Cardiovascular Diabetology

RESEARCH

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Interleukin-1β in circulating mononuclear cells predicts steatotic liver disease improvement after weight loss in subjects with obesity and prediabetes or type 2 diabetes

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Abstract

Background Metabolic dysfunction-associated steatotic liver disease (MASLD) is a major cardiovascular risk (CV) factor. Interleukin-1β (IL-1β), a cytokine involved in the pathogenesis of obesity-associated inflammation and type 2 diabetes (T2D), promotes hepatic steatosis. The Canakinumab Anti-inflammatory Thrombosis Outcome (CANTOS) trial showed that the inhibition of the IL-1β pathway was associated with a reduction of CV events in high-risk patients. The present study was designed to determine: (i) whether an equal degree of weight loss by liraglutide or lifestyle changes has a different impact on MASLD extent and IL-1β expression in peripheral blood mononuclear cells from obese subjects with prediabetes or early T2D; (ii) whether baseline IL-1β levels may predict the extent of weight loss and related metabolic changes.

Methods Thirty-two obese subjects with prediabetes (n = 16) or newly diagnosed T2D (n = 16), were randomized to the glucagon-like peptide receptor agonist (GLP1-RA) liraglutide or lifestyle counselling until achieving a comparable weight loss. Visceral adipose tissue (VAT) and gene expression of IL-1β in peripheral blood mononuclear cells were assessed by magnetic resonance and real time PCR, respectively.

Results At baseline, IL-1β was positively correlated to body mass index (BMI), fasting plasma glucose, HbA1c, VAT, MASLD extent, platelet count, chemerin and interleukin-1 receptor antagonist (IL1-RA). After achievement of the weight loss target in the two groups, a significant but comparable reduction of IL-1β (p for difference = 0.56) was observed in both arms, in parallel with a comparable improvement in glycaemic control, C reactive protein (CRP), BMI and MASLD. Furthermore, basal IL-1β levels independently predicted the extent of MASLD decrease (p = 0.030);

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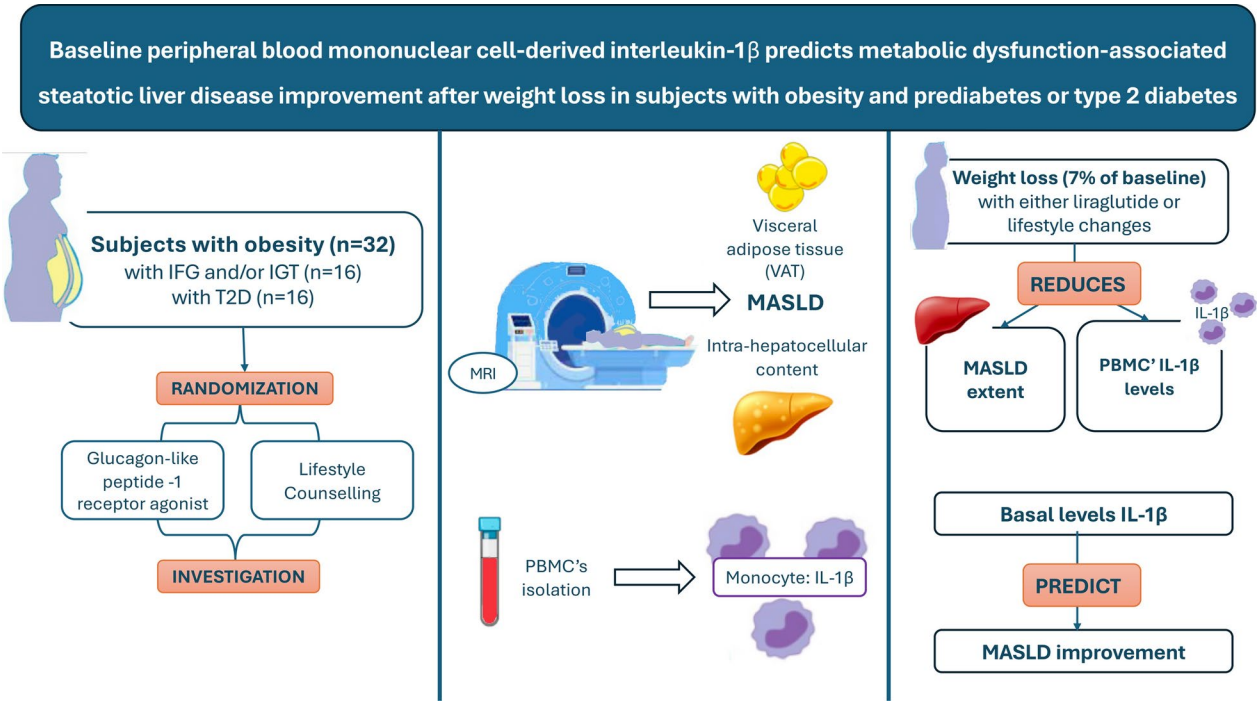
subjects in the highest tertile showed a median decrease of – 8.0 (95% CI – 12.3 to – 4.8) compared with – 23.0 (95% CI – 39.5 to – 16.3) in the lowest tertile.

Conclusion In patients with obesity with initial impairment of glucose metabolism successful weight loss is associated with a reduction of both IL-1β levels and MASLD degree. Of interest, basal levels of IL-1β predict the extent of MASLD improvement, regardless of the intervention. Our results may set the stage for ad-hoc studies investigating the usefulness of baseline IL-1β a level as a drug-response biomarker.

Keywords Interleukin-1β, Obesity, MASLD, Liver disease, Prediabetes, Type 2 diabetes, Inflammation, Biomarker, Weight-loss, Adipose tissue

Graphical abstract



Research insights

What is currently known about this topic?

MASLD is a major cardiovascular risk factor. Interleukin-1β is involved in the pathogenesis of obesity and type 2 diabetes. Interleukin-1β promotes hepatic steatosis.

What is the key research question?

Could an equal degree of weight loss have a different impact on MASLD extent and IL-1β levels?.

What is new?

Weight loss (7% of initial body weight), with either liraglutide or lifestyle changes, reduces MASLD and PBMC IL-1β. PBMCs IL-1β is able to predict MASLD improvement response after weight loss. PBMC IL-1β levels may be a drug response biomarker in MASLD.

How might this study influence clinical practice?

Our findings could lead to personalized treatment strategies for obese, prediabetic and diabetic patients.

Background

Metabolic dysfunction-associated steatotic liver disease (MASLD) refers to a spectrum of liver damage ranging from simple steatosis to nonalcoholic steatohepatitis, advanced fibrosis and cirrhosis and hepatocarcinoma [1]. It is often associated with other dysmetabolic conditions such as obesity and diabetes and is a recognized leading cause of cardiovascular mortality and morbidity [2–4], cognitive decline [5] with a significant impact on healthcare systems [6]. Nowadays, MASLD It is the most

common chronic liver disease in many developed countries [7, 8], and its prevalence reaches 57% in patients with obesity, 70% in patient with diabetes and 90% in patients with severe obesity [9].

Due to the complex pathogenesis of MASLD, no direct pharmacological therapy is currently available [10, 11], except for Resmetirom, an oral thyroid hormone receptor-β (THR- β) agonist, recently approved in the USA for use in conjunction with diet and exercise for the treatment of adults with noncirrhotic NASH with moderate to advanced liver fibrosis (consistent with stages F2 to F3 fibrosis) [12]. Weight loss combined with dietary and lifestyle changes are the only acknowledged interventions, although with an extremely heterogeneous response [10]. It is also still debated whether different types of weight loss intervention (i.e. diet or GLP1-RA [13, 14]) have similar or different effects on MASLD status. For all these reasons, the identification of biomarkers able to predict the success rate of interventions would be extremely valuable.

Research has focused on the pathway involving the nucleotide-binding oligomerization domain leucine-rich repeat-containing receptor-containing pyrin domain 3 (NLRP3) inflammasome and its final mediator, interleukin (IL)-1β, which is critical in the development of NASH [15, 16]. However, although directly supported by in vivo evidence and indirectly endorsed by clinical trials demonstrating the strong association of IL-1β with cardiovascular risk [17], no study has investigated the potential role of IL-1β as a disease-defining biomarker, with a particular focus on response to intervention.

