Oxidative Medicine and Cellular Longevity

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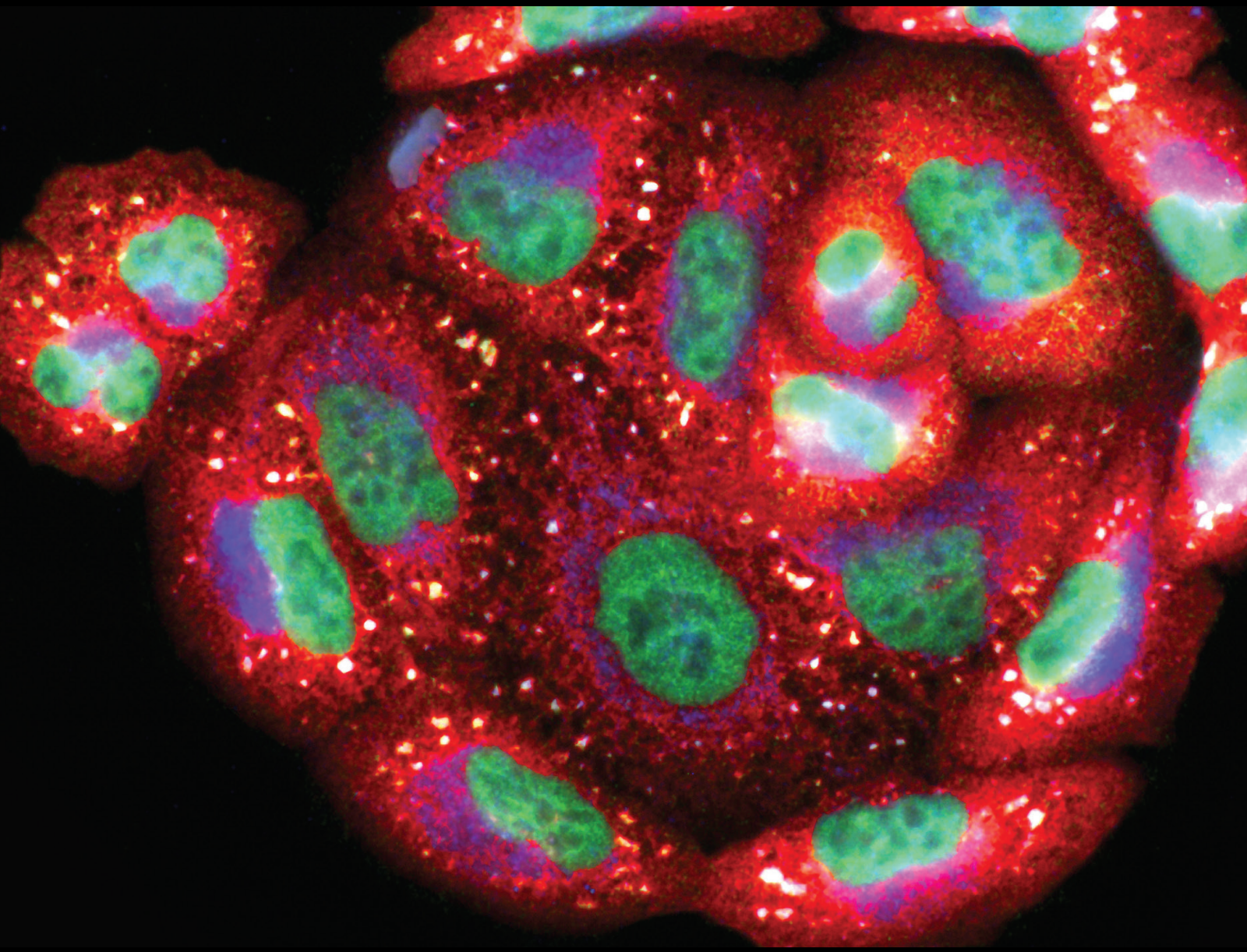

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Research Article

IL-6 and Leptin Are Potential Biomarkers for Osteoporotic Fracture Risk Assessment and Prediction of Postmenopausal Women with Low Bone Mass: A Follow-Up Study Using a Regional Sample Cohort

Xu Wang,[1] **Yili Zhang**,[2] **Baoyu Qi**,[1] **Kai Sun**,[1] **Chuanrui Sun**,[1] **Ning Liu**,[1] **Shengjie Fang**,[1] **Xu Wei**,[1] **Yanming Xie**,[3] **and Liguo Zhu**[1]

[1]*Wangjing Hospital, China Academy of Chinese Medical Sciences, Beijing, China*
[2]*School of Traditional Chinese Medicine & School of Integrated Chinese and Western Medicine, Nanjing University of Chinese Medicine, Nanjing, China*
[3]*Institute of Basic Research in Clinical Medicine, China Academy of Chinese Medical Sciences, Beijing, China*

Correspondence should be addressed to Xu Wei; weixu.007@163.com, Yanming Xie; ktzu2018@163.com, and Liguo Zhu; tcmspine@163.com

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Osteoporotic fracture, a major complication which is known as the outcome postmenopausal osteoporosis, seriously threatens the health of postmenopausal women. At present, the traditional osteoporotic fracture prediction methods are characterized by inconvenient application and time-consuming statistical results, while predictive serum biomarkers can make up for this shortcoming. Accurate and advanced risk prediction of osteoporotic fracture is meaningful to early prevention and intervention, effectively avoiding the risk of this disease and the secondary fracture in the surgical treatment. In this study, based on the BEYOND cohort, a 2-year follow-up study was conducted after subjects participated to survey if OF occurred. Independent sample t -test and Mann–Whitney U-test were used to analyze the differences of bone metabolism biomarkers between the OF and non-OF group. Cox proportional hazard model was used to screen the potential biomarkers might be used to predict OF risk. ROC curves and AUCs were used to analyze the predictive accuracy, and the Delong's test was used to compare the differences between the AUCs. 15 postmenopausal women with low bone mass and OF were found, and other 60 subjects without OF were matched with 1 : 4, age, and BMI classification as control group. The serum IL-6 (OR = 1.139, 95%CI = 1.058 – 1.226) and leptin (OR = 0.921, 95%CI = 0.848 – 1.000) were found as OF risk predictive biomarkers for postmenopausal women with low bone mass with high accuracy (IL – 6 = 0.871) (leptin = 0.813) and accuracy enhanced when they were combined (AUC = 0.898). The results of Delong's test showed that the difference of AUC between leptin and IL-6&Leptin was meaningful ($P = 0.024$) but meaningless between IL-6 and leptin ($P = 0.436$), IL-6 and IL-6&Leptin ($P = 0.606$). To sum up, IL-6 and leptin are the predictive biomarkers of OF for postmenopausal women with low bone mass. The IL-6 can improve the prediction accuracy of leptin ($P = 0.024$), but not vice versa ($P = 0.606$). *Trial Information*. Registered on the Chinese Clinical Trial Registry already. (Registration Number: ChiCTR-SOC-17013090).

1. Introduction

Postmenopausal osteoporosis (PMOP) [1], a serious public health problem, is the major type of osteoporosis (OP) characterized by bone tissue microstructure damaged and bone fragility increased, resulting in a higher fracture risk [2] for postmenopausal women. The prevalence of this condition has been reported to be 29.0% in women over 50, equating to 49 million people in China [3], which is one of the most prevalent metabolic bone diseases of Chinese elderly women. OF, being the final outcome and the most serious complication of PMOP, seriously threatens the health of

postmenopausal women. Due to many difficulties in the treatment of the disease such as high risk of secondary fractures [4], it is necessary to accurately predict the risk of OF and prevent it in advance. Early prediction of the disease prior to the occurrence of OF followed by effective prevention of appropriate treatment can reduce fracture risk.

Currently, dual-energy X-ray absorptiometry (DEXA) [5] is the normal approach of obtaining bone mineral density (BMD) to assess the risk of OF. Meanwhile, some established risk factors like T-scores [6] and prior fracture [7] also took part in predicting imminent risk of fracture, as falls [8], physical functioning [9], lifestyle [10, 11], and general health [12]. Nevertheless, the radiation, high cost of imaging equipment and the time-consuming, recall bias of questionnaire survey cannot be ignored when these methods are used to collect the prediction information. A faster and more efficient predictive method that is easier to screen on a large scale should be found, which is beneficial to the prevention of this high-risk disease. Serum biomarkers detection is an effective way to solve the abovementioned urgent problems.

At present, various pathogenesis of POMP and OF has been proposed, such as oxidative stress [13], inflammatory response [14], lipid metabolism [15], and angiogenesis [16]. Meanwhile, many relevant biomarkers have shown the correlation between osteoporosis and already been used to auxiliary diagnose OP and assess bone metabolism, such as leptin, Interleukin-6 (IL-6), insulin-like growth factor 1 (IGF-1), and vascular endothelial growth factor (VEGF) [17–19]. However, whether these biomarkers could be used to predict the risk of OF have no explicit evidence. Therefore, based on the BEYOND cohort [20] conducted by our team from 2017 to 2018 and its follow-up work on October, 2019, 15 patients with OF and 60 participants with low bone mass matched by age and BMI classification were selected to screen for predictive serum biomarkers of OF.

2. Materials and Methods

In this study, based on a cross-sectional and prospective follow-up study, we used Cox proportional hazards model to examine the relationships between the serum biomarkers and risk for OF among postmenopausal women with low bone mass. We focused on a 2-year period to evaluate the OF risk in this study.

The Ethics Committee of Wangjing Hospital of China Academy of Chinese Medical Sciences (approval number: WJEC-KT-2017-020-P001) approved this research.

This study had been registered on the Chinese Clinical Trial Registry already. (website: http://www.chictr.org.cn) (Registration Number: ChiCTR-SOC-17013090).

2.1. Data Source. This study used data from the study of BEYOND cohort, a cross-sectional and prospective study established from December 2017 to July 2018. It included 1540 participants from 10 communities in Chaoyang District and Fengtai District of Beijing as prospective data that had served as the basis for studies of OP and OF. Participants accepted examinations and questionnaire survey when they joined in this cohort and they were accepted a telephone interview on October 2019. Data on BMD, serum biomarkers, body mass index (BMI), lifestyle, medical history, medication use, and physical function were collected at the first examination visit. Participants reported on fractures and time, causes, locations, and treatments during the follow-up study.

Among 1,540 participants of BEYOND cohort, we included postmenopausal women with low bone mass (including osteopenia and osteoporosis) who were not diagnosed with diabetes, thyroid disease, kidney disease, and rheumatoid disease (disease cause secondary OP) as our follow-up interviewees ($n = 712$). Among these individuals, we selected participants suffered OF in these two years ($n = 15$) as OF group.

Then, the OF group was matched 1 : 4 with participants in the 712 who had no suffered OF in these two years. The non-OF group was selected from the postmenopausal women with low bone mass who were interviewed. Matching was performed based on their age (±2 year) and BMI classification (low weight: BMI < 18.5, normal: 18.5 ≤ BMI < 24, overweight: 24 ≤ BMI < 28, obesity: BMI ≥ 28). Finally, 1 : 4 matching resulted in the inclusion of 15 OF patients and 60 non-OF participants (Figure 1).

2.2. Diagnostic Criteria. Dual-energy X-ray absorptiometry device (Hologic, WI, USA) was used to assess the value of BMD (g/cm^2). The diagnosis of OP was based on the criteria outlined by the WHO and Chinese guidelines [21], T value > -1.0 was common; −2.5 ≤ T value ≤ −1.0 was osteopenia; T value < -2.5 was osteoporosis.

2.3. Study Outcomes. The primary outcome was OF. According to the definition of OF in Primary Osteoporosis Diagnosis and Treatment Guidelines published by Chinese Journal of Osteoporosis and Bone Mineral Research in 2017 [22], low bone mass ($T \leq -1.0$) combined with low energy fractures of the proximal humerus, pelvis, and distal forearm all belong to OF. Therefore, low-energy fractures in patients with osteoporosis and osteopenia in the BEYOND cohort will be included in this study.

2.4. Biomarker Detection. Fasting blood samples of the participants were collected between 8 a.m. and 9 a.m. in sitting position. The measurements were conducted through automated electrochemiluminescence immunoassay system (Roche, Cobas E601, Germany). In addition, the serum segregated for detection was stored at -80°C. Detected biomarkers include serum calcium, serum phosphorus, serum magnesium, 25-hydroxy-vitamin D [25(OH)VitD$_3$], β isomer of C-terminal telopeptide of type I collagen (β-CTx), osteocalcin (OST), parathyroid hormone (PTH), alkaline phosphatase (ALP), type I procollagen amino-terminal peptide (P1NP), IGF-1, IL-6, leptin, and VEGF.

