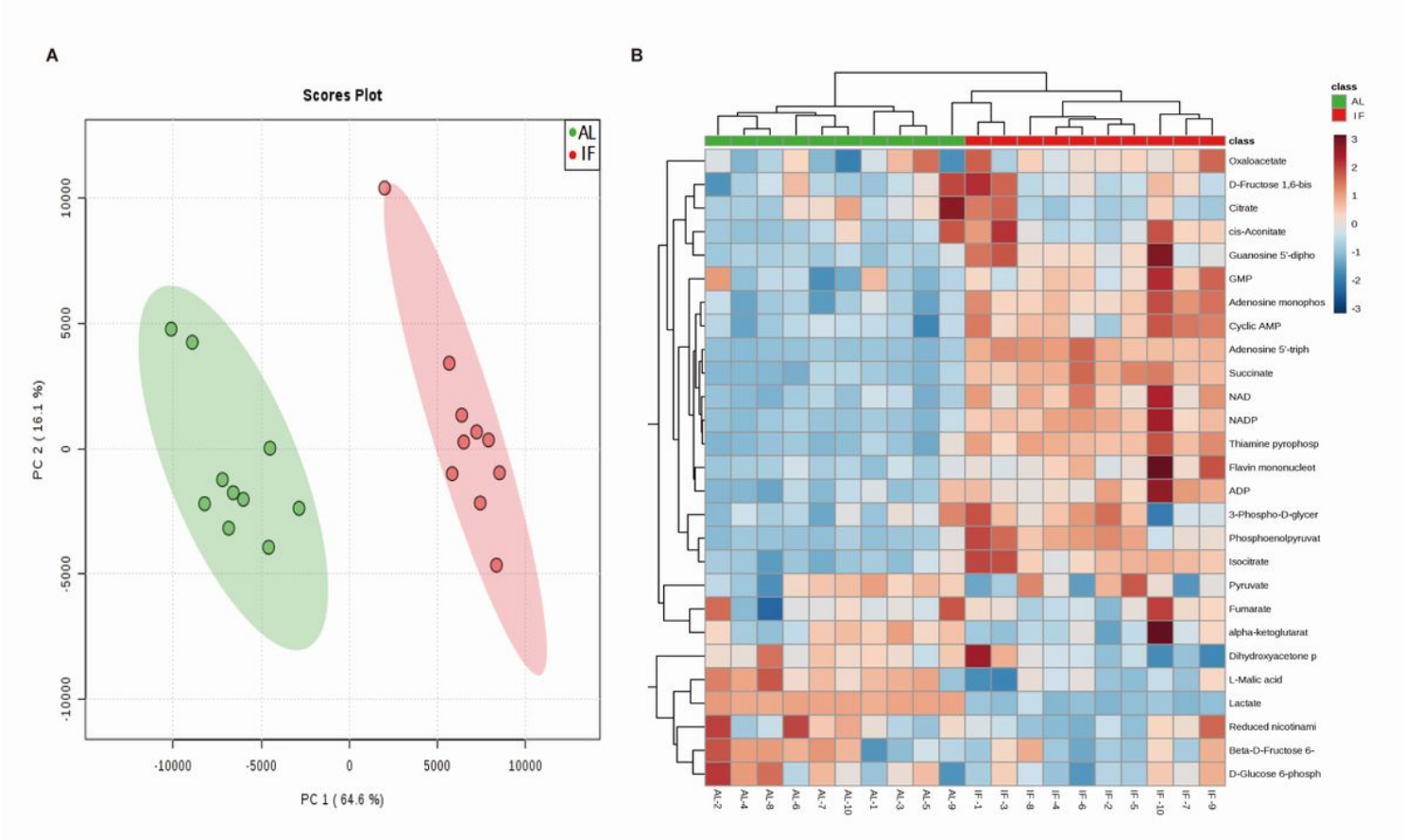

Figure 4

Targeted metabolomics analysis of the mouse liver. (A) Principle component analysis (PCA) plots was generated based on diet patterns and the difference was identified; (B) The 27 metabolites were analyzed by using LC-MS/MS and the heatmap showed the differences between intermittent fasting and ad libitum mice.

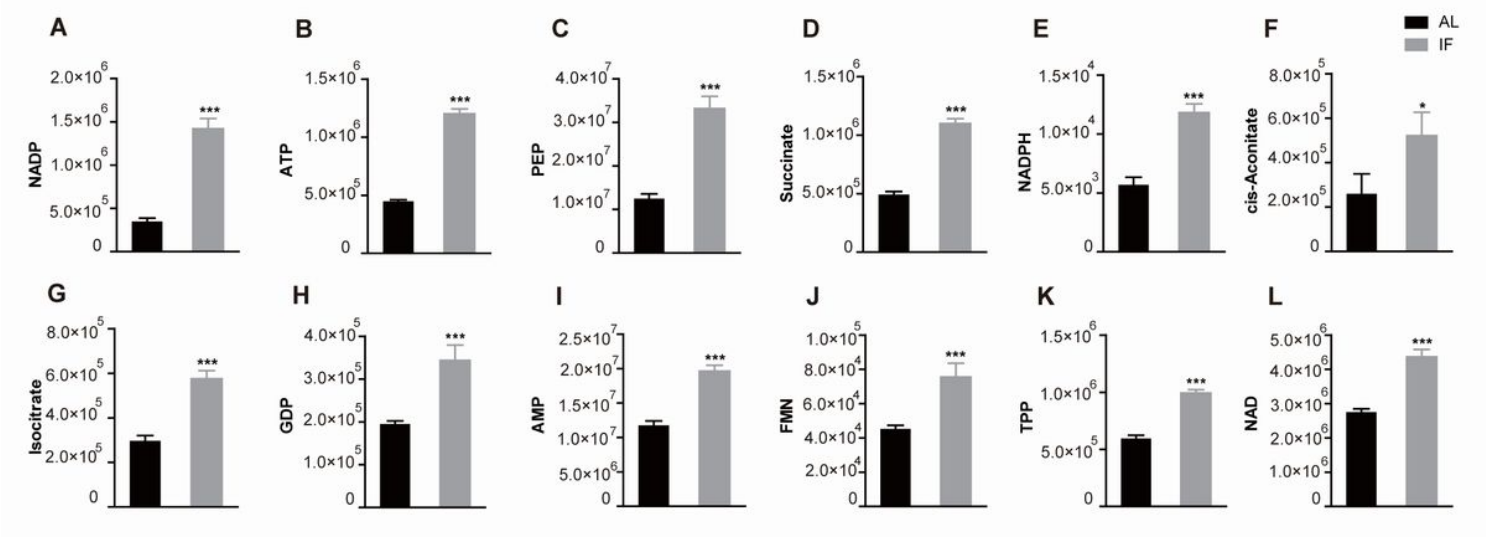

Figure 5

Twelve metabolites were significantly increased by intermittent fasting. (A) NADP: nicotinamide adenine dinucleotide phosphate; (B) ATP: adenosine triphosphate; (C) PEP: phosphoenolpyruvate (D)Succinate; (E) NADPH: reduced nicotinamide adenine dinucleotide phosphate; (F) cis-Aconitate; (G) Isocitrate; (H) GDP: guanosine diphosphate; (I) AMP: adenosine monophosphate; (J) FMN: flavin mononucleotide; (K) TPP: thiamine pyrophosphate; (L) NAD: nicotinamide adenine dinucleotide. (mean ± SEM, n = 10, *P < 0.05, **P < 0.01). AL: the ad libitum group; IF: intermittent fasting group.

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