We designed the present study to investigate whether the same degree of weight loss by GLP1-RA treatment or lifestyle changes has a different effect on the extent of MASLD in patients with obesity and prediabetes and/or diabetes, and whether baseline IL-1β levels measured in peripheral blood mononuclear cells (PBMCs) can predict response in terms of MASLD status.

Methods

Study population and protocol

This study was part of a longitudinal, randomized, controlled, parallel-arm study designed to assess, in obese subjects with impaired glucose tolerance (IGT) and/or impaired fasting glucose (IFG) or early T2DM, the effects of an equal degree of weight loss, achieved by either lifestyle changes or liraglutide, on cardiometabolic variables [18]. The main study included 62 patients. In the current study, we show the results of a post-hoc analysis in which the expression of inflammatory cytokines in PBMCs was assessed before and at the end of treatment in 32 (52%) of the study participants (Fig. 1). The number of participants included in the analysis (32 out of 62) was determined by the feasibility of isolating PBMCs from blood samples at the time of scheduled visits in the main study.

As previously published [18], we enrolled subjects with obesity (BMI ≥ 30), with a diagnosis of IGT or IFG or T2DM for less than 12 months, according to the American Diabetes Association (ADA) Guidelines [19], under diet therapy associated with ongoing metformin treatment at the highest tolerated dose (up to 3000 mg/day) at the time of enrolment. Exclusion criteria were: (i) type 1 DM, diagnosed with islet autoantibodies evaluation (islet cell cytoplasmic, and islet antigen 2 (IA-2) antibodies, anti-glutamic acid decarboxylase), when one of the following applied: age lower than 40, family history of type 1 DM, lean phenotype, early requirement for insulin; (ii) MODY (Maturity Onset Diabetes of the Young); (iii) BMI < 30 kg/m^2; (iv) DM diagnosis longer than 12 months; (v) oral antidiabetic agents (except metformin) or insulin treatment in the last three months; (vi) uncontrolled hypertension (systolic/diastolic blood pressure > 160/90 mmHg); (vii) significant comorbidities including kidney disease (glomerular filtration rate below 60 mL) or liver disease (aspartate aminotransferase (AST) or alanine aminotransferase (ALT) twice above the upper normal range), pregnancy or lactation; (viii) sexually active women of child-bearing potential not using adequate contraceptive methods; (ix) chronic non-steroidal anti-inflammatory drug therapy; (x) any contraindication to liraglutide (known or suspected hypersensitivity to GLP-1RA, previous acute or chronic pancreatitis, inflammatory bowel disease, gastrointestinal surgery, heart failure class NYHA III-IV); (xi) personal or family history of medullary thyroid carcinoma or of multiple endocrine neoplasia type 2 (MEN2); (xii) claustrophobia; metal implants or other contraindications for magnetic resonance imaging (MRI); (xiii) recent participation in other research projects within the last 3 months or participation in 2 or more projects within one year. After a baseline evaluation, the patients were randomized in a 1:1 ratio to receive liraglutide or lifestyle counselling. Study medication (liraglutide 6.0 mg/mL in 3-mL prefilled pen injectors) was supplied by Novo Nordisk. Liraglutide was administered by daily subcutaneous injection at bedtime and titrated over a 3-week period: 0.6 mg per day during the first week, 1.2 mg daily (second week), and 1.8 mg daily (third week), based on the clinical response and side effects. Failure to achieve the 1.8 mg dose was not a withdrawal criterion. Patients in the liraglutide arm received some advises on physical activity and diet, without a structured intervention program. The dietary recommendations adhered to the principles of the Mediterranean Diet, with a calorie variable from 1200 to 1800 kcal in relation to age, sex, body weight, physical activity and occupational activity, an average content of 30% lipids, of which less 10% as saturated fats,

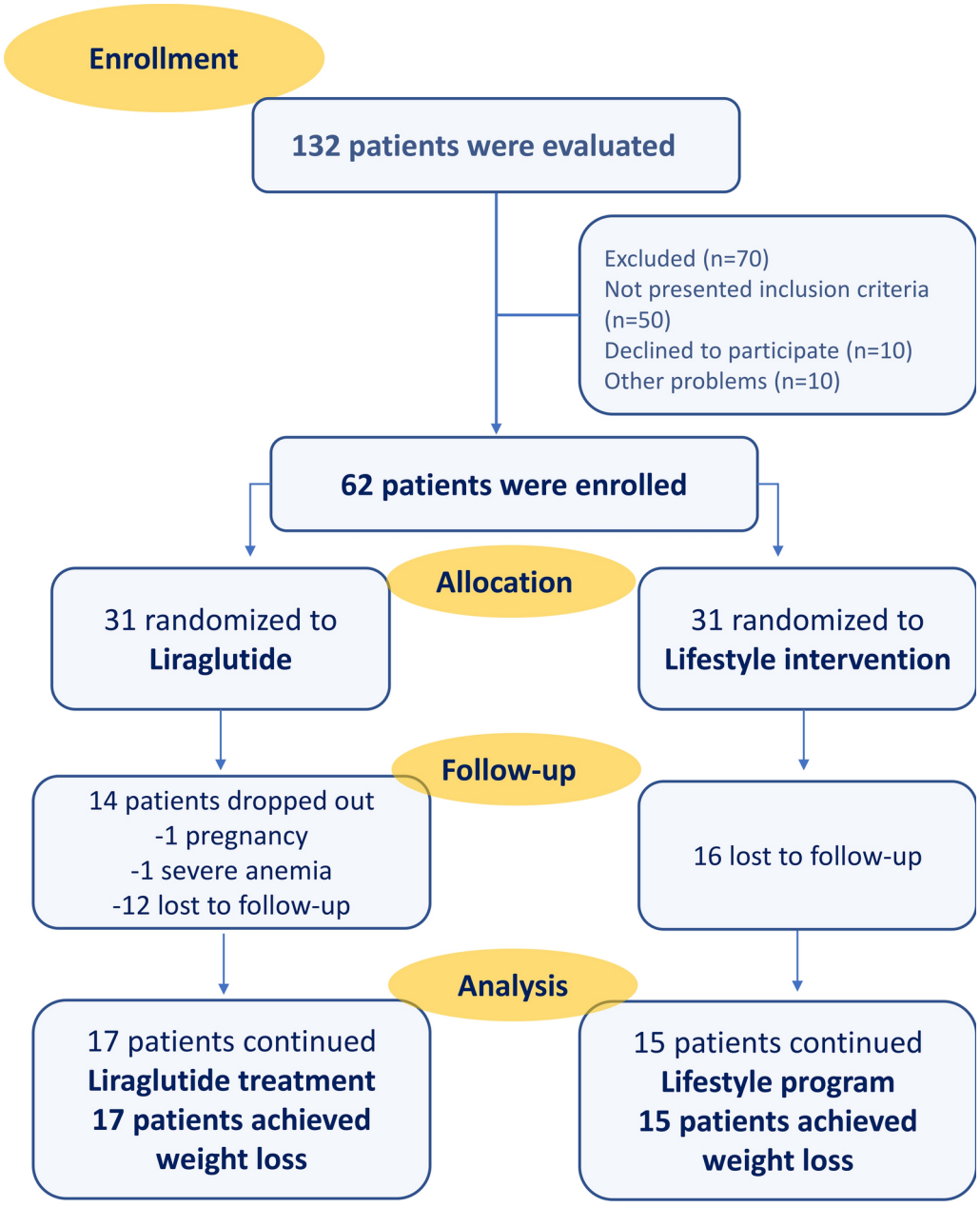


Fig. 1 Flow chart. Flow chart of enrolment of participants in the study

10% as monounsaturated and polyunsaturated fats, and proteins of 15%. Simple sugars fraction was always below 10% to allow better glycemic control. Dietary recommendations included use of food rich in insoluble and soluble fiber that help to avoid rapid blood glucose levels fluctuations. Patients practiced moderate activity, starting from light intensity activity and gradually increasing physical effort. It began with walks to medium-fast pace for about 30 min until you reach 1 h of brisk walking.