2.5. Statistical Analysis and Accuracy Assessment. Kolmogorov-Smirnov test was used to test continuous variables for a normal distribution and was presented as the median with an interquartile range. t-test or Mann–Whitney U-test was used to analyze continuous detection indexes and the variables which $P < 0.2$ were included in the model [23]. Furtherly, the Cox proportional hazards model was used to explore the predictive biomarkers associated with OF. Based

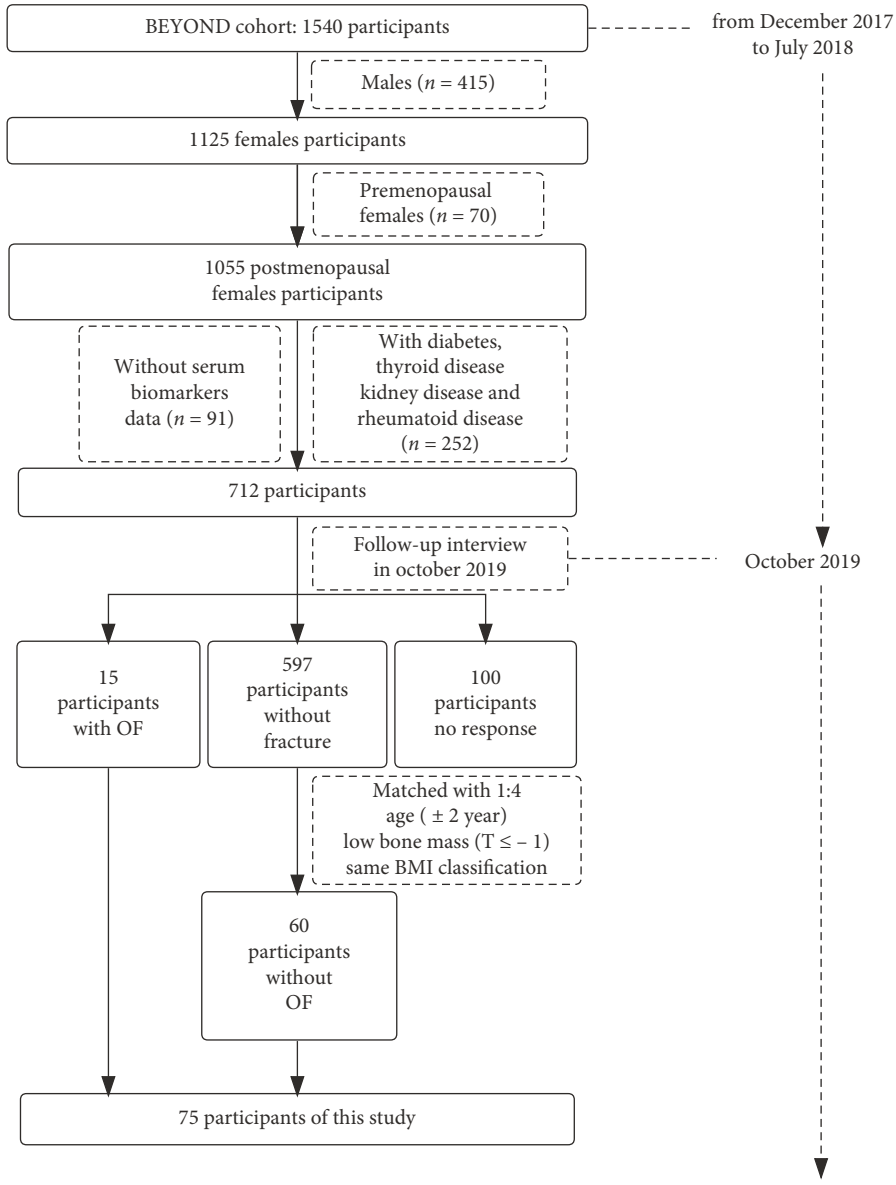

Figure 1: Participants screening process.

on the principle of modeling and variable selection, ROC curve and area under curve (AUC) were used to verify the accuracy of the model. $P < 0.05$ indicated that the difference between the two groups was statistically significant.

All statistical analysis was carried out with SPSS Statistics 22.0 software. ROC curves were obtained by Medclac 21.0 software. Figures were created in GraphPad Prism 8.0 (GraphPad Software, CA, USA).

3. Results

3.1. Study Population and Participant Characteristics. 15 OF patients and 60 non-OF participants were selected in this study. Among the 15 patients with OF, 7 participants were osteopenia, and the others were osteoporosis; 14 were due to fall, and one person was due to cough. There were 8 limb fractures, 5 spine fractures, and 2 hip fracture (Table 1). The oldest participant was 74 years old, the youngest was 51, median age of 15 patients was 61.00 (55.00, 70.00) years. Among the 15 patients, 5 were of normal weight, 6 were overweight, and 4 were obesity.

The median years of non-OF group were 61.50 (55.00, 69.75) years, the oldest was 75 years old, and the youngest was 50 years old. Meanwhile, their BMI classification was consistent with 15 patients completely.

3.2. Normality Test. According to the results of Kolmogorov-Smirnov and Shapiro-Wilk test, the ages, menopausal age, pregnancies times, delivery times, serum 25(OH)VitD3, β-CTx, ALP, P1NP, IL-6, and VEGF did not obey the normal distribution; the serum phosphorus, serum calcium, serum magnesium, OST, PTH, IGF-1, and leptin obeyed the normal distribution. The results of the normality test were shown in Table 2. Mann–Whitney U test was used to analysis the date

Table 1: The characteristics of 15 OF patients.

Number	Ages	BMI	Classification	Recruited time	Fracture time	Reason	Location
1	55	26.839	Overweight	June, 2018	August, 2019	Fall down	Ankle
2	52	21.484	Normal weight	June, 2018	December, 2018	Fall down	Ankle
3	58	28.444	Obesity	June, 2018	June, 2019	Fall down	Wrist
4	55	27.142	Overweight	May, 2018	December, 2018	Fall down	Hip
5	74	25.781	Overweight	May, 2018	February, 2019	Fall down	Wrist
6	61	25.631	Overweight	May, 2018	November, 2018	Fall down	Hip
7	58	23.225	Normal weight	May, 2018	September, 2018	Fall down	Lumbar spine
8	66	29.997	Obesity	May, 2018	October, 2018	Fall down	Lumbar spine
9	71	20.937	Normal weight	April, 2018	December, 2018	Fall down	Wrist
10	59	28.134	Obesity	April, 2018	February, 2019	Fall down	Wrist
11	70	25.537	Overweight	April, 2018	September, 2019	Cough	Lumbar spine
12	71	28.228	Obesity	March, 2018	November, 2018	Fall down	Ankle
13	51	21.414	Normal weight	December, 2017	October, 2019	Fall down	Lumbar spine
14	68	23.335	Normal weight	December, 2017	August, 2018	Fall down	Thoracic spine
15	65	27.392	Overweight	December, 2017	October, 2018	Fall down	Elbow

Table 2: Normality test of population information and biomarkers.

Group	Kolmogorov-Smirnova			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Ages	0.117	75	0.013	0.947	75	0.003
Menopause age	0.219	75	<0.001	0.890	75	<0.001
Delivery times	0.289	75	<0.001	0.777	75	<0.001
Pausimenia age	0.138	75	0.001	0.949	75	0.004
Serum phosphorus	0.094	75	0.097	0.929	75	<0.001
Serum calcium	0.080	75	0.200*	0.973	75	0.104
Serum magnesium	0.095	75	0.093	0.980	75	0.284*
25(OH)VitD3	0.145	75	0.001	0.898	75	<0.001
β-CTx	0.155	75	<0.001	0.936	75	0.001
OST	0.070	75	0.200*	0.975	75	0.136
PTH	0.090	75	0.200*	0.953	75	0.007
ALP	0.124	75	0.006	0.913	75	<0.001
P1NP	0.111	75	0.023	0.962	75	0.024
IGF-1	0.055	75	0.200*	0.994	75	0.987*
IL-6	0.344	75	<0.001	0.500	75	<0.001
Leptin	0.101	75	0.056	0.955	75	<0.001
VEGF	0.243	75	<0.001	0.659	75	<0.001

*$P > 0.05$, the simple is normally distributed.

did not obey the normal distribution, and t-test was used to analysis the date obey the normal distribution.

3.3. Univariate Analysis of Population Information and Biomarkers. Mann–Whitney U test was used to analyze the nonnormally distributed data, and t-test was used to analyze the normally distributed data. According to the results of univariate analysis, the differences of age, menopause age, and delivery times between the two groups have no statistically significant. Meanwhile, the differences of serum IGF-1($P = 0.004$), IL-6 ($P < 0.001$), and leptin ($P < 0.001$) between the OF group and the non-OF group are statistically significant (Table 3). In addition, six markers are found that the P value was less than 0.2, including serum calcium, OST, IGF-1, IL-6, leptin, and VEGF.

3.4. OF Risk Factor Analysis. Cox proportional hazard model was established to find the biomarkers could be used to predict OF. The variables whose P value was less than 0.2 (serum calcium, OST, IGF-1, IL-6, leptin, and VEGF) were

Table 3: Univariate analysis of population information and biomarkers.

Characteristics	Total ($n = 75$)	OF group ($n = 15$)	Non-OF group ($n = 60$)	t/Z	P
Age	61.00 (55.00, 70.00)	61.00 (55.00, 70.00)	61.50 (55.00, 69.75)	-0.073	0.942
Menopause age	50.00 (48.00, 52.00)	50.00 (48.00, 52.00)	50.00 (47.00, 52.00)	0.481	0.631
Pregnancies times	2.00 (1.00, 3.00)	2.00 (2.00, 3.00)	2.00 (1.00, 3.00)	0.414	0.679
Delivery times	1.00 (1.00, 2.00)	1.00 (2.00, 2.00)	1.00 (1.00, 2.00)	0.903	0.367
Serum phosphorus (mmol/L)	1.47 ± 0.26	1.50 ± 0.24	1.46 ± 0.28	-0.502	0.617
Serum magnesium (mmol/L)	0.95 ± 0.07	0.94 ± 0.05	0.96 ± 0.07	1.067	0.294
Serum calcium (mmol/L)	2.35 ± 0.07	2.38 ± 0.07	2.35 ± 0.07	-1.815	0.074
25(OH)VitD3 (pg/ml)	15.00 (11.50, 17.20)	16.60 (13.40, 18.60)	14.65 (10.85, 17.05)	1.020	0.308
β-CTx (ng/ml)	0.29 (0.21, 0.39)	0.30(0.25, 0.34)	0.29 (0.21, 0.39)	-0.020	0.984
OST (ng/ml)	16.14 ± 5.05	15.51 ± 3.52	16.29 ± 5.38	-0.384	0.192
PTH (pmol/L)	3.25 ± 1.07	3.05 ± 1.09	3.30 ± 1.07	-0.874	0.538
ALP (U/L)	83.00 (72.00, 98.00)	83.00 (70.00, 100.00)	83.00 (70.00, 97.75)	-0.388	0.735
P1NP (ng/ml)	56.87 (46.13, 77.17)	62.55 (53.41, 77.94)	56.03 (45.94, 76.87)	0.748	0.454
IGF-1 (ng/ml)	57.46 ± 21.06	73.38 ± 28.34	52.48 ± 16.88	-3.517	0.004*
IL-6 (pg/ml)	1.74 (1.02, 2.70)	2.90 (2.18, 21.74)	0.82 (1.46, 2.18)	4.426	<0.001**
Leptin (ng/ml)	22.35 ± 11.35	12.94 ± 7.83	24.71 ± 10.90	3.861	<0.001**
VEGF (pg/ml)	123.55 (73.66, 147.72)	137.53 (110.88, 178.20)	119.46 (65.93, 143.46)	1.497	0.134

Data are presented as Mean ± S.D. or Median (q_{25}, q_{75}). *$P < 0.05$, **$P < 0.001$.

Table 4: The result of Cox proportional hazards model.

Factor	β	SE	Wald	P	OR (95% CI)
OST	-0.085	.084	1.024	0.311	0.919 (0.780-1.083)
Serum calcium	10.511	5.551	3.585	0.058	36733.088 (0.691-1951531307.714)
IGF-1	0.009	0.014	0.403	0.525	1.009 (0.981-1.038)
IL-6	0.130	0.038	11.834	0.001*	1.139 (1.058-1.226)
Leptin	-0.082	0.042	3.860	0.049*	0.921 (0.848-1.000)
VEGF	0.000	0.002	0.004	0.950	1.000 (0.996-1.004)

*$P < 0.05$, **$P < 0.001$.

