

**Time-restricted feeding increases fat oxidation rate but not affect  
postprandial lipemia: a crossover trial**

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## Abstract

**Background:** Studies have revealed that time-restricted feeding affects the fat oxidation rate; however, its effects on the fat oxidation rate and hyperlipidemia following high-fat meals are unclear. This study investigated the effects of 5-day time-restricted feeding on the fat oxidation rate and postprandial lipemia following high fat meals. **Methods:** In this random crossover experimental study, eight healthy male adults were included each in the 5-day time-restricted feeding trial and the control trial. The meals of the time-restricted feeding trial were provided at 12:00, 16:00, and 20:00. The meals of the control trial were provided at 08:00, 14:00, and 20:00. The contents of the meals of both trials were the same, and the calories of the meals met the 24-hour energy requirement of the participants. After 5 days of the intervention, the participants consumed high-fat meals on the sixth day, and their physiological changes were determined.

**Key words:** intermittent fasting, oral fat tolerance test, fat oxidation

## Introduction

Consuming high-fat meals increases the triglyceride (TG) level in blood plasma. Studies have discovered that large increases in postprandial TG concentration lead to high risks of cardiovascular diseases and metabolic syndrome <sup>1</sup>. Compared with the fasting TG concentration, the postprandial TG concentration is a more precise predictor of the risks of cardiovascular diseases and metabolic syndrome <sup>2</sup>. Consuming high-fat meals increases the levels of biochemical substances in blood plasma, such as TG, free fatty acids, and remnant cholesterol. Studies have reported that these biochemical substances are major risk factors for metabolic syndrome, atherosclerosis, myocardial infarction, and coronary heart disease, all of which are associated with high mortality <sup>3,4</sup>. The high TG level after the consumption of high-fat meals can last for 6–8 hours. As three meals daily are typically consumed by the general population, high levels of TG may be constantly occurring in the body. Therefore, investigating methods to reduce the high TG level after eating high-fat meals is crucial for reducing the development of metabolic syndrome.

Studies have demonstrated that a single session of endurance exercise can effectively reduce the TG level after the consumption of high-fat meals <sup>5-8</sup>. In addition, performing a single session of endurance exercise 8 hours before meals can effectively reduce the increase in TG after the consumption of high-fat meals, and the effect can last for 48–60 hours <sup>9,10</sup>. Endurance exercises effectively reduce the increase in postprandial TG because they increase lipoprotein lipase activity and insulin sensitivity, and they reduce the release of very low-density lipoprotein by the liver <sup>10</sup>. Recent studies have shown that increasing the fat oxidation rate after eating high-fat meals is crucial for reducing the postprandial TG level <sup>11,12</sup>; however, the results have remained inconsistent. Some studies have described that performing high-intensity interval

training is positively correlated with decreases in the postprandial TG level <sup>13</sup>, whereas other studies have discovered that the increased fat oxidation rate following high-fat meals did not affect the postprandial TG level <sup>14</sup>.

Studies have discovered that time-restricted feeding can improve insulin sensitivity <sup>15</sup>, increase the fat oxidation rate <sup>16</sup>, and decrease the fasting TG level <sup>17</sup>. However, whether time-restricted feeding can exert health benefits in terms of effectively reducing the increase in the TG level following high-fat meals remains unclear. This study investigated the effects of 5-day time-restricted feeding on the fat oxidation rate and postprandial lipemia after the consumption of high-fat meals.

## Methods

### Participants

This study recruited eight healthy male adults as research participants (age:  $22 \pm 1.3$  yr, height:  $170.1 \pm 4.7$  cm, weight:  $75.4 \pm 17.5$  kg). All the participants had not undergone physical training; they did not exercise regularly; and they did not have any diseases that would prevent them from performing exercises, such as high blood pressure, hyperlipidemia, heart disease, joint disease, and osteoporosis. All the participants fully understood the experimental process before experiment initiation and were notified of the possible risks; they agreed to the terms of the experiment and provided their written consent. A similar number of participants and a similar

recruitment method have been employed by this research team in the past. This study was approved by the Institutional Review Board of Jen-Ai Hospital (110-10) in Taiwan.

## Design

This study used a crossover design for the experiment. The participants were divided into the time-restricted feeding trial and the control trial; the participants in the control trial did not practice intermittent fasting methods. The participants of both trials consumed the same meals for 5 days. The time-restricted feeding group used the 16:8 methods to practice intermittent fasting. The meals were provided at 12:00, 16:00, and 20:00. The meals of the control group were provided at 08:00, 14:00, and 20:00, but the consumption time was not limited. On the morning of the sixth day, all participants returned to the laboratory to consume high-fat meals, and their TG blood levels after the meal was determined. The participants were randomly assigned to different arms of the study to receive different treatments, and an interval of at least 14 days was maintained between the tests to avoid any effects of the preceding test on the succeeding test. Studies have discovered that 4 days of intermittent fasting effectively increased the fat oxidation rate and reduced blood glucose <sup>18,19</sup>. Therefore, 5 days of intermittent fasting should provide sufficient intervention time to stimulate fat oxidation rate changes.