Block randomization was performed by computer-generated random allocation sequence. The subjects were assigned a consecutive random number based on the order of inclusion in the study and were then allocated to one of the two treatment groups. The weight loss goal for all the participating subjects was to lose 7% of initial body weight (calculated at the time of randomization).

This weight loss goal was set based on the observation that this amount of weight loss is associated with improved metabolic outcomes [20]. Patients not achieving the weight loss goal within 15 months of the initiation of the randomized treatment, as well as those not completing the study for decision of the patient and/or of the investigator, were considered withdrawn from the study.

Each subject signed written informed consent to participate, and the Protocol was approved by the Ethics Committee of the University of Chieti (Approval n. 10 (protocol 20,131)23.05.2013). This study was performed under the Good Clinical Practice regulations (Good Clinical Practice for Trialon Medicinal Product-CPMP/European Commission-July 1990; Decreto Ministeriale 27.4.1992-Ministero della Sanità) and the Declaration of Helsinki (Hong Kong 1989). By signing the protocol, the participants in the study committed to adhere to local legal requirements. Enrolment took place at the Obesity and Diabetes Clinics of Chieti University Hospital. All study visits were performed at the Clinical Research Center (CRC) within the Center for Advanced Studies and Technology (CAST), "G. d'Annunzio" University Foundation. All subjects underwent, at baseline and after the end of the study, clinical evaluation, blood sample collection, and abdominal MRI for the assessment of adipose tissue (AT) distribution in terms of visceral AT (VAT) [21]. Periodic visits, every 3 weeks, were planned to reinforce the motivation to achieve the weight loss goal, by monitoring compliance to liraglutide (by pill counting) or to lifestyle changes (see below). At each visit, participants completed questionnaires and underwent physical examination. Each patient was carefully monitored for adverse events.

Lifestyle intervention program

Participants in the lifestyle intervention arm were encouraged to achieve the 7% weight loss in the first 6 months, based on previous studies suggesting that most subjects achieve their maximum weight loss within the first 20–24 weeks of a lifestyle program. Recommendations were provided as written information and periodic 20–30-min individual sessions were prescribed to emphasize the importance of a healthy lifestyle. Visits with the staff of nutritionists were planned once a week during the first 4 weeks, then once every 2 weeks for the following 20 weeks, then once a month. Participants were encouraged to follow the Food Guide Pyramid and a healthy low-calorie, low-fat diet, the equivalent of a National Cholesterol Education Program Step 1 diet [22], to lose weight, and to increase the intensity and frequency of their physical activity to moderate intensity (such as brisk walking) for at least 150 min per week, to achieve at least 700 kcal/week expenditure.

Magnetic resonance imaging (MRI) quantification of visceral fat and intra-hepatocellular content (MASLD)

A Achieva Philips 1.5 Tesla body scanner (Amsterdam, The Netherlands), available at the Institute for Advanced Biomedical Technologies (ITAB), a neuroscience and imaging research center within the University of Chieti "G. d'Annunzio", was employed to obtain magnetic resonance (MR) images. All acquisitions were obtained through a spin-echo sequence with a 500-ms repetition time and 20-ms echo time. To plan the data acquisition, a transverse and sagittal image of the abdomen region were taken to identify the intervertebral space between the lumbar fourth (L4) and fifth (L5) vertebrae. Transverse slices [10] were then acquired every 50 mm, from the L4–L5 space toward the feet. The optimal threshold for adipose tissue was 110 (on a scale of 256). Adipose tissue area and volume were calculated as previously described [21].

The lipid concentration of the liver was calculated according to the method reported by Folch et al. [23]. This approach delineates the triglyceride concentration in liver specimens (mg of triglyceride/g of liver tissue) and was used as a gold standard to make a comparison with both MRI data and histology. The multi-echo MRI technique to assess the adipose tissue content is set on a three-dimensional multi-echo gradient sequence obtained in axial orientation with 12 distinct echoes (TE min = 1.04 ms, δTE = 0.78 ms, TE Final = 25.14 ms, TR = 72 ms, Flip Angle = 25°, FOV 375/328 mm, matrix resolution 232/129). Images were executed for spectral analysis of the MRI signal to differentiate between adipose and water content in each image pixel. The software automatically produces the water and adipose intensity maps, the water and fat R2* (reciprocal of T2*) maps, and fat fraction maps. Water and fat signal maps are then examined by the radiologist as a traditional parametric map (area of interest (ROI) analysis) to determine the definitive fat fraction calculated as a percentage. The analysis of three ROIs was performed in the fat fraction maps manually dressed in segment III, close to the lower border of the liver, avoiding vascular vessels. The medium of three measured fat fragment values was determined for each patient to provide the definitive fat fraction content.

Magnetic resonance imaging is considered the gold standard to quantify hepatic steatosis.

Analytical measurements

Biological material collection

At admission to the study and after the achievement of the weight loss goal, venous blood samples were collected after an overnight fasting and frozen at – 20 °C for subsequent biochemical measurements.

Isolation of peripheral blood mononuclear cells

Blood was collected in Ficoll tubes (Vacutainer CPT, BD Diagnostics) and centrifuged for 20 min at 1800 g and room temperature. The turbid white layer above the Ficoll containing the mononuclear blood cells was transferred to a clean tube and washed twice with PBS. Subsequently, monocytes were isolated using magnetic

CD14–coated beads and magnetic-activated cell sorting (Miltenyi Biotec).

Real-time polymerase chain reaction

Conversion of total cellular RNA to cDNA was carried out with Moloney murine leukemia virus reverse transcriptase and random hexamers (Amersham Bioscience, Piscataway, NJ) in a final volume of 33 μL, using 1 μg of cDNA according to the manufacturer recommendations. Real-time polymerase chain reaction (PCR) amplification was performed in an MX3000P PCR cycler (Stratagene) using the SYBR Green JumpStart kit (Sigma Aldrich, St Louis, MO) in 25 μL of final reaction volume containing 2 μL cDNA, 10 pmol of each primer, 0.25 μL of internal reference dye, and 12.5 μL of JumpStart Taq ReadyMix (buffer, dNTP, stabilizers, SYBR Green, Taq polymerase, and JumpStart Taq antibody). TATA binding protein was used as an endogenous control for normalizing RNA concentration. The amplification program consisted of 1 cycle at 95 °C for 10 min, followed by 40 cycles with a denaturing phase at 95 °C for 30 s, an annealing phase at 60 °C for 30 s, and an elongation phase at 72 °C for 30 s. Differences in Ct values between test gene and endogenous control (TATA binding protein, ΔCt) were calculated and used for statistical analysis. PBMCs' levels of IL-1β, MCP-1, ICAM-1, VCAM-1, IL-6, TNFα, P-selectin, Toll-like receptor-4 (TLR-4) were assessed by real time PCR.

Biochemical measurements

Plasma glucose concentration was determined by the glucose oxidase method. The HbA1c was measured by automated high-performance liquid chromatography (HPLC) [24]. Serum hs-CRP concentrations were measured using highly sensitive immunoassay. IL-1RA (DRA00B, Quantikine® R&D Systems, Minneapolis, MN) and chemerin (DCHM00, Quantikine® R&D Systems, Minneapolis, MN), were measured by high-sensitivity enzyme-linked immunosorbent assays (ELISA) following the manufacturer's instructions.

Statistical analysis

This is a post-hoc analysis of a published study [18]. In the present analysis of the study, the outcome of interest was the change in (delta) MASLD extent and IL-1β expression in PBMC after achievement of 7% of initial weight loss. The sample size of 32 subjects included in this sub-study was determined by the feasibility of isolating PBMCs from blood samples during the scheduled visits of the main study. Although no a priori (prospective) power analysis was performed for this sub-study, a post hoc power analysis indicated that with 32 participants we had approximately 90% power (α = 0.05) to detect a mean difference of 1 standard deviation (SD) in the changes in MASLD extent and IL-1β expression between the liraglutide and lifestyle intervention groups. To illustrate, if one were to conduct a prospective power analysis based on an expected effect size of 1 SD, an α of 0.05, and a desired power of 80%, the calculation would yield a requirement of approximately 16 subjects per group (total n ≈ 32). This confirms that, had the sample size been determined solely on statistical grounds, the number of subjects available for the analysis would have been adequate to detect the pre-specified effect size.