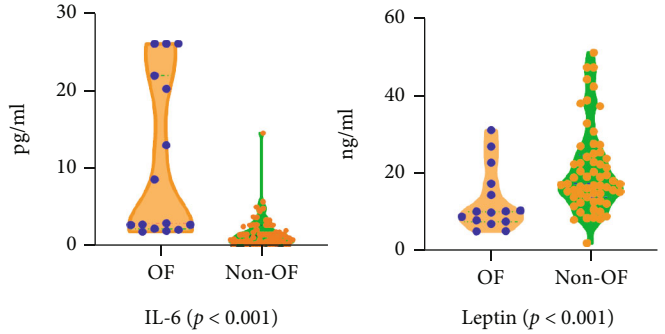

Figure 2: The comparison of IL-6 and leptin between OF and non-OF group. The blue points are the data of OF group participants, and the orange points are the data of non-OF group participants.

included in the Cox proportional hazards model, and the time was set as the number of month from they joined the cohort to follow-up study visited. The results showed that the differences between IL-6 and leptin were statistically significant. IL-6 was predictive risk biomarkers for OF in this population ($P < 0.001$, OR = 1.139), while leptin was a protective factor for OF ($P < 0.049$, OR = 0.921) (Table 4).

3.5. Serum IL-6, Leptin between OF and Non-OF Group. According to the results of Cox proportional hazards model,

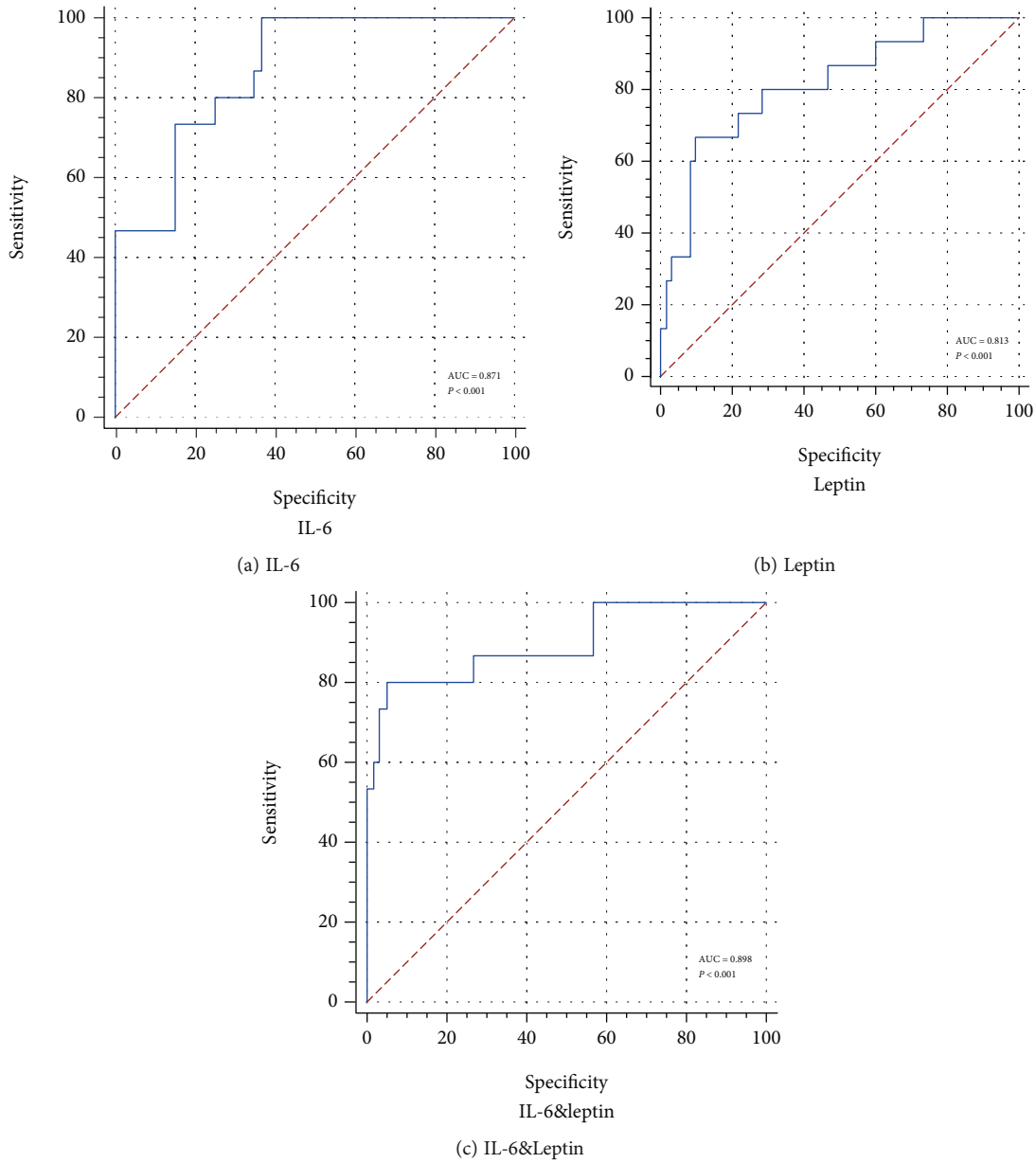

Figure 3: ROC curves of IL-6 and leptin. (a) ROC curve of IL-6 (AUC = 0.871, $P < 0.001$). (b) ROC curve of leptin (AUC = 0.813, $P < 0.001$). (c) ROC curve of IL-6&Leptin (AUC = 0.898, $P < 0.001$).

Table 5: Predictive characteristics of IL-6 and leptin.

Biomarkers	Cutoff	Specificity	Sensitivity	AUC	95% CI for AUC
IL-6	0.633	63.33	100.00	0.871	(0.774-0.937)
Leptin	0.567	90.00	66.67	0.813	(0.707-0.894)
IL-6&Leptin	0.750	95.00	80.00	0.898	(0.806-0.956)

IL-6 and leptin were found as a OF risk prediction model. All serum concentration of these biomarkers is shown in Figure 2. We could find that the serum IL-6 level of OF group was significantly higher than that in the non-OF group ($P < 0.001$), while the result of leptin was opposite ($P < 0.001$).

3.6. ROC Curve and AUC Analysis. The ROC curves showed an accurate discrimination performance for these two biomarkers. Herein, we showed the OF prediction accuracy of IL-6 (Figure 3(a)), leptin (Figure 3(b)), and IL-6&Leptin (Figure 3(c)). The AUCs for different types were shown in Figure 3 and Table 5. The AUC value of IL-6 (AUC = 0.871) was close to leptin (AUC = 0.813). And the OF predictive accuracy increased when they were applied together (AUC = 0.898).

Furthermore, the Delong's test was used to analyze the differences of AUCs between every biomarkers group. We found that the difference of AUC between leptin and IL-6&Leptin was meaningful ($P = 0.024$), which showed the OF prediction accuracy of IL-6&Leptin group was higher than leptin. However, the difference of AUC between IL-6

Table 6: The results of Delong's test.

Biomarkers group	Delong's test	P	95% CI
IL-6 vs. leptin	0.779	0.436	-0.0877 - 0.203
IL-6 vs. IL-6&Leptin	0.516	0.606	-0.0747 - 0.128
Leptin vs. IL-6&Leptin	2.260	0.024*	0.0112 - 0.158

*$P < 0.05$, **$P < 0.001$.

and leptin ($P = 0.436$) and IL-6 and IL-6&Leptin ($P = 0.606$) was all meaningless (Table 6).

4. Discussion

OF is the most serious complication of postmenopausal osteoporosis [24] and active prevention of it can be beneficial to prolong life expectancy and improve quality of life for the elderly [25]. Based on the characteristics of OF, early prevention is particularly more important than treatment [26]. In this study, based on the information and data from follow-up study and the results of Cox proportional hazards model, we focused on the predictive effect of IL-6 and leptin. These two biomarkers are associated with inflammatory response, oxidative stress, lipid metabolism, and bone tissue formation and destruction.

Inflammatory microenvironment mediated by the immune system in vivo is considered to be a major reason of abnormal bone metabolism. It may lead to osteoclast activation to accelerate bone loss [27, 28], increase the risk of fracture [29, 30], and slow down the healing rate of fracture [31, 32]. Many medicines are used to treat osteoporosis and repair the bone tissue by alleviating the expression of tissue inflammatory factors [33–35]. Meanwhile, inflammatory factors infiltration caused by many inflammatory diseases can also lead to abnormal bone metabolism, resulting in increased fracture probability, such as chronic pancreatitis [36, 37], chronic enteritis [38], hepatitis [39], and chronic obstructive pulmonary disease [40]. IL-6 is a common inflammatory factor whose effect on bone metabolism has been confirmed already [41]. A randomized controlled clinical trial led by Saribal et al. [42] included 40 patients with hip fractures due to osteoporosis and 40 age-matched nonosteoporotic healthy controls and found that the difference of IL-6 levels between this two group was statistically significant, which considered the relevance of IL-6 and OF. In 2014, based on a cohort with a total of 9704 Caucasian women, Barbour et al. [43] found that women in the highest quartile of IL-6 had a significantly higher risk of hip fractures compared to women in the lowest quartiles, which suggested that IL-6 can predict fractures of women. However, the association between IL-6 and OF still needs more evidence.

Leptin is a hormone with multiple functions that can act locally and systemically. It not only involved in lipid metabolism but also associated with inflammatory response [44] and oxidative stress [45]. In recent years, the relationship between leptin and bone metabolism has been confirmed gradually. Leptin stimulates the differentiation of stromal cells to osteoblasts [46], increases proliferation of osteoblasts [47], and inhibits osteoclastogenesis. Deficiency in leptin signaling, through knockout of the Leptin receptor gene, decreases bone volume and BMD [48], indicating the important role of leptin in bone homeostasis. However, there is no consensus on whether leptin can be used to predict OF. Based on a cohort of 1167 postmenopausal women and a 25-year follow-up interview, an epidemiological survey [49] conducted in Japan showed that leptin levels and postmenopausal women were significantly independent risk factors for long bone fractures and vertebral fractures. However, another research [50] found that there was no significant difference in serum leptin between osteoporosis patients and nonosteoporosis patients. In fact, the effect of leptin on bone metabolism is related to factors such as weight and obesity [51]. Some scholars believe that the high expression of leptin in obese patients (BMI > 28) may have a lower effect on bone metabolism than those with normal weight [52]. Therefore, when using leptin to predict fracture risk, it cannot be discussed separately from weight or obesity. In this study, we match subjects by same BMI classification, so it can be considered that the relationship between fracture and leptin is reliable.

In addition, IL-6 and leptin interact with each other too. Both these two biomarkers can be produced by adipose tissue [53] and mediated many pathological reaction together. Hoffmann et al. [54] found that leptin administration within the subphysiological to physiological range diminished circulating proinflammatory IL-6 in female mice and reduction of IL-6 gene expression in adipose tissue, as well as decreased adipose tissue macrophage infiltration might contribute. However, Wueest and Konrad [55] found that IL-6 could induce the release of leptin from adipocytes, which was contrary to the conclusion of the previous article. In fact, there are few studies on the relationship between IL-6, leptin, and OF, but it cannot be ignored.

Meanwhile, according to the results of Delong's test, IL-6 can improve the prediction accuracy of leptin ($P = 0.024$), but not vice versa. It can be considered that the prediction accuracy of IL-6&Leptin is not better than IL-6, and we should use IL-6 to predict OF separately due to economical. However, prediction accuracy of leptin is reliable (AUCs = 0.813), and these two biomarkers have different predictive directionalities to OF, which may have implications for accurate prediction of different populations in the future. Therefore, we preserve the role of leptin in the predictive model.

This study has the following advantages: based on the BEYOND cohort, all 15 patients of OF group suffered this disease after entry into the cohort and before follow-up study, ensuring the reliability of the results. Then, a Cox disease risk prediction method was used to construct a prediction model of OF risk with IL-6 and leptin as the main risk prediction indicators.

This study also has certain limitations. First, only Beijing community subjects were included in our study, which limited the extrapolation of the results of our study. In addition, only two-year follow-up interview had been developed, and the incidence of outcome indicators was low (15/612, 2.45% in 2 years). So as to solve the problem, we matched these 15 patients in a ratio of 1 : 4 with ages and BMI classification. Meanwhile, a latest prevalence study [56] in China found that the clinical fracture prevalence of women over 40 years old in 5 years is 4.3%, as well as the prevalence in 5 years of women in urban is

4.4%. Due to the similar participants and research design, we believe that the OF prevalence of our study is similar to the results of the latest prevalence study in China. Notwithstanding these limitations, the main progress of our study was the follow up interview for a relatively large sample size and established a fracture risk prediction model suitable for the clinical characteristics of Chinese postmenopausal women with low bone mass.

5. Conclusion

Overall, evidence based on current findings suggests that serum IL-6 and leptin can be the predictive factors of OF risk for postmenopausal women with low bone mass, but it needs to be proved by long-term follow-up studies with large sample. We will continue to improve the relevant programs and increase the sample size, so as to find higher quality evidences.