## Protocol

### Pretest

In the pretest, gas analyzers (Vmax Series 29C, Sensor Medics, CA, USA) were used to assess the energy consumption of the participants while they were resting and performing nonmaximal intensity exercises for precisely calculating the daily calorie consumption of each participant. After the participants arrived at the laboratory, they wore heart rate monitors (Polar, Finland), and their energy consumption was examined using gas analyzers. Next, they quietly rested for 20 minutes in the supine position, and their resting heart rate and energy consumption were recorded. They then performed nonmaximal intensity exercises for measuring their energy consumption during low-intensity activities. The participants stood on a treadmill with a slope of 0° for 10 minutes to record their energy consumption during standing. The participants then walked or ran on the treadmill at five speeds, namely 1, 2, 3, 4, and 5 miles per hour. The participants maintained each speed for 3 minutes for examining the relationship between the energy consumption and heart rate of the participants during low-intensity activities.

After the pretest, the participants were asked to perform their normal daily life activities while wearing the heart rate monitor; their heart rates were recorded for 24

hours. The 24-hour heart rate, the regression curve of the heart rate, and energy consumption calculated in the pretest were used to calculate the total energy consumption of the participants. In the experiment, meals were adjusted so that the total calories of each meal met the 24-hour energy requirement of each participant. The method used to record energy consumption and the brand of heart rate monitors (Polar) used in this study have been described elsewhere <sup>12,20,21</sup>. Moreover, 24-hour energy consumption was recorded the day before the experiment, and the calories of meals were adjusted to meet the 24-hour energy requirement of each patient. The same methods were repeated on the fifth day of the experiment to confirm the 24-hour energy consumption changes of the two trials.

### Formal Experiment

The experiment was conducted over 6 days. On the first day, the participants arrived at the laboratory at 08:00 and quietly rested for 20 minutes in the supine position; gas analyzers were used to record their energy consumption. Subsequently, the participants were randomly allocated to the time-restricted feeding trial or the control trial. The meals of the time-restricted feeding trial were provided at 12:00, 16:00, and 20:00, and the participants were required to consume all the food during this time. The meals of the control trial were provided at 08:00, 14:00, and 20:00, but the consumption

time was not limited. In addition to regular meals, a snack with approximately 200 calories was provided to the participants for consumption. The participants in the time-restricted feeding trial were only allowed to consume the snack from 12:00 to 20:00, whereas no restrictions were imposed on the control trial for snack consumption. The meals of the participants were provided by dieticians. Based on the results of the pretest, the calories of each meal met the daily energy requirement of the participants. The macronutrient consumption for TRF and CON were listed in table 1.

After experiment completion on the fifth day, the participants returned to the laboratory on the eighth day from 08:00 to 09:00. They rested for 10 minutes in the supine position, and gas analyzers were used to collect the gas data of the participants for 20 minutes. Next, a catheter was inserted into the forearm of each participant to collect fasting blood samples. After blood sample collection, the participants were provided with a specific high-fat meal. The participants rested quietly in the laboratory for 4 hours, and their blood lipid changes during this period were observed.

#### Oral fat tolerance test (OFTT)

All oral fat tolerance test (OFTT) meals were designed and provided by dieticians, as previously described <sup>12,22,23</sup>. The meals included toast, butter, cheese, muesli, and

cream. For every kg of the body weight of the participant, the meal provided 1.2 g of fat, 1.1 g of carbohydrate, 0.33 g of protein, and 16.5 kcal of energy. The nutritional information was obtained from the nutritional facts on food packages. During the experiment, the participants were required to consume the OFTT meal within 15 minutes.

#### Blood collection

In the experiment, a catheter (Venflon 20G, Sweden) was inserted into the vein of the forearm, and a three-way stopcock (Connecta Ltd., Sweden) was used to collect 10 mL of blood each time. Blood was collected before meals, 30 minutes after meals, and every hour after meals up to the fourth hour. After each session of blood collection, 10 mL of isotonic saline water was used to clean the catheter to avoid blood clotting in the catheter.

The collected blood was immediately placed in blood collection tubes containing ethylenediaminetetraacetic acid. A cell counter was used to analyze the hematocrit (Sysmax KX-21N, Kobe, Japan). After the analysis, the blood was centrifuged for 20 minutes at  $500 \times g$  at 4°C. Blood plasma was obtained and was immediately placed in a -80°C refrigerator for preservation and future biochemical analysis.

#### Blood biochemical analysis

TG, blood glucose, free fatty acid, and glycerol levels in blood were analyzed using an automated biochemistry analyzer (7020, Hitachi, Japan) and commercial reagents (GOD-PAP method, Randox, Ireland). The insulin concentration in blood plasma was analyzed using a chemiluminescence immunoassay analyzer (Elecsys 2010, Roche Diagnostics, Basel, Switzerland) and commercial reagents (Roche Diagnostics). The fat and carbohydrate oxidation rates were calculated using the following formula<sup>24</sup>:

$$\text{Fat oxidation (g/min)} = 1.695 \times \text{VO}_2 - 1.701 \times \text{VCO}_2$$

$$\text{Carbohydrate oxidation (g/min)} = 4.585 \times \text{VCO}_2 - 3.226 \times \text{VO}_2$$

### Statistical Analysis

All the data in this study are presented as average  $\pm$  standard deviation. First, the normality of the data was tested using the Shapiro–Wilk test. The fasting fat oxidation rate, blood biochemical values, and areas under the fat oxidation rate curve and the TG curve were analyzed using the paired sample t test. The postprandial fat oxidation rate and blood biochemical values were analyzed using two-way ANOVA with repeated measures. If the data were significant, the Bonferroni method was used to perform post hoc comparisons. Using G\*power 3, to achieve an alpha value of 5% and a power of 0.8, the sample size of eight was considered sufficient for this study. The significance level was set at  $\alpha < 0.05$ .