The Kolmogorov–Smirnov test and examination of residual distribution were used to determine whether each variable had a normal distribution. Where necessary, natural-log transformation was or appropriate non-parametric tests were used. Comparisons of baseline data between the groups were performed by chi-squared statistics, Fisher exact tests, unpaired Student's *t*-tests or Mann–Whitney *U*-tests. Delta values were calculated as post-treatment minus pre-treatment values. Univariable correlation test and multivariable linear regression analysis were used to assess the relationship between continuous variables at baseline and to characterize predictors of change in MASLD. For comparison between arms, we used a linear mixed-effects model for repeated measures over time, with changes in MASLD or IL-1β as the dependent variable, study group and time-by-group interaction as fixed effects, time-to-weight loss (month), basal weight, waist as fixed effect covariates and patients and error as random effects. Within the mixed model, we obtained least-squares estimates of the treatment differences and standard errors and estimated 95% confidence intervals (CIs) and *p*-values for the two pre-specified intergroup contrasts (liraglutide and lifestyle intervention) for baseline and end of study within each group. The p-values were calculated for the various statistical tests performed and interpreted as quantitative measures of the precision of the estimates.

The data analysis was generated using SAS/STAT software, Version 9.1.3 of the SAS System for Windows©2009 (SAS Institute Inc. Cary, NC, USA).

Results

Study population baseline characterization

Thirty-two metformin-treated subject with obesity and prediabetes [impaired fasting glucose (IFG), impaired glucose tolerance (IGT) or both (n = 16)] or newly diagnosed T2D (n = 16), randomized to the glucagon-like peptide receptor agonist (GLP-RA) liraglutide (1.8 mg/d) or lifestyle counselling until achieving a modest and comparable weight loss (7% of baseline body weight), were enrolled to the outpatient Diabetes Clinic and Obesity Centre of our University Hospital, from October 2013 to July 2015. Median time-to-weight loss was 4 months (4 (3.0–6.0) months in the liraglutide and lifestyle arms

Table 1 Baseline clinical and analytical features of obese patients randomized to liraglutide- or lifestyle-induced weight-loss intervention

Variable	Pre-lifestyle (n = 15)	Pre-liraglutide (n = 17)	P value*
Age (years)	53.00 (51.00–55.00)	55.00 (48.50–63.50)	0.502
Gender (male), n (%)	8 (53.3)	9 (52.9)	0.982
Smoke, n (%)	0 (0.0)	4 (23.5)	**0.045**
Weight (Kg)	96.10 (88.60–124.00)	109.00 (97.55–116.35)	0.165
BMI (Kg/m^2)	35.35 (30.90–40.52)	38.86 (34.26–42.31)	0.350
Waist circumference (cm)	116.0 (110.0–132.0)	118.0 (112.0–129.0)	0.089
WHR	0.95 (0.90–1.01)	0.98 (0.93–1.05)	0.433
Hypertension, n (%)	9 (60.0)	14 (82.4)	0.160
Systolic arterial pressure (mmHg)	131.00 (123.00–146.00)	144.00 (129.00–152.00)	0.295
Diastolic arterial pressure (mmHg)	80.00 (70.00–85.00)	82.00 (77.50–86.00)	0.278
Glycated hemoglobin (HbA1c) (mmol/mol)	43 (37–50)	42 (38–52)	0.682
Fasting plasma glucose (mmol/L)	5.33 (5.06–5.72)	5.33 (4.97–6.0)	0.882
Fasting plasma insulin (uU/mL)	10.60 (8.70–23.50)	15.40 (9.75–21.30)	0.331
Total cholesterol (mmol/L)	4.44 (4.03–4.65)	4.34 (3.59–5.27)	0.970
High density lipoprotein (HDL) cholesterol (mmol/L)	1.09 (0.98–1.37)	1.21 (0.98–1.47)	0.823
Triglycerides (mmol/L)	0.98 (0.81–1.32)	1.46 (0.89–2.37)	**0.059**
Dyslipidemia, n (%)	6 (40.0)	8 (47.1)	0.688
Meabolic syndrome (ATPIII), n (%)	6 (40.0)	10 (58.8)	0.288
Metabolic syndrome (IDF), n (%)	7 (46.7)	10 (58.8)	0.492
Aspartate aminotransferase (AST) (U/L)	33.00 (27.00–51.00)	28.00 (24.50–39.00)	0.350
Alanine aminotransferase (ALT) (U/L)	51.00 (32.00–71.00)	44.00 (35.50–47.50)	0.682
Total bilirubin (mg/dL)	0.70 (0.50–0.90)	0.60 (0.40–0.85)	0.455
MASLD (mm^2)	31.00 (19.00–46.00)	30.00 (13.00–46.00)	0.970
SAT (cm^2)	404.00 (273.00–516.20)	431.51 (312.09–520.21)	0.576
VAT (cm^2)	253.01 (180.17–307.50)	336.00 (258.71–401.29)	**0.089**
hs-C-reactive protein (mg/dL)	0.32 (0.27–0.71)	0.37 (0.20–0.80)	0.951
HOMA-IR	2.70 (1.95–6.31)	3.74 (2.53–5.40)	0.526
Serum creatinine (mg/dL)	0.80 (0.67–0.90)	0.70 (0.62–0.85)	0.295
IL-1β (PBMCs)	1.13 (1.05–1.15)	1.14 (1.03–1.37)	0.526
MCP-1 (PBMCs)	3.37 (3.32–3.65)	3.52 (3.42–3.71)	0.114
TNFa (PBMCs)	1.57 (1.51–1.67)	1.65 (1.53–1.73)	0.176
IL6 (PBMCs)	6.68 (6.30–7.26)	6.50 (6.40–6.98)	0.710
ICAM-1 (PBMCs)	1.63 (1.50–1.75)	1.56 (1.41–1.75)	0.455
VCAM-1(PBMCs)	4.79 (4.50–5.00)	4.72 (4.53–4.93)	0.628
SELP (PBMCs)	1.02 (0.95–1.17)	1.06 (0.96–1.16)	0.602
TLR4 (PBMCs)	1.86 (1.65–2.18)	1.81 (1.56–1.93)	0.278
Chemerin (ng/mL)	66.36 (59.31–79.38)	71.44 (64.15–86.84)	0.331
IL1Ra (ng/mL)	566.62 (371.71–1294.88)	454.82 (318.39–752.68)	0.331
Cardiovascular disease, n. (%)			
ALL CVD	2 (13.3)	1 (5.9)	0.471
Carotid stenosis (> 50%)	2 (13.3)	0 (0.0)	0.120
Stroke, TIA or revascularization	0 (0.0)	1 (5.9)	0.340
Peripheral artery disease	0 (0.0)	1 (5.9)	0.340
Diabetic microvascular disease			
Nephropathy	1 (6.7)	0 (0.0)	0.279
Therapy, n (%)			
Metformin	15 (100)	17 (100)	–
ACE-I	3 (20)	4 (23.5)	0.810
ARBs	3 (20)	5 (29.4)	0.539
Diuretics	4 (26.7)	6 (35.3)	0.599
β-blockers	3 (20)	6 (35.3)	0.337
CCA	1 (6.7)	0 (0.0)	0.279

Table 1 (continued)

Variable	Pre-lifestyle (n = 15)	Pre-liraglutide (n = 17)	P value*
Other antihypertensives	0 (0.0)	2 (11.8)	0.170
Statins	2 (13.3)	2 (11.8)	0.893
Omega 3	0 (0.0)	1 (5.9)	0.340
Proton pump inhibitors	2 (13.3)	2 (11.8)	0.893
ASA	1 (6.7)	1 (5.9)	0.927

BMI Body mass index, *WHR* Waist to Hip ratio, *HDL* High-density lipoproteins, *AST* Aspartate Aminotransferase, *ALT* Alanine amino Transferase, *MASLD* Metabolic dysfunction associated steatotic liver disease, *HOMA-IR* Homeostatic Model Assessment of Insulin Resistance, *eGFR* Estimated glomerular filtration rate, *IMT* Intima-media thickness, *CAD* Coronary artery disease, *CCA* Common carotid artery, *MI* Myocardial infarction, *TIA* Transient ischemic attack, *PPAR-Y* Peroxisome proliferator-activated receptor gamma, *SGLT2* Sodium-glucose co-transporter-2, *GLP1 RA* Glucagon-like peptide 1 receptor agonist, *DPP-IV* Dipeptidyl peptidase IV, *ACE-I* ACE-inhibitors, *ARBs* Angiotensin receptor blockers, *CCA* Calcium channel antagonists, *ASA* Acetylsalicylic acid

Data are median (25–75th percentile). [†]Determined by Chi-Square or Mann–Whitney or x^2 test, as appropriate; bold significance p<0.05

respectively. Table 1 shows the clinical and biochemical characteristics of the 32 patients in the study, stratified by treatment arm. Of the whole population, 16 subjects had prediabetes and 16 newly diagnosed T2DM (Table 2).