Abbreviations

OF:	Osteoporotic fracture
AUC:	Area under curve
PMOP:	Postmenopausal osteoporosis
BMI:	Body mass index
OP:	Osteoporosis
DEXA:	Dual-energy X-ray absorptiometry
BMD:	Bone mineral density
IGF-1:	Insulin-like growth factor 1
IL-6:	Interleukin-6
VEGF:	Vascular endothelial growth factor
25(OH)VitD$_3$:	25-Hydroxy-vitamin D3
OST:	Osteocalcin
PTH:	Parathyroid hormone
β-CTx:	β isomer of C-terminal telopeptide of type I collagen
ALP:	Alkaline phosphatase
P1NP:	Type I procollagen amino-terminal peptide.

Data Availability

All data reported in this study are available upon request by contact with the corresponding author.

Ethical Approval

The study was approved by the medical ethics committee of Wangjing Hospital, and all procedures were performed in accordance with the Declaration of Helsinki.

Disclosure

The funders had no role in writing this paper.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Xu Wei, Liguo Zhu, and Yanming Xie contributed to the study design. Xu Wang, Yili Zhang, and Baoyu Qi were responsible for the statistical analysis and the article writing. Kai Sun, Chuanrui Sun, Ning Liu, and Shengjie Fang made a contribution on study design, execution, and acquisition of data. All authors participated in interpretation of the data and critical review of the manuscript and approved of this manuscript to be submitted for publication. Xu Wang, Yili Zhang, and Baoyu Qi contributed equally to this work and share first authorship.

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References

[1] J. Blake, F. A. Cosman, E. M. Lewiecki, M. R. McClung, J. Pinkerton, and M. Shapiro, "Management of osteoporosis in postmenopausal women: the 2021 position statement of The North American Menopause Society," *Menopause*, vol. 28, no. 9, pp. 973–997, 2021.

[2] J. E. Compston, M. R. McClung, and W. D. Leslie, "Osteoporosis," *Lancet*, vol. 393, no. 10169, pp. 364–376, 2019.

[3] X. Cheng, K. Zhao, X. Zha et al., "Opportunistic screening using low-dose CT and the prevalence of osteoporosis in China: a nationwide, multicenter study," *Journal of Bone and Mineral Research*, vol. 36, no. 3, pp. 427–435, 2021.

[4] Y. A. Li, C. L. Lin, M. C. Chang, C. L. Liu, T. H. Chen, and S. C. Lai, "Subsequent vertebral fracture after vertebroplasty," *Spine (Phila Pa 1976)*, vol. 37, no. 3, pp. 179–183, 2012.

[5] J. A. Kanis, "Diagnosis of osteoporosis and assessment of fracture risk," *Lancet*, vol. 359, no. 9321, pp. 1929–1936, 2002.

[6] "Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. Report of a WHO study group," *World Health Organization Technical Report Series*, vol. 843, pp. l–129, 1994.

[7] J. A. Kanis, H. Johansson, N. C. Harvey et al., "The effect on subsequent fracture risk of age, sex, and prior fracture site by recency of prior fracture," *Osteoporosis International*, vol. 32, no. 8, pp. 1547–1555, 2021.

[8] M. Nilsson, J. Eriksson, B. Larsson, A. Odén, H. Johansson, and M. Lorentzon, "Fall risk assessment predicts fall-related injury, hip fracture, and head injury in older adults," *Journal of the American Geriatrics Society*, vol. 64, no. 11, pp. 2242–2250, 2016.

[9] R. Furrer, N. M. van Schoor, A. de Haan, P. Lips, and R. T. de Jongh, "Gender-specific associations between physical functioning, bone quality, and fracture risk in older people," *Calcified Tissue International*, vol. 94, no. 5, pp. 522–530, 2014.

[10] S. Rozenberg, O. Bruyère, P. Bergmann et al., "How to manage osteoporosis before the age of 50," *Maturitas*, vol. 138, pp. 14–25, 2020.

[11] L. Zhang, Q. Liu, X. Zeng et al., "Association of dyslipidaemia with osteoporosis in postmenopausal women," *The Journal of International Medical Research*, vol. 49, no. 3, p. 300060521999555, 2021.

[12] N. Almutlaq, A. Neyman, and L. A. DiMeglio, "Are diabetes microvascular complications risk factors for fragility fracture?," *Current Opinion in Endocrinology, Diabetes, and Obesity*, vol. 28, no. 4, pp. 354–359, 2021.

[13] D. M. Black and C. J. Rosen, "Postmenopausal osteoporosis," *New England Journal of Medicine*, vol. 374, no. 3, pp. 254–262, 2016.

[14] R. Bijelic, S. Milicevic, and J. Balaban, "Risk factors for osteoporosis in postmenopausal women," *Medicinski Arhiv*, vol. 71, no. 1, pp. 25–28, 2017.

[15] J. S. Kimball, J. P. Johnson, and D. A. Carlson, "Oxidative stress and osteoporosis," *The Journal of Bone and Joint Surgery. American Volume*, vol. 103, no. 15, pp. 1451–1461, 2021.

[16] W. Ding, C. Xu, Y. Zhang, and H. Chen, "Advances in the understanding of the role of type-H vessels in the pathogenesis of osteoporosis," *Archives of Osteoporosis*, vol. 15, no. 1, p. 5, 2020, Published 2020 Jan 2.

[17] Y. Zhang, X. Huang, K. Sun et al., "The potential role of serum IGF-1 and leptin as biomarkers: towards screening for and diagnosing postmenopausal osteoporosis," *Journal of Inflammation Research*, vol. Volume 15, pp. 533–543, 2022.

[18] T. Wang and C. He, "TNF-α and IL-6: the link between immune and bone system," *Current Drug Targets*, vol. 21, no. 3, pp. 213–227, 2020.

[19] E. C. Watson and R. H. Adams, "Biology of bone: the vasculature of the skeletal system," *Cold Spring Harbor Perspectives in Medicine*, vol. 8, no. 7, p. a031559, 2018.

[20] M. Sun, Y. Zhang, H. Shen et al., "Prevalence of and risk factors for community-based osteoporosis and associated fractures in Beijing: study protocol for a cross-sectional and prospective study," *Front Med (Lausanne).*, vol. 7, no. 7, article 544697, 2020.

[21] E. S. Siris, R. Adler, J. Bilezikian et al., "The clinical diagnosis of osteoporosis: a position statement from the National Bone Health Alliance Working Group," *Osteoporosis International*, vol. 25, no. 5, pp. 1439–1443, 2014.

[22] W. B. Xia, Z. L. Zhang, H. Lin, X. L. Jin, W. Yu, and Q. Fu, "Guidelines for diagnosis and treatment of primary osteoporosis (2017)," *Chinese journal of osteoporosis and bone mineral research*, vol. 33, no. 9, pp. 413–444, 2017.

[23] S. Greenland, "Modeling and variable selection in epidemiologic analysis," *American Journal of Public Health*, vol. 79, no. 3, pp. 340–349, 1989.

[24] M. A. Clynes, N. C. Harvey, E. M. Curtis, N. R. Fuggle, E. M. Dennison, and C. Cooper, "The epidemiology of osteoporosis," *British Medical Bulletin*, vol. 133, no. 1, pp. 105–117, 2020.

[25] M. Viswanathan, S. Reddy, N. Berkman et al., "Screening to prevent osteoporotic fractures," *Journal of the American Medical Association*, vol. 319, no. 24, pp. 2532–2551, 2018.

[26] R. Eastell, C. J. Rosen, D. M. Black, A. M. Cheung, M. H. Murad, and D. Shoback, "Pharmacological management of osteoporosis in postmenopausal women: an endocrine society∗ clinical practice guideline," *The Journal of Clinical Endocrinology and Metabolism*, vol. 104, no. 5, pp. 1595–1622, 2019.

[27] K. Yokota, K. Sato, T. Miyazaki et al., "Combination of tumor necrosis factor α and interleukin-6 induces mouse osteoclast-like cells with bone resorption activity both in vitro and in vivo," *Arthritis & Rhematology*, vol. 66, no. 1, pp. 121–129, 2014.

[28] B. Zhao, S. N. Grimes, S. Li, X. Hu, and L. B. Ivashkiv, "TNF-induced osteoclastogenesis and inflammatory bone resorption are inhibited by transcription factor RBP-J," *The Journal of Experimental Medicine*, vol. 209, no. 2, pp. 319–334, 2012.

[29] V. Fischer and M. Haffner-Luntzer, "Interaction between bone and immune cells: Implications for postmenopausal osteoporosis," *Seminars in Cell & Developmental Biology*, vol. 123, pp. 14–21, 2020.

[30] D. Wu, A. Cline-Smith, E. Shashkova, A. Perla, A. Katyal, and R. Aurora, "T-cell mediated inflammation in postmenopausal osteoporosis," *Frontiers in Immunology*, vol. 12, 2021.

[31] W. Zhou, Z. Lin, Y. Xiong et al., "Dual-targeted nanoplatform regulating the bone immune microenvironment enhances fracture healing," *ACS Applied Materials & Interfaces*, vol. 13, no. 48, pp. 56944–56960, 2021.

[32] S. K. Feng, T. H. Chen, H. M. Li et al., "Deficiency of omentin-1 leads to delayed fracture healing through excessive inflammation and reduced $CD31^{hi}Emcn^{hi}$ vessels," *Molecular and Cellular Endocrinology*, vol. 534, no. 534, article 111373, 2021.

[33] J. Wang, M. He, G. Wang, and Q. Fu, "Organic gallium treatment improves osteoporotic fracture healing through affecting the OPG/RANKL ratio and expression of serum inflammatory cytokines in ovariectomized rats," *Biological Trace Element Research*, vol. 183, no. 2, pp. 270–279, 2018.

[34] V. Vijayan, M. Khandelwal, K. Manglani, S. Gupta, and A. Surolia, "Methionine down-regulates TLR4/MyD88/NF-κB signalling in osteoclast precursors to reduce bone loss during osteoporosis," *British Journal of Pharmacology*, vol. 171, no. 1, pp. 107–121, 2014, Erratum in: Br J Pharmacol. 2014 Jul; 178 (13): 2747-2748.

[35] Y. J. Jo, H. I. Lee, N. Kim et al., "Cinchonine inhibits osteoclast differentiation by regulating TAK1 and AKT, and promotes osteogenesis," *Journal of Cellular Physiology*, vol. 236, no. 3, pp. 1854–1865, 2021.

[36] M. Vujasinovic, L. Nezirevic Dobrijevic, E. Asplund et al., "Low bone mineral density and risk for osteoporotic fractures in patients with chronic pancreatitis," *Nutrients*, vol. 13, no. 7, p. 2386, 2021.

[37] S. N. Duggan, C. Purcell, M. Kilbane et al., "An association between abnormal bone turnover, systemic inflammation, and osteoporosis in patients with chronic pancreatitis: a case-matched study," *The American Journal of Gastroenterology*, vol. 110, no. 2, pp. 336–345, 2015.

[38] J. Bartko, B. Reichardt, R. Kocijan, K. Klaushofer, J. Zwerina, and M. Behanova, "Inflammatory bowel disease: a nationwide study of hip fracture and mortality risk after hip fracture," *Journal of Crohn's & Colitis*, vol. 14, no. 9, pp. 1256–1263, 2020.

[39] E. Biver, A. Calmy, and R. Rizzoli, "Bone health in HIV and hepatitis B or C infections," *Ther Adv Musculoskelet Dis*, vol. 9, no. 1, pp. 22–34, 2017.

[40] M. Blaschke, R. Koepp, J. Cortis et al., "IL-6, IL-1β, and TNF-α only in combination influence the osteoporotic phenotype in Crohn's patients via bone formation and bone resorption," *Advances in Clinical and Experimental Medicine*, vol. 27, no. 1, pp. 45–56, 2018.

[41] C. Scheidt-Nave, H. Bismar, G. Leidig-Bruckner et al., "Serum interleukin 6 is a major predictor of bone loss in women specific to the first decade past menopause," *The Journal of Clinical Endocrinology and Metabolism*, vol. 86, no. 5, pp. 2032–2042, 2001.

[42] D. Saribal, F. S. Hocaoglu-Emre, S. Erdogan, N. Bahtiyar, S. Caglar Okur, and M. Mert, "Inflammatory cytokines IL-6 and TNF-α in patients with hip fracture," *Osteoporosis International*, vol. 30, no. 5, pp. 1025–1031, 2019.

[43] K. E. Barbour, L. Y. Lui, K. E. Ensrud et al., "Inflammatory markers and risk of hip fracture in older white women: the study of osteoporotic fractures," *Journal of Bone and Mineral Research*, vol. 29, no. 9, pp. 2057–2064, 2014.

[44] A. La Cava, "Leptin in inflammation and autoimmunity," *Cytokine*, vol. 98, pp. 51–58, 2017.

