Official Title of the study: Effects of Intermittent Fasting in Metabolic Dysfunction Associated Fatty Liver Disease

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Study Protocol

Patients

This study was approved by the Ethics Committee of Marmara University School of Medicine (No: 09.2022.707) Informed consent was obtained from each patient included in the study. This is a prospective non-drug intervention study conducted by the same gastroenterologist on patients diagnosed with MAFLD. Individuals with fatty liver disease diagnosed by vibration-controlled transient elastography are diagnosed with MAFLD if at least one of the following three conditions is present: overweight/obesity, type 2 diabetes mellitus (T2DM), or underweight or normal weight with at least two metabolic risk abnormalities [waist circumference, blood pressure, triglycerides, high-density lipoprotein cholesterol (HDL-C), prediabetes, insulin resistance, high-sensitivity C reactive protein (CRP)]. All of our patients were diagnosed with MAFLD because their BMI was above 25 kg/m2 in addition to the detection of fatty liver.

Exclusion criteria were patients with an average daily alcohol consumption of >20 g for females and >30 g for males; pregnant or lactating women; patients with ischemic heart disease or heart failure, chronic inflammatory diseases, chronic viral infections, cancer, moderate-to-severe kidney disease, uncontrolled hypertension, and eating disorders; those with a history of bariatric surgery; and those on insulin due to increased risk of hypoglycemia.

Design

Patients who voluntarily agreed to participate in the study were randomly assigned into two groups. Patients enrolled in the study were referred for an initial consultation with a dietitian following their diagnosis by a gastroenterologist. The energy-restricted diet group followed an energy-restricted diet for 8 weeks, while the energy + time-restricted diet group followed the same diet and a 16:8 eating pattern and restricted food intake to an 8 h window each day. The effectiveness of the intervention was assessed using anthropometric and biochemical measurements, and Fibroscan investigations were performed before the intervention (T0) and after the intervention (T8).

Measurements

Blood samples were collected from patients after 12 h of fasting. The samples were centrifuged for 15 min and stored at −80 ◦C after serum separation until analysis. Serum FGF-21, Beclin-1, and ATG-5 were analyzed using enzyme-linked

immunosorbent assay (ELISA) kits, following the manufacturer's protocols (Human FGF-21 ELISA, Biovendor, Brno, Czech Republic; Human BECN1 [Beclin-1] ELISA Kit, ElabScience, Houston, TX, USA; Human Autophagy protein 5 [ATG5] ELISA Kit, MyBioSource, Inc., San Diego, USA) and in duplicate.

- The extent of hepatic steatosis and fibrosis was determined using transient elastography on a Fibroscan 502 TouchR device(Echosens SA, Paris, France). All Fibroscan measurements were performed following the manufacturer's instructions as specified previously.

- Patients' heights were measured using a stadiometer (Seca 769), barefoot, feet side by side, and head in the Frankfort plane. Body composition was determined using the bioelectrical impedance method (Tanita MC 780 P).