Baseline correlation between PBMCs' IL-1β levels and metabolic status

At baseline, no difference was observed in PBMCs' peripheral monocyte IL-1β levels in the two treatment arms or between subjects with prediabetes and diabetes (Tables 1 and 2).

IL-1β was positively correlated with body mass index (BMI) (rho = 0.42, p = 0.016), fasting plasma glucose (rho = 0.42, p = 0.018), HbA1c (rho = 0.35, p = 0.050), VAT (rho = 0.39, p = 0.028), MASLD (rho = 0.45, p = 0.009), platelet count (rho = 0.51, p = 0.003), chemerin (rho = 0.46, p = 0.009) and interleukin-1 receptor agonist (IL1-RA) (rho = 0.52, p = 0.002) (Fig. 2).

Effects of liraglutide and lifestyle interventions

After achievement of the weight loss target in the two groups, a comparable reduction of IL-1β was observed in both arms (Fig. 3, p for difference = 0.56), in parallel with a comparable improvement in glycaemic control, C reactive protein (CRP), BMI and MASLD as previously assessed [18]. The characteristics of the subjects after the intervention, stratified by treatment arm, are reported in Table 3. The tertiles of basal levels of IL-1β directly correlated with delta MASLD (p = 0.030 in the multivariable analysis adjusted for treatment, age, sex, CRP, and basal levels of MASLD) (Fig. 4). Specifically, delta MASLD was higher [median – 8.00; 95%CI – 12.25 to – 4.75] *vs.* [median – 23.00; 95%CI: – 39.50 to – 16.25] in the third *vs.* first tertile of IL-1β. Furthermore, baseline MASLD levels differed slightly by sex, with women (n = 15) showing a mean baseline MASLD of 39.3 ± 23.2 versus 26.2 ± 15.6 in men (p = 0.069). However, the change in MASLD did not differ between sexes, with women showing a mean delta MASLD of – 17.9 ± 14.4 and men – 12.6 ± 10.7 (p = 0.24). Additionally, delta MASLD was higher in the third vs. first tertile of IL-1β in both men (– 17.0; – 78.2 to 33.5 vs. – 9.0; – 4.6 to – 13.4) and women (– 27.0; – 40.6 to – 16.5 vs. – 6.0; – 22.5 to 9.8; P = 0.83 for difference).

Discussion

In the present study, we report for the first time that: (i) baseline peripheral monocyte IL-1β levels correlates with the extent of MASLD and related metabolic status; (ii) a 7% weight loss, regardless of the intervention adopted, reduces PBMCs' IL-1β levels along with the improvement in metabolic control; (iii) baseline PBMCs' IL-1β is positively associated with improved MASLD status upon weight loss. Our findings point out that IL-1β assessed at PBMCs' level may be a relevant biomarker in MASLD, able to detect disease severity as well as predict the efficacy of therapeutic intervention.

We found that baseline IL-1β is related to the MASLD status as well as to the metabolic characterization. IL-1 family cytokines are major drivers of inflammation in MASLD [25]. In particular, IL-1β is involved in all the stages of the disease [26]. It promotes liver steatosis, inflammation and fibrosis by signaling through the IL-1 receptor [27]. Inflammasomes serve as sensors for both endogenous and exogenous pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), regulating the processing of the proinflammatory factor pro-IL-1 β [28]. The NLRP3-related pathway plays a key role in adipose tissue-induced inflammation, adipocyte inflammation, and hepatocyte injury, particularly through the NLRP3-IL-1β signaling pathway [29]. Loss of NLRP3 inflammasome function protects mice from diet-induced MASLD, contrasting with the outcomes observed when the inflammasome is active [30]. The correlation we found between IL-1β and adipose tissue parameters is consistent with the notion that NLRP3-IL-1β pathway drives inflammation in obesity, and at the hepatic level IL-β is overexpressed in patients with obesity with or without diabetes [29].

The underlying cause of metabolic inflammation is dysregulated immunity, with macrophage polarization imbalance playing a crucial role. Under normal

Table 2 Baseline clinical and biochemical features of obese patients with prediabetes or early type 2 diabetes mellitus

Variable	Pre_T2DM (n = 16)	T2DM (n = 16)	P value*
Age, (years)	53.00 (49.25—62.50)	53.50 (49.50—57.50)	0.897
Male gender, n. (%)	9 (56.3)	8 (50.0)	0.723
Smoke, n. (%)	2 (12.5)	2 (12.5)	1.000
Weight, (Kg)	100.30 (88.70–121.80)	105.60 (96.35–114.73)	0.590
BMI, n. (Kg/m^2)	37.85 (32.61–40.19)	37.36 (32.36–42.70)	0.564
Waist circumference (cm)	112.0 (102.0–128.0)	119.0 (111.0–127.0)	0.381
WHR	0.96 (0.92–1.01)	0.96 (0.90–1.04)	0.616
Hypertension, n. (%)	13 (81.3)	10 (62.5)	0.238
Systolic arterial pressure, (mmHg)	130.50 (120.50–144.00)	133.00 (125.50–142.00)	**0.023**
Diastolic arterial pressure, (mmHg)	76.00 (70.00–81.75)	82.00 (75.25–85.00)	**0.003**
Glycated hemoglobin, (mmol/mol)	40.0 (36.0–43.0)	50.0 (41.0–60.0)	**0.002**
Fasting plasma glucose (mmol/L)	5.14 (5.01–5.67)	5.56 (5.21–6.10)	0.171
Fasting plasma insulin (uU/mL)	11.15 (8.88–22.72)	14.05 (9.43–21.45)	0.669
Total cholesterol, (mmol/L)	4.24 (3.64–4.48)	4.69 (3.82–5.48)	**0.035**
HDL cholesterol, (mmol/L)	1.19 (0.98–1.39)	1.09 (0.99–1.45)	0.926
Triglycerides, (mmol/L)	0.89 (0.78–1.22)	1.46 (1.31–2.02)	**0.004**
Dyslipidemia, n. (%)	5 (31.3)	9 (56.3)	0.154
ATPIII, n. (%)	7 (43.8)	9 (56.3)	0.480
IDF, n. (%)	7 (43.8)	10 (62.5)	0.288
AST, (U/L)	29.50 (22.00–43.50)	34.50 (25.75–40.50)	0.539
ALT, (U/L)	37.50 (32.25–59.25)	46.00 (39.25–70.25)	0.210
Total bilirubin, (mg/dL)	0.64 (0.50–0.82)	0.65 (0.40–0.88)	0.956
MASLD (mm^2)	24.00 (12.00–47.50)	31.00 (15.25–45.75)	0.160
SAT (cm^2)	416.57 (305.37–521.10)	427.32 (300.40–506.25)	0.985
VAT (cm^2)	253.80 (176.76–307.32)	336.83 (255.27–410.44)	**0.008**
hs-C-reactive protein, (mg/dL)	0.45 (0.29–0.94)	0.74 (0.63–0.95)	0.713
HOMA-IR	3.08 (2.13–5.83)	3.90 (2.29–5.60)	0.752
Serum Creatinine (mg/dL)	0.72 (0.65–0.82)	0.74 (0.63–0.88)	0.221
IL-1β (PBMCs)	1.12 (1.03–1.15)	1.14 (1.05–1.33)	0.468
MCP-1 (PBMCs)	3.54 (3.38–3.70)	3.42 (3–33–3.66)	0.381
TNFα (PBMCs)	1.57 (1.51–1.69)	1.61 (1.55–1.71)	0.669
IL6 (PBMCs)	6.43 (6.26–7.08)	6.82 (6.43–7.25)	**0.056**
ICAM-1 (PBMCs)	1.64 (1.50–1.77)	1.53 (1.44–1.71)	0.224
VCAM-1(PBMCs)	4.75 (4.56–4.92)	4.78 (4.50–5.09)	0.897
SELP (PBMCs)	1.05 (0.94–1.15)	1.04 (0.96–1.17)	0.696
TLR4 (PBMCs)	1.87 (2.05–1.69)	1.78 (1.62–1.98)	0.423
Chemerin (ng/mL)	69.56 (62.47–80.12)	69.87 (59.99–89.89)	0.926
IL1Ra (ng/mL)	422.07 (279.25–741.74)	487.55 (367.66–955.07)	0.361
Cardiovascular disease, n. (%)			
ALL CVD	2 (12.5)	1 (6.3)	0.544
Carotid stenosis (> 50%)	1 (6.3)	1 (6.3)	1.000
Stroke, TIA or revascularization	1 (6.3)	0 (0.0)	0.310
Peripheral artery disease	1 (6.3)	0 (0.0)	0.310
Diabetic microvascular disease, n			
Nephropathy	0 (0.0)	1 (6.3)	0.310
Therapy, n. (%)			
Metformin	16 (100)	16 (100)	–
ACE-I	3 (18.8)	4 (25.0)	0.669
ARBs	5 (31.3)	3 (18.8)	0.414
Diuretics	7 (43.8)	3 (18.8)	0.127
β-blockers	7 (43.8)	2 (12.5)	**0.049**
CCA	1 (6.3)	0 (0.0)	0.310
Other antihypertensives	1 (6.3)	1 (6.3)	1.000