[45] V. Tisato, A. Romani, E. Tavanti et al., "Crosstalk between adipokines and paraoxonase 1: a new potential axis linking oxidative stress and inflammation," *Antioxidants (Basel).*, vol. 8, no. 8, p. 287, 2019.

[46] B. Zhang, L. Yang, Z. Zeng et al., "Leptin potentiates BMP9-induced osteogenic differentiation of mesenchymal stem cells through the activation of JAK/STAT signaling," *Stem Cells and Development*, vol. 29, no. 8, pp. 498–510, 2020.

[47] W. R. Holloway, F. M. Collier, C. J. Aitken et al., "Leptin inhibits osteoclast generation," *Journal of Bone and Mineral Research*, vol. 17, no. 2, pp. 200–209, 2002.

[48] D. Bao, Y. Ma, X. Zhang et al., "Preliminary characterization of a leptin receptor knockout rat created by CRISPR/Cas9 system," *Scientific Reports*, vol. 5, no. 1, p. 15942, 2015.

[49] Y. Nakamura, M. Nakano, T. Suzuki et al., "Two adipocytokines, leptin and adiponectin, independently predict osteoporotic fracture risk at different bone sites in postmenopausal women," *Bone*, vol. 137, article 115404, 2020.

[50] J. Mohiti-Ardekani, H. Soleymani-Salehabadi, M. B. Owlia, and A. Mohiti, "Relationships between serum adipocyte hormones (adiponectin, leptin, resistin), bone mineral density and bone metabolic markers in osteoporosis patients," *Journal of Bone and Mineral Metabolism*, vol. 32, no. 4, pp. 400–404, 2014.

[51] V. Mpalaris, P. Anagnostis, A. D. Anastasilakis, D. G. Goulis, A. Doumas, and I. Iakovou, "Serum leptin, adiponectin and ghrelin concentrations in post-menopausal women: is there an association with bone mineral density?," *Maturitas*, vol. 88, pp. 32–36, 2016.

[52] K. Gkastaris, D. G. Goulis, M. Potoupnis, A. D. Anastasilakis, and G. Kapetanos, "Obesity, osteoporosis and bone metabolism," *Journal of Musculoskeletal & Neuronal Interactions*, vol. 20, no. 3, pp. 372–381, 2020.

[53] S. W. Coppack, "Pro-inflammatory cytokines and adipose tissue," *The Proceedings of the Nutrition Society*, vol. 60, no. 3, pp. 349–356, 2001.

[54] A. Hoffmann, T. Ebert, N. Klöting et al., "Leptin decreases circulating inflammatory IL-6 and MCP-1 in mice," *BioFactors*, vol. 45, no. 1, pp. 43–48, 2019.

[55] S. Wueest and D. Konrad, "The role of adipocyte-specific IL-6-type cytokine signaling in FFA and leptin release," *Adipocytes*, vol. 7, no. 3, pp. 226–228, 2018.

[56] L. Wang, W. Yu, X. Yin et al., "Prevalence of osteoporosis and fracture in China: the China osteoporosis prevalence study," *JAMA Network Open*, vol. 4, no. 8, article e2121106, 2021.

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Review Article

Plin5 Bidirectionally Regulates Lipid Metabolism in Oxidative Tissues

Xinqing Zhang,[1] Wu Xu,[2] Rui Xu,[2] Zhen Wang,[3] Xinyan Zhang,[4] Peng Wang,[3] Ke Peng,[3] Meiling Li,[3] Jing Li,[3] Yanfei Tan,[5] Xiong Wang,[3] and Haifeng Pei[1,3]

[1]*College of Medicine, Southwest Jiaotong University, Chengdu 610031, China*
[2]*School of Medicine and Life Sciences/Reproductive and Women-Children Hospital, Chengdu University of Traditional Chinese Medicine, Chengdu 611137, China*
[3]*Department of Cardiology, The General Hospital of Western Theater Command, Chengdu 610083, China*
[4]*Department of Medical Information Service, The General Hospital of Western Theater Command, Chengdu 610083, China*
[5]*National Engineering Rsearch Center for Biomaterials, Sichuan University, Chengdu 610065, China*

Correspondence should be addressed to Yanfei Tan; tanyf@scu.edu.cn, Xiong Wang; wangxiong5210@foxmail.com, and Haifeng Pei; web2010@foxmail.com

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Cytoplasmic lipid droplets (LDs) can store neutral lipids as an energy source when needed and also regulate the key metabolic processes of intracellular lipid accumulation, which is associated with several metabolic diseases. The perilipins (Plins) are a family of proteins that associate with the surface of LDs. As a member of Plins superfamily, perilipin 5 (Plin5) coats LDs in cardiomyocytes, which is significantly related to reactive oxygen species (ROS) production originated from mitochondria in the heart, consequently determining the progression of diabetic cardiomyopathy. Plin5 may play a bidirectional function in lipid metabolism which is in a state of dynamic balance. In the basic state, Plin5 inhibited the binding of comparative gene identification-58 (CGI-58) to adipose triglyceride lipase (ATGL) by binding CGI-58, thus inhibiting lipolysis. However, when the body is under stress (such as cold, fasting, exercise, and other stimuli), protein kinase A (PKA) phosphorylates and activates Plin5, which then causes Plin5 to release the binding site of CGI-58 and ATGL, prompting CGI-58 to bind to ATGL and activate ATGL activity, thus accelerating the lipolysis process, revealing the indispensable role of Plin5 in lipid turnover. Here, the purpose of this review is to summarize the present understanding of the bidirectional regulation role of Plin5 in oxidative tissues and to reveal its potential role in diabetic cardiomyopathy protection.

1. Introduction

Obesity, diabetes mellitus, dyslipidemia, and hypertension often cluster together as the most significant risk factors for cardiovascular diseases [1]. Excess accumulation of intracellular lipid is believed to cause several metabolic diseases like obesity cardiomyopathy, inducing irreversible damage to cardiovascular systems [2, 3]. The rising trend in the lipid-associated metabolic diseases has drawn people's attention to the pathobiological functions of cytosolic LDs which are subcellular structures to store neutral lipid [4, 5]. Medical treatment to prevent against excess accumulation of intracellular lipid alleviated the adverse development of cardiovascular diseases [6], which appeared to be controlled by LDs-associated proteins. Cytosolic LDs are comprised of a core of neutral lipids, triacylglycerols (TAG), and/or cholesteryl ester (CE) and surrounded by a phospholipid monolayer [7]. The contrasting chemical natures of hydrophilic lipid metabolic enzymes and their hydrophobic substrates have directed people's attention to LDs surface which was viewed as regulatory interface between the aqueous cytosol and the hydrophobic lipid core [8]. Thus, LDs may have essential protective roles in the sequestration of cytotoxic fatty acids (FAs) in nonadipose tissues. Specifically, perilipin

proteins (Plins) are the definitive abundant proteomic markers of LDs surfaces in both adipose and non-adipose cells and function as primary mediators for neutral lipid storage/hydrolysis [9]. LDs are coated by several proteins, including Plins and other structural proteins, membrane-trafficking proteins, lipases, and lipogenic enzymes [10]. Especially, Plins are known as representation of LDs and necessarily associated with LDs formation [11]. The mammalian genome has encoded five Plins genes, with unique tissue-dependent patterns of transcription and splice variation [12]. Perilipin 1 (Plin1) is abundant in white adipose tissue (WAT) and brown adipose tissue (BAT). Perilipin 2 (Plin2) and perilipin 3 (Plin3) are widely distributed, with Plin2 highly expressing in hepatocytes. Perilipin 4 (Plin4) is observed in adipocytes, cardiomyocytes, and myocytes. However, Plin5 is highly expressed in oxidative tissues and generally restricted to tissues/cells that utilize lipids for energy through mitochondrial β-oxidation. [13, 14]. Overall, Plins take important roles in the formation and degradation of LDs.

As a member of the perilipin superfamily, Plin5 is central to lipid homeostasis in these tissues by promoting association of LDs with mitochondria [15]. Besides the expression on surface of LDs, Plin5 also appears in nucleus, cytoplasm, and mitochondria. Plin5 is closely related to the generation of ROS originated from mitochondria, consequently determining the progression of oxidative stress [16]. Recently, the data from *in vivo* and *in vitro* indicate an important role of Plin5 in the regulation of cardiac lipid storage and function. Previous studies have suggested that lipolysis was mainly due to PKA -mediated phosphorylation of hormone-sensitive lipase (HSL), but recent studies have shown that it is mainly regulated by the translocation of lipase from the cytosol to the surface of LDs. And Plin5 phosphorylation is necessary for HSL translocation and has become a hot topic in recent years due to the important role of Plins in lipolysis. Under basal conditions in cardiac tissues, Plin5 coats on the surface of LDs as a physical barrier to inhibit enzymatic activity, inhibiting the binding of CGI-58 to ATGL by binding CGI-58, thus inhibiting lipolysis, as well as a guarder to prevent excessive β-oxidation of free fatty acids. When phosphorylated in lipolysis following activation of PKA under β-adrenoceptor-stimulated condition or when energy demand is increased, Plin5 serves as a platform to facilitate lipolysis on LDs surface by promoting the interaction of ATGL with CGI-58, thus accelerating the lipolysis process [17]. Moreover, besides the promotion of β-oxidation, phosphorylated Plin5 can migrate to nucleus to promote peroxisome proliferator-activated receptor gamma coactivator 1-α (PGC-1α) function by disinhibiting sirtuin 1 (SIRT1) deacetylase activity, enhancing transcription of mitochondrial function, and reinforcing fatty acid metabolism. Therefore, Plin5 may serve as a bidirectional regulator for lipid turn over by phosphorylation modification and content variation in cardiomyocytes and act as a potential molecular target for the treatment of dyslipidemia in diabetic cardiomyopathy. However, in skeletal muscle, the phosphorylation level of Plin5 remains unchanged when facing with either contractile or adrenergic stimulation, revealing that Plin5 may share different functions in different tissues (Figure 1). The focus of this review was to summarize the reported roles of Plin5 in oxidative tissues.

2. Special Function of Plin5 in Oxidative Tissues

2.1. Cardiac Tissues. In contrast to other perilipins, Plin5 has unique ability to store lipid droplet in O_2 abundant organelles. Studies have shown that free fatty acid (FFA) can be used as ligand to activate peroxisome proliferator-activated receptors (PPARs) and induce the expression of Plin5. For example, exogenous FFA can stimulate the expression of Plin5 in cultured rat cardiomyocytes [18]. Conversely, Plin5 can also affect the metabolic process of FFA. It is found that the FFA uptake decreased and glucose uptake increased after the knockout of Plin5 in myocardium, which thus maintained the normal energy requirement of the heart [19], and that the expression of FFA uptake-related enzymes such as lipoprotein lipase (LPL) and CD36 was decreased in myocardial overexpressing Plin5 [20]. This result suggested that the increase in TAG during Plin5 overexpression is not likely due to increased FFA uptake. Besides, Plin5 could regulate the β-oxidation of FFA by acting on the spatial action of mitochondria and affecting FFA-related enzymes. Research found that the expression of mitochondrial oxidation-related genes and Plin5 were negatively correlated in myocardium [20, 21]. And Plin5 ablation could enhance the oxidation of FFA in the myocardium [22]. Further research found that the mRNA expression of mitochondrial cytochrome C and cytochrome C oxidase subunit IV (COX IV) in myocardium of $Plin5^{-/-}$ mice increased significantly compared with WT mice, indicating that mitochondrial function was enhanced [19]. On the other hand, overexpression of Plin5 decreased the expression of PPARs target gene in myocardium [20], and the gene expressions of mitochondrial energy metabolism, oxidative phosphorylation, and utilization of FFA were all downregulated, which had been reported as PPAR target genes [23]. In another report, myocardial overexpression of Plin5 reduced the expression and activity of carnitine palmitoyltransferase 1 (CPT-1), a key enzyme that regulates FFA entry into mitochondria, showing that mitochondrial uptake of FFA was decreased [21]. In summary, recent studies have shown that Plin5 deletion could increase myocardial FFA absorption and enhance mitochondrial oxidation of FFA; in contrast, overexpression of Plin5 could limit mitochondrial oxidation of FFA.