Table 2 (continued)

Variable	Pre_T2DM (n = 16)	T2DM (n = 16)	P value*
Statins	2 (12.5)	2 (12.5)	1.000
Omega 3	0 (0.0)	1 (6.3)	0.310
Proton pump inhibitors	3 (18.8)	1 (6.3)	0.285
ASA	1 (6.3)	1 (6.3)	1.000

BMI Body mass index, *WHR* Waist to Hip ratio, *HDL* High-density lipoproteins, *AST* Aspartate Aminotransferase, *ALT* Alanine amino Transferase, *MASLD* Metabolic dysfunction associated steatotic liver disease, *HOMA-IR* Homeostatic Model Assessment of Insulin Resistance, *eGFR* Estimated glomerular filtration rate, *IMT* Intima-media thickness, *CAD* Coronary artery disease, *CCA* Common carotid artery, *MI* Myocardial infarction, *TIA* Transient ischemic attack, *PPAR-Y* Peroxisome proliferator-activated receptor gamma, *SGLT2* Sodium-glucose co-transporter-2, *GLP1RA* Glucagon-like peptide 1 receptor agonist, *DPP-IV* Dipeptidyl peptidase IV, *ACE-I* ACE-inhibitors, *ARBs* Angiotensin receptor blockers, *CCA* Calcium channel antagonists, *ASA* Acetylsalicylic acid

Data are median (25–75th percentile). †Determined by Chi-Square or Mann–Whitney or x^2 test, as appropriate; bold significance $p < 0.05$

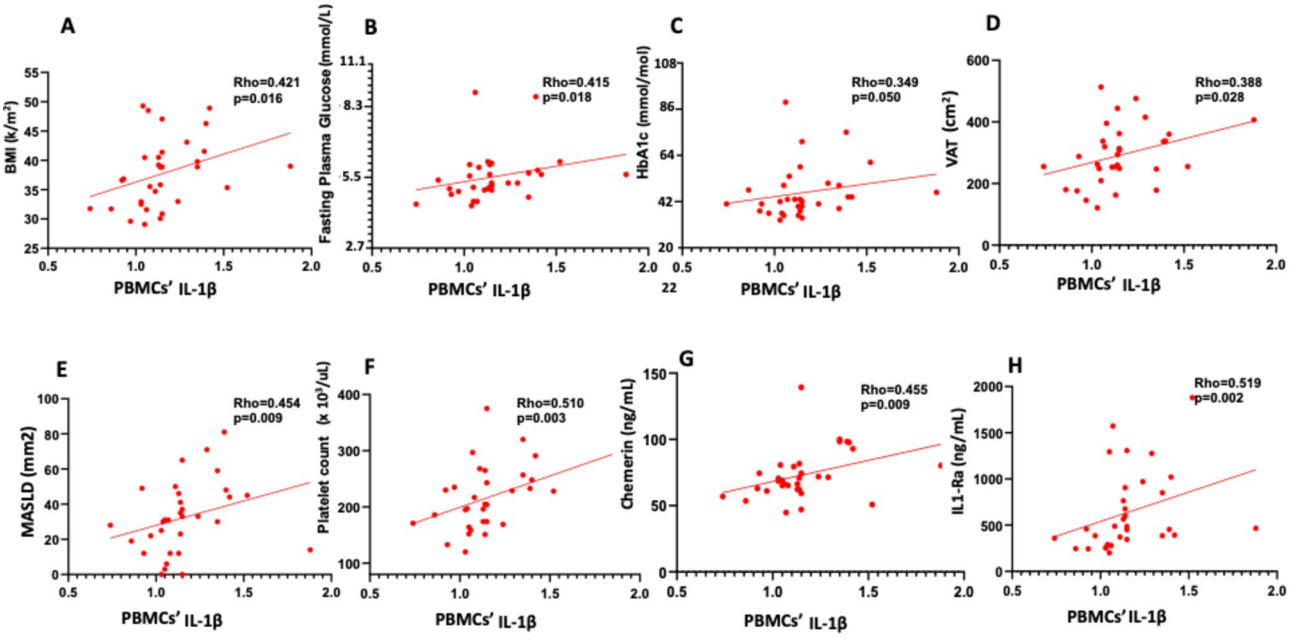


Fig. 2 Baseline correlation between IL-1β and glycaemic, inflammatory and metabolic characteristics. Correlation between IL-11β and BMI (**panel A**), fasting plasma glucose (**panel B**), HbA1c (**panel C**), VAT (**panel D**), MASLD (**panel E**), platelet count (**panel F**), chemerin (**panel G**), IL-1Ra (**panel H**)

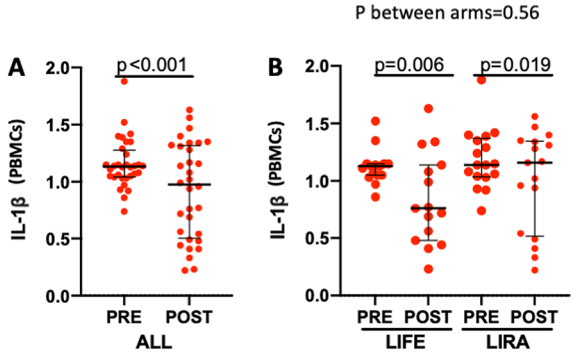


Fig. 3 Effects of liraglutide- or lifestyle-induced weight loss on IL-1β in obese subjects with pre-diabetes or early type 2 diabetes mellitus. Changes in IL-1β in obese subjects after weight loss in obese patients with prediabetes or diabetes diagnosed within one year (**panel A**), and after liraglutide- or lifestyle counseling-induced weight loss (**panel B**).

circumstances, M1 and M2 macrophages are in a balanced state. However, this balance is disrupted when free fatty acids and lipopolysaccharides accumulate in the body, leading to a shift toward increased M1 polarization. M1 macrophages primarily secrete proinflammatory cytokines like IL-1β which promote the inflammatory response [29]. The IL-1 system includes two agonists, IL-1α and IL-1β, a specific activation system (IL-1 converting enzyme, ICE), and a receptor antagonist (IL-1Ra) [31]. Molecules that regulate inflammation and immunity act in a coordinated manner to influence various components of the system [32]. IL-1Ra is produced by various cell types, including monocyte-macrophages, PMNs, and fibroblasts. Adherent human monocytes stimulated with LPS produce nearly equivalent amounts of IL-1β and IL-1Ra [31]. Among cytokines, granulocyte–macrophage colony-stimulating factor (GM-CSF) enhances the production of IL-1Ra in monocytes [32]. While IL-1α and IL-1β are weak inducers of IL-1Ra in monocytes, IL-1β