In cardiomyocytes, mitochondria comprise more than 30% of cell volume and generate about 90% of the ATP [24]. Accordingly, mitochondria are also the major source of ROS in the cardiovascular diseases [24, 25]. ROS is known to be implicated in the induction of cardiac hypertrophy in various pathologic states. Overexpression of Plin5 in cardiomyocytes can increase intracellular ROS levels and content of malonaldehyde (MDA) [21]. Meanwhile, overexpression of Plin5 triggers an upregulation of the NF-E2-related factor 2 (Nrf2) antioxidative pathway with specific increases in gene expression involved in glutathione metabolism [21]. Under the normal condition, Plin5 knockout had no significant effect on the

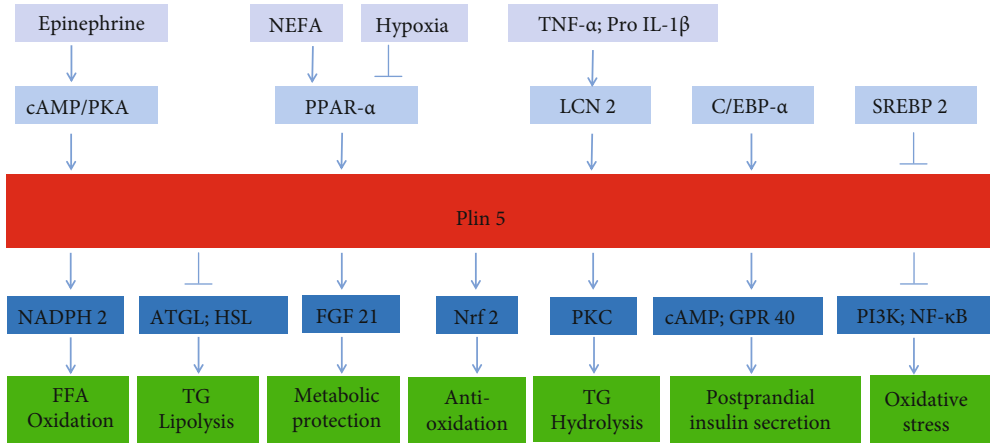

Figure 1: The upstream and downstream regulating factors for plin5.

contents of ROS, but in the ischemia-reperfusion injury, Plin5 knockout led to the increase of ROS [26]. Cultured cardiomyocytes from Plin5$^{-/-}$ mice had more actively oxidized FFAs and ROS production than those of WT mice, which was, however, reduced by the administration of an antioxidant N-acetylcysteine [22]. Other studies have found that Plin5 deficiency in hypoxic cardiomyocytes exposed to LDL dramatically increases the levels of unpacked FFA and ROS [27]. It was hypothesized that cardiac ROS production in Plin5$^{-/-}$ mice might result from a surplus flux of FA into the mitochondria. In a study involving type I diabetes, the results were just the opposite: compared with WT, Plin5$^{-/-}$ mice did not exhibit excessive ROS generation or cardiac dysfunction but had an improvement in heart function although LDs decomposition increased [27]. The authors have found that diabetic Plin5$^{-/-}$ mice are resistant to type 1 diabetes-induced heart malfunction due to the suppression of the diacylglycerol/ceramide-PKC pathway and of excessive ROS generation by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase [28]. These seemingly contradictory findings may be due to the specificity of the research models.

We find that excessive LDs are stored in nonfat tissues, such as the liver, pancreas, and coronary arteries, which are often associated with fatty liver, T2DM, and coronary atherosclerotic heart disease [29, 30]. Studies have shown that the increase of intracellular LDs can activate oxidative stress [31]. However, most of the data show that TGs mainly have storage function, and the main toxicity is caused by FFA and its metabolites, such as ceramide and diacylglycerol [32]. Therefore, LDs may improve cytotoxicity more than as a causative agent. In heart, LDs can isolate FFA in the form of TAG to prevent lipid damage induced by FFA and its derivatives [33]. Research shows that Plin5 plays an important role in the regulation of lipid metabolism by LDs in cardiomyocytes. In addition, it has been reported that Plin5 knockout has almost no effect on cardiac function under the basal state [19, 26, 28]. However, other studies have shown that Plin5$^{-/-}$ may aggravate age-related cardiac dysfunction, and this heart defect can be prevented by antioxidant therapy [22]. Meanwhile, in Plin5 overexpression mice, myocardial steatosis, increased heart weight, left ventricular hypertrophy, and mild cardiac function were observed [20, 21]. In the mouse model of myocardial ischemia reperfusion injury, Plin5 deficiency aggravates the heart dysfunction [34], and similar results also were found in another myocardial ischemia model [19]. In a study of Plin5 gene in patients with clinical myocardial infarction, Plin5 gene mutation is related to cardiac dysfunction after myocardial infarction [19]. In a word, these studies suggest that Plin5 plays an important role in maintaining normal cardiac function under normal physiological conditions or pathophysiological conditions.

In summary, the studies on various gene models of Plin5 indicate that Plin5 is required for normal cardiac metabolism and function, but too much Plin5 may lead to cardiomyopathy [17]. And both ablation and overexpression of Plin5 have given rise to harmful results; its concrete function may depend on the progression of cardiac diseases and different damage factors. Therefore, we need to further validate the role of Plin5 in the heart on human diseases.

2.2. Hepatic Tissues. Hepatocytes are parenchymal cells of the liver responsible for mobilizing lipids for energy and storing excess lipids in the form of LDs. Excessive accumulation of LDs is the cause of hepatic steatosis; meanwhile, accumulative evidence has suggested that LDs proteins were involved in the pathophysiology of liver diseases characterized by excessive lipid accumulation in hepatocytes, such as alcoholic liver disease, nonalcoholic fatty liver disease (NAFLD), and hepatitis C virus infection [11]. Plin5 is closely related to lipid metabolism, since the abnormal lipid metabolism plays an important role in the liver cell lesions. Previous work has indicated that there was an increase of Plin5 expression in mouse models of NAFLD [35]. Another study shows that cells overexpressing Plin5 release lower amounts of FFAs in basal conditions [36]. All those indicate that Plin5 may contribute to the formation of liver steatosis possibly through inhibition of the release of FFAs from LDs [37]. In Plin5 deficient mice, TG content was found to decrease both in primary mouse hepatocytes and in the liver, implying the involvement of Plin5 in TG accumulation [38]. The above data suggest that Plin5 can prevent the FFA and its metabolites over accumulation, thus preventing liver toxicity damage, but in other Plin5$^{-/-}$ mice did not show up liver injury [39]. Therefore, Plin5 is likely to be critically involved

in the development of pathologies associated with fatty liver. However, whether it is in agreement with the situation in human should be further explored.

2.3. Muscle Tissues. Skeletal muscle stores significant amounts of TG within intracellular LDs, which are near the mitochondria and endoplasmic reticulum [40]. Plin5 is highly expressed in skeletal muscle [41] and plays a critical role in coordinating skeletal muscle TG metabolism and LDs accumulation [13], which impacts sphingolipid metabolism, and is requisite for the maintenance of skeletal muscle insulin action [42, 43]. At present, there are many studies to explore the role of Plin5 in human muscle tissue. Although Plin5 expression is increased in the muscle and liver of mice fed on high-fat diet (HFD) [35, 44, 45], there is no change of Plin5 expression in skeletal muscle of humans fed on HFD for 12 weeks [46], and most studies report normal Plin5 protein content in obese humans [47, 48]. Consistent with these observations, the expression or content of Plin5 in skeletal muscle does not differ between T2DM patients and normal glycemic individuals [48]. In rat muscle, Plin5 content is correlated with mitochondrial respiration rates on a lipid-derived substrate [49]. The overexpression of Plin5 in skeletal muscle promoted expression of a cluster of genes under control of PPAR-α and PGC-1α involved in FFAs catabolism and mitochondrial oxidation [50], suggesting the role of Plin5 in mediating the skeletal muscle oxidative gene expression, either directly or indirectly.

2.4. Adipose Tissues. There are two kinds of adipose tissue in the human body, including WAT and BAT. WAT acts as an energy depot by storing lipids that are released into circulation when required. In contrast, BAT uses its stored fat to generate heat by oxidation of FFAs to maintain the body temperature [51]. In published studies, Plin5 is highly expressed in BAT but barely detectable in WAT [13]. The changes in LDs-protein gene expression included Plin1-5, suggesting that LDs experience a different adaptation to cold exposure in WAT and BAT cells. Prolonged cold exposure, which also induces the appearance of brown-like adipocytes in mice WAT depot, was accompanied with the enhancement in Plin5 expression [52]. However, arguing against an important role of Plin5 in BAT thermogenesis is the finding that Plin5$^{-/-}$ and WT littermates have similar tolerance to the cold [22]. In the research of Tansey et al.., it consumed equal amounts of food, but the adipose tissue mass in the null animals was reduced to approximately 30% of that in WT. Meanwhile, the Plin5$^{-/-}$ adipocyte showed higher basal lipolysis rate, thus proving that the perilipin knockout reduced the protective effect on LDs [53]. The biochemical pathways involved in obesity resistance in Plin5$^{-/-}$ mice may provide a potential direction for obesity treatment.

3. The Concrete Role of Plin5 in Mitochondria

3.1. Pathophysiological Role of Plin5 in Mitochondria. Lipid droplet and mitochondria are important organelles involved in lipid metabolism and energy homeostasis. Cumulative research has shown that physical contact between the two organelles is important for their function, and Plin5 has been found to mediate this contact [54]. Phosphorylated Plin5 can migrate to nucleus to promote PGC-1α co-activator function by disinhibiting SIRT1 deacetylase activity, enhancing transcription of mitochondrial function, and reinforcing fatty acid metabolism [55]. Furthermore, it is suggested that Plin5 can recruit mitochondria to lipid droplets through a C-terminal region in a variety of cell and tissue types, including Chinese hamster ovary cells, AML12, HL-1 cells, primary brown fat cells, INS1 cells, and mouse heart cells [56], and Plin5 may mediate the interaction between lipid droplets and mitochondrial oxidative tissue [42]. The contact site between mitochondria and lipid droplet usually called peridroplet mitochondria (PDM) is an important location for mitochondria to regulate lipid droplet storage and oxidation, and it can promote expansion of lipid droplets [57]. Plin5 increased the number of PDM by recruiting mitochondria to lipid droplets, which indirectly proved that Plin5 could promote the expansion of lipid droplets through PDM. To sum up, Plin5 can enhance mitochondrial function by increasing the role of PGC-1α. Moreover, Plin5 can promote the structural connection between lipid droplets and mitochondria, promoting the expansion of lipid droplets.

3.2. The Relationship of Plin5 and Mitophagy. Autophagy is a process for degradation of long-lived or injured organelles and proteins which involves vacuolar isolation of intracellular components and their targeted lysosomal degradation, promoting cellular responses to stress conditions including starvation and pathological stresses such as oxidative stress [58, 59]. There is growing evidence that autophagy, in addition to being a massive nonselective degradation mechanism, can selectively remove damaged mitochondria in order to promote mitochondrial turnover, a process known as "mitophagy" [60, 61]. The term "mitophagy" was first coined in 2005, and it can be divided into three types: type 1 usually involves phosphatidylinositide 3-kinases (PI3K) and is closely related to mitochondrial division. Under starvation conditions, preautophagic vesicles form cup-shaped phagocytic vesicles and then wrap mitochondria, resulting in depolarization of mitochondrial outer membrane and hydrolysis of autophagic vesicles in lysosomes. Type 2, in which damaged mitochondria bind to autophagosomes containing microtubule-associated protein 1 light chain 3(LC3), depolarization occurs without the involvement of PI3K and has nothing to do with mitochondrial division. Type 3 is also called micromitochondrial autophagy; oxidized mitochondrial protein forms mitochondria-derived vesicles (MDVs) through budding, and the vesicles are gradually fused into poly-vesicles, which are hydrolyzed by lysosomes into mitochondrial fragments [62, 63]. Mitophagy is involved in the occurrence and development of many diseases. Serine/threonine kinases, PTEN-induced putative kinase 1(PINK1), and E3 ubiquitin ligases, Parkin, are the most classical mechanisms in the study of mitophagy; PINK1 and Parkin mutations can lead to the accumulation of damaged mitochondria, which further promotes the occurrence of neuronal degeneration and ultimately leads

to Parkinson's disease [64]. Expression of Parkin is also lost in many types of cancer, and overexpression of Parkin in breast and glioma cells inhibits cellular proliferation. Similar to Parkin, overexpression of PINK1 is thought to attenuate the growth of glioblastoma [65]. Saito et al. showed that Rab9 gene-mediated mitophagy maintained mitochondrial homeostasis through the Ulk1/Rab9/Rip1/Drp1 pathway in ischemic environment. However, knockout of Rab9 gene can inhibit mitophagy and aggravate myocardial injury caused by ischemia [66]. Mitophagy is involved in the occurrence of many diseases, but its mechanism is still unclear and needs further exploration. In a murine model under both fasting and refeeding conditions, Plin5 was required for the induction of autophagy during fasting, which contributed to its anti-inflammatory effects. The ability of Plin5 to promote autophagy and prevent inflammation was dependent upon signaling through SIRT1, which is known to be activated in response to nuclear Plin5 under fasting conditions [67]. Plin5 is a chaperone-mediated autophagy (CMA) substrate; its degradation through CMA is required for LD breakdown. The reduced activity of CMA failed to degrade Plin5 and other perilipin proteins (which are substrates of CMA), inhibited LD breakdown, and caused steatosis in hepatocytes [68]. What is more, mitochondrial autophagy can balance lipid generation by regulating lipid biosynthesis and decomposition to prevent the development of fatty liver, so effective activation of mitochondrial autophagy can be developed to treat fatty liver disease [69].