Table 3 Baseline and post-intervention clinical and analytical features of obese patients randomized to liraglutide- or lifestyle-induced weight-loss intervention

Variable	Pre-lifestyle (n = 15)	Post-lifestyle (n = 15)	P value	Pre-liraglutide (n = 17)	Post-Liraglutide (N = 17)	P value
Weight (Kg)	96.10 (88.60–124.00)	90.5 (89.0–117.0)	< 0.001	109.00 (97.55–116.35)	101.00 (94.40–103.70)	< 0.001
BMI (Kg/m^2)	35.35 (30.90–40.52)	36.1 (32.8–37.6)	< 0.001	38.86 (34.26–42.31)	34.00 (32.95–39.03)	< 0.001
Waist circumference (cm)	116.0 (110.0–132.0)	110.0 (105.0–120.0)	0.009	118.0 (112.0–129.0)	110.00 (107.00–122.50)	< 0.001
WHR	0.95 (0.90–1.01)	0.93 (0.90–1.0)	0.363	0.98 (0.93—1.05)	0.98 (0.92–1.01)	0.733
Systolic arterial pressure (mmHg)	131.00 (123.00–146.00)	141.00 (131.00–153.00)	0.683	144.00 (129.00—152.00)	139.00 (120.00–146.00)	0.037
Diastolic arterial pressure (mmHg)	80.00 (70.00–85.00)	85.00 (80.00–94.00)	0.382	82.00 (77.50–86.00)	81.00 (69.00–90.00)	0.149
Glycated hemoglobin (HbA1c) (mmol/mol)	43.0 (37.0–50.0)	36.0 (36.0–40.0)	0.013	42.00 (38.0–52.0)	38.0 (34.0–43.0)	< 0.001
Fasting plasma glucose (mmol/L)	5.33 (5.06–5.72)	4.78 (4.72–5.22)	0.053	4.97 (4.97–6.00)	4.83 (4.33–5.44)	< 0.001
Fasting plasma insulin (uU/mL)	10.60 (8.70–23.50)	7.7 (5.4–19.7)	0.002	15.40 (9.75–21.30)	11.0 (5.7–20.5)	0.025
Total cholesterol (mmol/L)	4.44 (4.03–4.65)	4.21 (3.80–4.55)	0.233	4.34 (3.59–5.27)	3.67 (3.02–5.12)	0.001
High density lipoprotein (HDL) cholesterol (mmol/L)	1.09 (0.98–1.37)	0.96 (0.80–1.24)	0.333	1.21 (0.98—1.47)	1.21 (1.01–1.42)	0.049
Triglycerides (mmol/L)	0.98 (0.81–1.32)	1.33 (0.89–1.81)	0.209	1.46 (0.89–2.37)	1.46 (0.87–1.72)	0.408
Aspartate aminotransferase (AST) (U/L)	33.00 (27.00–51.00)	33.00 (28.00–36.00)	0.003	28.00 (24.50–39.00)	22.00 (20.00–26.00)	0.001
Alanine aminotransferase (ALT) (U/L)	51.00 (32.00–71.00)	33.00 (28.00–36.00)	0.009	44.00 (35.50–47.50)	28.00 (23.00–34.00)	< 0.001
Total Bilirubin (mg/dL)	0.70 (0.50–0.90)	0.70 (0.60–1.00)	0.440	0.60 (0.40–0.85)	0.57 (0.47–0.78)	0.977
MASLD (mm^2)	31.00 (19.00–46.00)	18.00 (10.00–25.00)	< 0.001	30.00 (13.00–46.00)	17.00 (5.00–24.00)	< 0.001
SAT (cm^2)	404.00 (273.00–516.20)	403.77 (365.00–636.47)	< 0.001	431.51 (312.09–520.21)	419.67 (205.81–482.20)	0.003
VAT (cm^2)	253.01 (180.17–307.50)	226.25 (165.88–233.74)	0.011	336.00 (258.71–401.29)	289.73 (186.31–338.87)	< 0.001
hs-C-reactive protein (mg/dL)	0.32 (0.27–0.71)	0.23 (0.22–0.32)	0.021	0.37 (0.20–0.80)	0.29 (0.07–0.56)	0.003
HOMA-IR	2.70 (1.95- 6.31)	1.63 (1.25–3.99)	< 0.001	3.74 (2.53—5.40)	2.39 (1.48–5.31)	0.068
Serum creatinine, (mg/dL)	0.80 (0.67–0.90)	0.80 (0.72–1.00)	0.944	0.70 (0.62–0.85)	0.76 (0.60–0.94)	0.610
IL-1β (PBMCs)	1.13 (1.05–1.15)	0.72 (0.47–1.34)	0.006	1.14 (1.03–1.37)	1.02 (0.48–1.35)	0.019
MCP-1 (PBMCs)	3.37 (3.32–3.65)	2.70 (1.24–3.05)	< 0.001	3.52 (3.42–3.71)	3.11 (0.72–4.00)	0.177
TNFα (PBMCs)	1.57 (1.51–1.67)	1.07 (0.73–1.18)	0.002	1.65 (1.53–1.73)	0.78 (0.58–1.26)	0.002
IL6 (PBMCs)	6.68 (6.30–7.26)	3.43 (2.39–5.24)	0.001	6.50 (6.40–6.98)	5.60 (2.24–8.04)	0.177
ICAM-1 (PBMCs)	1.63 (1.50–1.75)	1.44 (0.68–1.89)	0.017	1.56 (1.41–1.75)	0.85 (0.67–1.78)	0.055
VCAM-1 (PBMCs)	4.79 (4.50–5.00)	2.70 (2.60–6.44)	0.047	4.72 (4.53–4.93)	3.64 (2.74–5.70)	0.013
SELP (PBMCs)	1.02 (0.95–1.17)	0.53 (0.46–1.48)	0.008	1.06 (0.96–1.16)	0.68 (0.37–1.22)	0.035
TLR4 (PBMCs)	1.86 (1.65–2.18)	1.62 (1.27–2.17)	0.017	1.81 (1.56–1.93)	0.97 (0.62–1.61)	0.068
Chemerin (ng/mL)	66.36 (59.31–79.38)	49.05 (46.19–67.88)	0.191	71.44 (64.15–86.84)	70.57 (60.98 -81.83)	0.035
IL1Ra (ng/mL)	566.62 (371.71–1294.88)	698.26 (396.45–1019.77)	0.394	454.82 (318.39–752.68)	494.41 (356.86–689.10)	0.831

BMI Body mass index, *WHR* Waist to Hip ratio, *HDL* High-density lipoproteins, *AST* Aspartate Aminotransferase, *ALT* Alanine amino Transferase, *MASLD* Metabolic dysfunction associated steatotic liver disease, *HOMA-IR* Homeostatic Model Assessment of Insulin Resistance, *eGFR* Estimated glomerular filtration rate, *IMT* Intima-media thickness, *CAD* Coronary artery disease, *CCA* Common carotid artery, *MI* Myocardial infarction, *TIA* Transient ischemic attack, *PPAR-Y* Peroxisome proliferator-activated receptor gamma, *SGLT2* Sodium-glucose co-transporter-2, *GLP1 RA* Glucagon-like peptide 1 receptor agonist, *DPP-IV* Dipeptidyl peptidase IV, *ACE-I* ACE-inhibitors, *ARBs* Angiotensin receptor blockers, *CCA* Calcium channel antagonists, *ASA* Acetylsalicylic acid

Data are median (25–75th percentile). [†]Determined by Chi-Square or Mann–Whitney or x^2 test, as appropriate

potentiates IL-1Ra induction by IgG. The production of IL-1Ra by cells of the mononuclear phagocyte lineage is influenced by their differentiation state [31]. Levels of IL-1Ra are increased in conditions linked to liver fat accumulation, including obesity and T2DM [33]. Pihlajamäki et al. [34] found that serum levels of IL-1Ra, and liver mRNA expression of IL1Ra are associated with NASH and the degree of lobular inflammation in liver. The decrease in serum IL-1RA level and expression of IL1Ra after obesity surgery correlated with the improvement of lobular inflammation [34]. No study has, to the best of our knowledge, reported the close relationship between IL-1β measured at PBMCs' level and the impaired metabolic status which characterizes obesity-induced MASLD. Our results highlight the crucial role of IL-1β in MASLD pathogenesis and are supported by

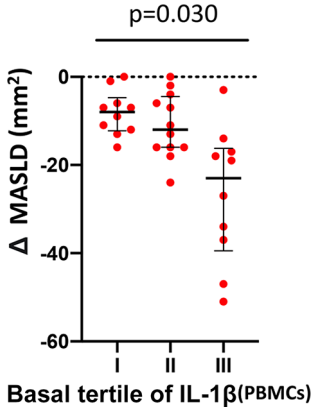


Fig. 4 Correlation between basal tertile of IL-1β and delta MASLD. Correlation between basal IL-1β tertiles and delta MASLD in obese patients with prediabetes or newly-diagnosed diabetes

in vivo experimental evidence: double knockout (caspase-1/11 knockout and neutrophil elastase/proteinase-3) mice unable to activate IL-1β are protected against obesity-related MASLD [35]. Indeed, targeting IL-1β has been proposed in MASLD [36], although no human studies have been conducted to date and no data on hepatic steatosis are available from the CANTOS trial [37]. Our findings support further investigation in this direction.

Weight loss is currently the cornerstone intervention for MASLD and NASH [38]. Specifically, 7% weight loss, as achieved in our study, can improve histological disease activity [39], [8]. The benefit obtained is independent of the type of intervention: in a phase II study, liraglutide-mediated histological resolution of steatohepatitis and fibrosis in NASH patients who were overweight was largely dependent on weight loss and glycaemic control [40]. A beneficial effect on metabolic associated steatohepatitis has been also recently reported in phase II trials with dual GIP and GLP-1RA [41] or dual glucagon and GLP-1RA [42].

It is well established that weight loss reduces plasma level of inflammatory markers via positive metabolic adaptation: calorie restricted diet improves mitochondrial function through AMPK activation, and the reduction in body fat mass is associated with decreased cytokine mRNA levels [43]. Consistently, IL-1β release from visceral adipose tissue decreases after gastric bypass surgery [44]. With regard to GLP-1RAs, several mechanisms, on top of concurrent weight loss, may justify the benefit on liver steatosis. We previously showed that liraglutide treatment was associated with a significantly greater reduction on circulating soluble suppression of tumorigenesis-2 (ST-2) levels, a marker of liver and myocardial fibrosis, as compared to lifestyle-induced weight loss [45]. It has also been proposed that GLP1RA may directly affect circulating immune cells [46, 47]. In 24 patients with obesity and diabetes patients treated with exenatide for 12 weeks, there was a reduced mRNA expression of TNF-a and IL-1β in PBMCs [48]. A similar effect was observed for liraglutide [48]. However, considering the lack of in vitro evidence for GLP-1R expression in PBMCs, these effects could also be indirect and mediated by weight loss [48]. Consistently, in our study we found that both intervention arms achieved similar results in terms of MASLD improvement, reducing as well PBMC's IL-1β levels. Although indirect evidence is already available, our study is the first to rigorously evaluate two matched weight loss interventions and assess the predictors of their impact on MASLD.

The change in MASLD did not differ between sexes. Moreover, the association between IL-1β tertiles and delta MASLD was consistent across sexes, suggesting that sex does not substantially modify the relationship between IL-1β and MASLD change. However, prior research has shown that MASLD pathophysiology may differ by sex due to hormonal, metabolic, and inflammatory factors [49, 50]. For example, estrogen is thought to exert protective effects on hepatic metabolism, potentially contributing to sex-based differences in MASLD prevalence and severity [50]. Conversely, after menopause, the risk of MASLD and related metabolic dysfunction in women tends to increase, approaching that of men. These factors highlight the need for further studies to explore sex-specific mechanisms in MASLD progression and response to interventions [50].

The notion that PBMC's IL-1β levels predict MASLD improvement over a 7% weight loss period is novel and with relevant implications. MASLD is an extremely heterogeneous disease in terms of pathogenesis, with a significant interindividual variability in susceptibility to MASLD and its progression to liver-related complications [51]. This is coupled with an extreme variability in response to treatment: a recent study showed that, in subjects with 1–2%/year weight loss, the frequency of MASLD remission reached a plateau of 43% [52]. Similarly, current investigational therapies achieve suboptimal response rates (20–40%) [53]. This calls for the urgent identification of effective biomarkers [54] to detect the phenotype which can benefit the most from interventions, allowing to develop personalized approaches [55]. Some ongoing investigations have showed the potential use of MRI-based proton-density fat fraction (PDFF) [11] or plasma and hepatocytes-derived extracellular vesicles [56]. However, these are still techniques with limited availability and high cost. Artificial intelligence has also been proposed, but in the field of hepatology we are still far from its validation and therefore from its actual implementation [57]. Conversely, PBMC isolation and subsequent IL-1β dosage is widely available and carries relatively low costs [58]. Our findings might support

the implementation of PBMC's IL-1β as an intervention-driver biomarker in patients with MASLD.

Our study has some limitations. First, the relatively small sample size may have reduced our ability to detect modest associations between MASLD and other variables. However, this strengthens the robustness of the main findings. Second, the short duration of the study and the lack of long-term follow-up may have complicated the assessment of predictors of long-term benefit, as well as the presence or absence of a legacy effect regarding the predictive power of IL-1β. Nevertheless, this was a proof-of-concept study to set the stage for larger observations to validate our findings. Third, in our study we could not perform protein-level validation of IL-1β mRNA, however we provide additional data on IL-1β signalling by showing circulating levels of IL-1β receptor (IL-1Ra) and its direct correlation with PBMC's IL-1β. Fourth, we did not measure the inflammasome signature in plasma, but only in PBMC. However, this is a strength of our study. Indeed, circulating inflammatory monocytes better reflect the chronic low-grade inflammation observed in ageing and chronic cardiometabolic diseases such as obesity and T2D [59–61].

Conclusions

In the present study, we found that IL-1β measured at the level of PBMCs is able to predict MASLD response to weight loss, as well as identify a more MASLD-related impaired metabolic status. Due to the extreme heterogeneity of the disease, the lack of effective biomarkers to predict MASLD treatment response is a limiting factor in the implementation of proactive strategies to manage MASLD. This, in turn, promotes high-risk cardiovascular phenotypes that burden our healthcare systems. Our results may set the stage for larger ad hoc studies to investigate the utility of baseline PBMC IL-1β levels as a drug response biomarker in MASLD.

Abbreviations

GLP-1	Glucagon-like peptide-1
OGTT	Oral glucose tolerance test
T2DM	Type 2 diabetes mellitus
TNF	Tumor necrosis factor
IGT	Impaired glucose tolerance
IFT	Impaired fasting glucose
EDTA	Ethylenediaminetetraacetic acid
TG	Triglycerides
VAT	Visceral adipose tissue
SAT	Subcutaneous adipose tissue
BMI	Body mass index
LDL	Low-density lipoprotein
HDL	High-density lipoprotein
HOMA-IR	Homeostatic model assessment insulin resistance
CRP	C-reactive protein
MASLD	Metabolic dysfunction-associated steatotic liver disease
WHR	Waist–Hip ratio
CVD	Cardiovascular disease
IL-6	Interleukin 6
IL-1β	Interleukin-1β
IL-1Ra	Interleukin 1 receptor antagonist
PBMCs	Peripheral blood mononuclear cells

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Author contributions

Authors' contributions: Concept and design: FS, FP, AC. Acquisition, analysis, or interpretation of data: PS, SC, RL, AC, RT, AM. Drafting of the manuscript FS, FC, PS, AM, SC. Critical revision of the manuscript: FS, FC. Statistical analysis and figures: PS, RL, AdC. All authors read and approved the final manuscript. F.S. is the guarantor of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Availability of data and materials

No datasets were generated or analyzed during the current study.

Declarations

Ethics approval and consent to participate

The trial was approved by the Italian Ethics Committee of the University of Chieti (Approval n. 10 (protocol 20131) 23.05.2013). Each patient provided written informed consent before participation.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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