4. Upstream Factors Regulating Plin5

Previous studies have shown that many factors can influence the expression of Plin5 in the heart, liver, and skeletal muscle (Table 1). First, many molecules play crucial roles in Plin5 expression, such as peroxisome proliferator-activated receptor (PPAR)-α/δ [14], C/EBP-α [14], Curcumin, PGC-1α [42], lipocalin-2 (LCN2) [70], SREBP2 [71], miR-370, and ROS. PPAR-α agonist is able to increase the expression of Plin5 [16]. WY-14643, a PPAR-α agonist, can restore Plin5 expression in hypoxic cardiomyocytes [27]. C/EBP-α promotes the transcription of Plin5 gene in porcine [72]. Recent findings show that palmitate and PPAR agonists induced Plin5 expression in INS-1 cells in vitro [73]. Curcumin increases Plin5 gene expression to recover LD formation and lipid accumulation in activated hepatic stellate cells [74]. Basal Plin5 expression was significantly reduced in $LCN2^{-/-}$ hepatocytes. Plin4 ablation also reduces Plin5 expression in mice, leading to decreased cardiac TG accumulation [75]. Low-density lipoprotein (LDL) strongly increases Plin5 expression in cardiomyocytes. Nonesterified fatty acids (NEFAs) are strong inducers of Plin5 transcription through PPAR activation [44]. Estrogen receptor-associated receptor (ERR)-α increases Plin5 expression by interacting with PPAR. Sulforaphane (SFN) decreases LD-associated protein Plin 2 and Plin5 expression that may be achieved by downregulating PPARγ [76]. Oleic acid (OA) can induce Plin5 expression in HepG2 cells and in a dose- and time-dependent manner [77]. Overexpression of leptin in transgenic mice decreased Plin5 expression [78]. TNF-α decreased Plin5 expression and promoted lipolysis in the basal state. However, SREBP2 can inhibit the expression of Plin5 [71]. MiR-370 can downregulate Plin5, which in turn resulted in an increase in PPARα and Bcl-2 expression to promote cardiomyocyte proliferation and inhibit cardiomyocyte cycle arrest and apoptosis [79]. ROS via JNK-p38-ATF signaling upregulated Plin5 expression and increased Plin5 to enhance lipid synthesis and to promote LD contact with mitochondria, which help cells to modulate stress response [80]. Second, many drugs can affect the expression of Plin5. Statins decrease the levels of Plin5 in the livers and primary hepatocytes, paralleled by a significant reduction in TG content. The transcription of Plin5 could be directly inhibited by SREBP2, which was upregulated by the cholesterol depletion of statins. One of the statins, atorvastatin, can promote PKA-mediated phosphorylation of Plin5 to reduce lipid accumulation in the liver [71]. PKA stimulation can enhance Plin5 phosphorylation to increase TG hydrolysis and direct FFAs to mitochondrial oxidation [81]. A functionally conserved PPRE site maps to the first intron of Plin5, and Plin5 expression can be induced in myocardium, skeletal, and liver by PPAR-α agonists, but also in WAT by pioglitazone, a PPAR-γ agonist. Some agonists, however, are not exclusive but can cross-activate different PPAR family members [16]. Third, some exogenous environmental stimuli can also affect its expression. Such as hypoxia, it can aggravate intracellular TG accumulation promoted by electro-negative LDL in cardiomyocytes via impairing Plin5 pathway [27]. Cold conditions also increase Plin5 expression [82]. And as LDs target protein, Plin5 expression is enhanced under physiological or pharmacological conditions that promote systemic FA elevation, e.g., fasting (liver and heart), endurance exercise (skeletal muscle), and chronic β3-adrenergic stimulation (liver). Exogenous FAs can also stimulate Plin5 expression in cell culture. One primary pathway involves the transcription factor family of PPARs [16]. Recently, some experiments on pigs suggested that variations in the Plin5 sequence might be linked to LIPE expression through a still poorly known regulative molecular process [83]. In an article by Yamada and Honma et al., they identified that Plin5 was upregulated in mice force-fed with fructose compared with those force-fed with glucose [84].

5. Downstream Factors Regulated by Plin5

The change of Plin5 expression can affect many physiological processes and molecular expression. Early studies have shown that Plin5 can prevent uncontrolled TG mobilization and excessive release of FFA [20] (Table 2) and act as a barrier to lipolysis. However, recent studies have found that PLIN proteins do not serve as lipolytic barriers but rather are docking sites for proteins facilitating selective lipase access under a variety of lipolytic conditions [85]. First, Plin5 can affect the genes correlated with lipid metabolism, such as SIRT1, CGI-58, HSL, and ATGL/ABHD5. Monounsaturated fatty acids (MUFAs) can bind to plin5 and activate its downstream target gene SIRT1, thus becoming the first known endogenous allosteric regulator of SIRT1 [86]. It is reported that Plin2, 3, and 5 all interact with HSL and ATGL [87]. Plin5 blocks ATGL-mediated lipolysis by competitively binding to CGI-58 and disrupting the interaction between CGI-58 and ATGL

Table 1: Upstream factors regulating Plin5.

Upstream mediators	Effect target	Upstream regulation effect	First author, year, and reference no.
Molecules			
PPAR-α/δ	Muscle	Activation of PPAR-α/δ can increase the expression of Plin5	Bindesbøll C et al., 2012 [44]
C/EBPα	Adipocyte tissue, liver	C/EBPα promotes transcription of the porcine Plin5 gene	Zhou L et al., 2013 [72]
SREBP2	Liver	SREBP2 can inhibition the expression of Plin5	Asimakopoulou A et al., 2014 [70]
LCN2	Liver	Basal expression of Plin5 was significantly reduced in $Lcn2^{-/-}$ cells	Gao X et al., 2017 [93]
Plin4	Heart	Plin4 ablation can reduce Plin5 expression at both mRNA and protein levels	Chen W et al., 2013 [75]
PKA	Heart, liver	Protein kinase A (PKA)-stimulation can enhance Plin5 phosphorylation	Pollak NM et al., 2015 [81]
LDL	Cardiomyocytes	LDL (-) strongly induces Plin5 mRNA expression and protein levels	Bindesbøll C et al., 2012 [44]
Environmental			
Hypoxia	Cardiomyocytes	Hypoxia can impair Plin5 upregulation	Revuelta-López E et al., 2015 [27]
Fasting	Liver, heart	Plin5 expression is enhanced	Kimmel AR et al., 2014 [16]
Chronic β3-adrenergic stimulation	Liver	Plin5 expression is enhanced	Kimmel AR et al., 2014 [16]
Endurance exercise	Skeletal muscle	Plin5 expression is enhanced	Kimmel AR et al., 2014 [16]

Table 2: Downstream factors regulated by Plin5.

Downstream mediators	Effect target	Downstream regulation effect	First author, year, and reference no.
CGI-58/ ATGL	Cardiomyocyte, liver, adipocyte tissue, muscle	Plin5 competitively binds to CGI-58 and disrupting the interaction between CGI-58 and ATGL	Wang C et al.. 2015 [35] Pollak NM et al., 2013 [20] Sanders MA et al., 2015 [88] Mason RR et al., 2014 [39]
HSL	Adipocyte tissue	Plin5 interacts with HSL	Macpherson RE et al., 2013 [87]
PPARα/ PGC1-α	Muscle	Overexpression of Plin5 promotes expression of genes under control of PPARα and PGC-1α	Bosma M et al., 2013 [50]
	Liver, heart	Plin5 decreases expression of PPARα target genes	Trevino MB et al., 2015 [94] Wang H, et al.,2013 [21]
NF-E2-related factor 2	Heart	Plin5 increases expression of oxidative-induced genes via NF-E2-related factor 2 antioxidative pathway	Wang H et al., 2013 [21]
FGF21	Muscle	Upregulating the Plin5 level drives expression of the FGF21 gene	Harris LA et al., 2015 [41]
cAMP/ GPR40	Islet	Ad-Plin5 enhanced glucose-stimulated insulin secretion in GPR40- and cAMP-activated protein kinase- dependent manners	Trevino MB et al., 2015 [90]
PKC/ NAPDH	Heart	Plin5-KO suppresses diacylglycerol/ceramide-PKC pathway and NADPH oxidase	Kuramoto K et al., 2014 [28]
NF-κB	Artery	IκBα/NF-κB pathway was activated in $Plin5^{-/-}$	Zhou PL et al., 2017 [34]
MAPK	Aortic tissue	Plin5-/- activates PI3K/AKT and MAPKs pathways	Zhou PL et al., 2017 [34]
PI3K/AKT	Cardiomyocytes	Plin5-null decreases phosphorylation of PI3K/AKT	Zheng P et al., 2017 [26]

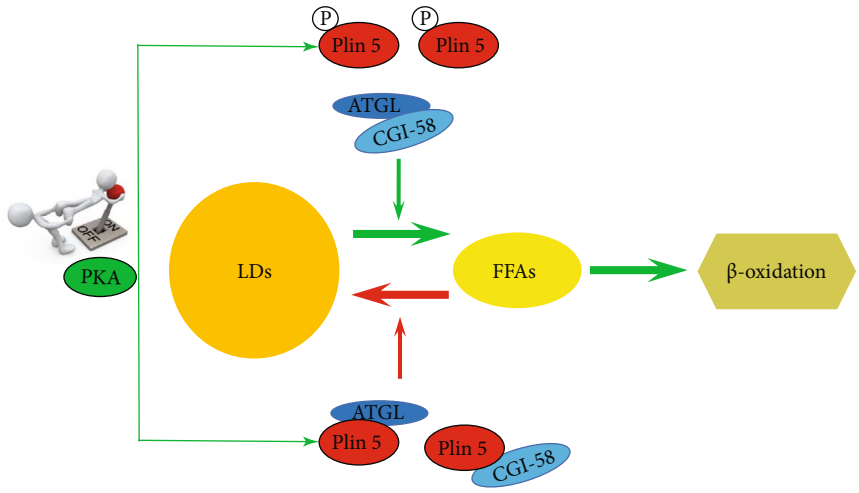

Figure 2: Plin5 serves as a bidirectional switch for FFAs metabolism.

in the liver, thus inhibiting lipolysis and improving hepatic lipotoxicity [87]. Plin5 also expresses in myocardium and has been shown to interact with ATGL and its coactivator CGI-58 [20]. ATGL and its protein activator, α-β-hydrolase domain-containing 5 (ABHD5), each can bind to Plin5. ABHD5 potently activates ATGL, but this lipase-promoting activity is suppressed when ABHD5 is bound to Plin proteins on LDs [88]. The association of Plin5-ABDH5 complexes on lipid droplet surfaces was more stable than Plin5-ATGL complexes [89]. Second, Plin5 can influence the expression of antioxidant genes, such as PPAR-α, PGC1-α, Nrf2, and NAPDH. Plin5 deficiency can reduce myocardial lipid accumulation and upregulate PPAR-α and PGC1-α levels. The genes they control are involved in FA catabolism and mitochondrial oxidation [50]. Therefore, Plin5 deficiency increases the mobilization of stored lipids [34]. Moreover, Plin5 regulates the formation and stabilization of cardiac LDs, and it promotes cardiac steatosis without major heart function impairment, which may have been prevented by a strongly increased expression of oxidative-induced genes via Nrf2 antioxidative pathway [20]. Specially, membrane translocation of protein kinase C (PKC) and the assembly of NADPH oxidase 2 complex on the membrane were also suppressed. Diabetic Plin5-ablation mice are resistant to type 1 diabetes-induced heart malfunction due to the suppression of diacylglycerol/ceramide-PKC pathway and excessive ROS generated by NADPH oxidase [28]. Third, Plin5 plays a vital role in some signal paths, such as cAMP/GPR40, NF-κb, phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT), phospho-Akt/phospho-glycogen synthase kinase-3β/nuclear factor erythroid 2-related factor 2Akt/GSK-3β/Nrf2, and MAPK. Upregulation of Plin5 in islets enhanced the augmentation of glucose-stimulated insulin secretion by FA and 8-Br-cAMP in G-protein-coupled receptor 40 (GPR40) and cAMP-activated protein kinase-dependent manners, respectively, implicating its role in the postprandial insulin secretion [90]. Interestingly, Plin5 deficiency promotes atherosclerosis progression through accelerating inflammation, apoptosis, and oxidative stress, which was linked with the activation of PI3K/AKT and mitogen-activated protein kinases (MAPKs) pathways [34]. In accordance with this, Plin5 plays an important role in protecting against HG-induced apoptosis, oxidative stress, and inflammation in podocytes via modulation of Akt/GSK-3β/Nrf2 signaling [91]. Moreover, Plin5 alleviates myocardial ischemia/reperfusion (MI/R) injury by reducing oxidative stress through increasing phosphorylation of PI3K/Akt to inhibit the lipolysis of LDs [26]. What is more, Plin5 can affect the fibroblast growth factor 21 (FGF21) expression. Plin5-driven LD accumulation in skeletal muscle stimulates the expression of FGF21. Upregulation of Plin5 drives the FGF21 gene expression in fast-twitch fibers and exhibits metabolically protective roles in skeletal muscle [91]. However, the detailed roles and mechanisms of Plin5 to regulate lipid turn over are far from clear yet. In particularly, we should pay more attention to the different modifications of Plin5 and the following changes in lipid homeostasis.

6. Prospect

Abnormal lipid metabolism plays an important role in the pathophysiology of diabetes, cardiovascular disease, liver disease, and its complications. Lipid metabolism and glucose metabolism disorders affect each other and together become the culprit of diabetic cardiovascular complications. Active lipid-lowering treatment in diabetic patients can significantly reduce the incidence of cardiac dysfunction and mortality. We proposed a hypothesis to connect all the pathways and to simulate the probable process of Plin5-related cardioprotection in diabetes. As one of the lipid-associated protein family members, Plin5 plays an important regulatory role in lipid deposition and orderly arrangement. In the basal state, perilipin anchored to the lipid droplet surface as a physiological barrier prevented soluble lipase from reaching the lipid droplet, rendering the TG unhydrolyzed by lipase, whereas phospholipidated by PKA upon lipolysis stimulation, leaving soluble lipase phosphoric acid translocated to the surface of LDs, at which point it colocalized with the phosphorylated perilipin on the surface of the LDs to stimulate lipolysis. It has been shown that ATGL plays a major role in basal lipolysis. Therefore, ATGL is speculated to be

related to lipofuscin. There may be some unknown interaction between the proteins which could be another starting point to study perilipin and lipid metabolism. Plin5 can be seen as a connecting bridge between the mitochondrion and FFAs metabolism. Further studies are needed to clarify the role of Plin5 in recruiting and attaching mitochondria to the surface of LD and to determine which signaling pathways are involved in the regulation of mitochondrial localization of Plin5. In particular, the relationship between Plin5 and mitophagy may provide new ideas for the treatment of many diseases. As residual products of excessive FFA β-oxidation, ROS contribute to the development of diabetic cardiomyopathy and diabetic vascular complications. Recent findings have supported that Plin5 reduces ROS by sequestering FFAs from excessive oxidation and even is a critical regulator of lipid uptake and lipolysis. Therefore, we suppose that Plin5 may therefore represent a novel therapeutic drug target for the treatment of those diseases related to elevated fat accumulation and steatosis, for further understanding of abnormal body fat distribution, insulin resistance, consequently, opening up a new direction, providing new ideas to explore the development of this kind of drugs. We think that most of the studies on Plin5 were undertaken in cell systems and transgenic mice, but some cell systems lack other lipid metabolism proteins which may be necessary to faithfully replicate the protein-protein interactions required for in vivo lipolysis and other metabolic functions. The role of Plin5 in metabolic disease remains perplexing, owing to the lack of concordance between studies using similar experimental designs and interspecies differences. We are only beginning to delineate Plin5 responses to environmental/physiological situations, and further studies will provide a clearer picture of Plin5's functions in physiological and pathophysiological states in vivo.

Overall, Plin5 serves as a bidirectional switch in oxidative tissues, and this knowledge could lead to new avenues of therapy and prevention of diabetic cardiomyopathy. At present, the data indicates that a complex network of signaling mechanisms is involved in Plin5 mediation (Figure 2). Its numerous regulators and signaling pathways provide researchers with many chances to explore its mechanism. However, there are many unsolved issues regarding the function of Plin5 in the heart. Undoubtedly, more work is needed to understand the role of Plin5 in cardiomyocyte biology before it can be considered as a valuable therapeutic target for translational studies of diabetic cardiomyopathy.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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References

[1] Y. Zhang, J. R. Sowers, and J. Ren, "Pathophysiological insights into cardiovascular health in metabolic syndrome," *Experimental Diabetes Research*, vol. 2012, Article ID 320534, 2012.

[2] E. Fabbrini and F. Magkos, "Hepatic steatosis as a marker of metabolic dysfunction," *Nutrients*, vol. 7, no. 6, pp. 4995–5019, 2015.

[3] J. Ren, N. N. Wu, S. Wang, J. R. Sowers, and Y. Zhang, "Obesity cardiomyopathy: evidence, mechanisms, and therapeutic implications," *Physiological Reviews*, vol. 101, no. 4, pp. 1745–1807, 2021.

[4] S. D. Kohlwein, M. Veenhuis, and I. J. van der Klei, "Lipid droplets and peroxisomes: key players in cellular lipid homeostasis or a matter of fat–store 'em up or burn 'em down," *Genetics*, vol. 193, no. 1, pp. 1–50, 2013.

[5] Z. Sun and M. A. Lazar, "Dissociating fatty liver and diabetes," *Trends in Endocrinology & Metabolism*, vol. 24, no. 1, pp. 4–12, 2013.

[6] R. S. Rosenson, M. H. Davidson, B. J. Hirsh, S. Kathiresan, and D. Gaudet, "Genetics and causality of triglyceride-rich lipoproteins in atherosclerotic cardiovascular disease," *Journal of the American College of Cardiology*, vol. 64, no. 23, pp. 2525–2540, 2014.

[7] J. Plakkal Ayyappan, A. Paul, and Y. H. Goo, "Lipid droplet-associated proteins in atherosclerosis," *Molecular Medicine Reports*, vol. 13, no. 6, pp. 4527–4534, 2016.

[8] N. Kory, A. R. Thiam, R. V. Farese Jr., and T. C. Walther, "Protein crowding is a determinant of lipid droplet protein composition," *Developmental Cell*, vol. 34, no. 3, pp. 351–363, 2015.

[9] P. E. Bickel, J. T. Tansey, and M. A. Welte, "PAT proteins, an ancient family of lipid droplet proteins that regulate cellular lipid stores," *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, vol. 1791, no. 6, pp. 419–440, 2009.

[10] Z. Sun, J. Gong, H. Wu et al., "Perilipin 1 promotes unilocular lipid droplet formation through the activation of Fsp 27 in adipocytes," *Nature Communications*, vol. 4, pp. 1–15, 2013.

[11] R. M. Carr and R. S. Ahima, "Pathophysiology of lipid droplet proteins in liver diseases," *Experimental Cell Research*, vol. 340, no. 2, pp. 187–192, 2016.

[12] X. Lu, J. Gruia-Gray, N. G. Copeland et al., "The murine perilipin gene: the lipid droplet-associated perilipins derive from tissue-specific, mRNA splice variants and define a gene family of ancient origin," *Mammalian Genome*, vol. 12, no. 9, pp. 741–749, 2001.

[13] K. T. Dalen, T. Dahl, E. Holter et al., "LSDP5 is a PAT protein specifically expressed in fatty acid oxidizing tissues," *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, vol. 1771, no. 2, pp. 210–227, 2007.

[14] N. E. Wolins, B. K. Quaynor, J. R. Skinner et al., "OXPAT/PAT-1 is a PPAR-induced lipid droplet protein that promotes fatty acid utilization," *Diabetes*, vol. 55, no. 12, pp. 3418–3428, 2006.

[15] C. Laurens, V. Bourlier, A. Mairal et al., "Perilipin 5 fine-tunes lipid oxidation to metabolic demand and protects against lipotoxicity in skeletal muscle," *Scientific Reports*, vol. 6, no. 1, pp. 1–12, 2016.

[16] A. R. Kimmel and C. Sztalryd, "Perilipin 5, a lipid droplet protein adapted to mitochondrial energy utilization," *Current Opinion in Lipidology*, vol. 25, no. 2, pp. 110–117, 2014.

[17] R. R. Mason and M. J. Watt, "Unraveling the roles of PLIN5: linking cell biology to physiology," *Trends in Endocrinology & Metabolism*, vol. 26, no. 3, pp. 144–152, 2015.

[18] J. B. Lockridge, M. L. Sailors, D. J. Durgan et al., "Bioinformatic profiling of the transcriptional response of adult rat cardiomyocytes to distinct fatty acids," *Journal of Lipid Research*, vol. 49, no. 7, pp. 1395–1408, 2008.

[19] C. Drevinge, K. T. Dalen, M. N. Mannila et al., "Perilipin 5 is protective in the ischemic heart," *International Journal Of Cardiology*, vol. 219, pp. 446–454, 2016.

[20] N. M. Pollak, M. Schweiger, D. Jaeger et al., "Cardiac-specific overexpression of perilipin 5 provokes severe cardiac steatosis via the formation of a lipolytic barrier[S]," *Journal of Lipid Research*, vol. 54, no. 4, pp. 1092–1102, 2013.

[21] H. Wang, U. Sreenivasan, D. W. Gong et al., "Cardiomyocyte-specific perilipin 5 overexpression leads to myocardial steatosis and modest cardiac dysfunction," *Journal of Lipid Research*, vol. 54, no. 4, pp. 953–965, 2013.

[22] K. Kuramoto, T. Okamura, T. Yamaguchi et al., "Perilipin 5, a lipid droplet-binding protein, protects heart from oxidative burden by sequestering fatty acid from excessive oxidation," *The Journal of Biological Chemistry*, vol. 287, no. 28, pp. 23852–23863, 2012.

[23] M. Rakhshandehroo, B. Knoch, M. Müller, and S. Kersten, "Peroxisome proliferator-activated receptor alpha target genes," *PPAR Research*, vol. 2010, 20 pages, 2010.

[24] E. Murphy and C. Steenbergen, "Preconditioning: the mitochondrial connection," *Annual Review of Physiology*, vol. 69, no. 1, pp. 51–67, 2007.

[25] D. B. Zorov, C. R. Filburn, L. O. Klotz, J. L. Zweier, and S. J. Sollott, "Reactive oxygen species (ROS)-induced ROS release: a new phenomenon accompanying induction of the mitochondrial permeability transition in cardiac myocytes," *The Journal of Experimental Medicine*, vol. 192, no. 7, pp. 1001–1014, 2000.

[26] P. Zheng, Z. Xie, Y. Yuan et al., "Plin 5 alleviates myocardial ischaemia/reperfusion injury by reducing oxidative stress through inhibiting the lipolysis of lipid droplets," *Scientific Reports*, vol. 7, pp. 1–10, 2017.

[27] E. Revuelta-López, R. Cal, J. Julve et al., "Hypoxia worsens the impact of intracellular triglyceride accumulation promoted by electronegative low-density lipoprotein in cardiomyocytes by impairing perilipin 5 upregulation," *The International Journal of Biochemistry & Cell Biology*, vol. 65, pp. 257–267, 2015.

[28] K. Kuramoto, F. Sakai, N. Yoshinori et al., "Deficiency of a lipid droplet protein, perilipin 5, suppresses myocardial lipid accumulation, thereby preventing type 1 diabetes-induced heart malfunction," *Molecular and Cellular Biology*, vol. 34, no. 14, pp. 2721–2731, 2014.

[29] J. M. Friedman, "Causes and control of excess body fat," *Nature*, vol. 459, no. 7245, pp. 340–342, 2009.

[30] N. A. Ducharme and P. E. Bickel, "Lipid droplets in lipogenesis and lipolysis," *Endocrinology*, vol. 149, no. 3, pp. 942–949, 2008.

[31] R. Sinha, S. Dufour, K. F. Petersen et al., "Assessment of skeletal muscle triglyceride content by (1) H nuclear magnetic resonance spectroscopy in lean and obese adolescents: relationships to insulin sensitivity, total body fat, and central adiposity," *Diabetes*, vol. 51, no. 4, pp. 1022–1027, 2002.

[32] A. E. Feldstein and G. J. Gores, "Apoptosis in alcoholic and nonalcoholic steatohepatitis," *Frontiers in Bioscience: A Journal and Virtual Library*, vol. 10, no. 1-3, pp. 3093–3099, 2005.

[33] J. E. Schaffer, "Lipotoxicity: when tissues overeat," *Current Opinion in Lipidology*, vol. 14, no. 3, pp. 281–287, 2003.

[34] P. L. Zhou, M. Li, X. W. Han et al., "Perilipin 5 deficiency promotes atherosclerosis progression through accelerating inflammation, apoptosis, and oxidative stress," *Journal of Cellular Biochemistry*, vol. 120, no. 11, pp. 19107–19123, 2019.

[35] C. Wang, Y. Zhao, X. Gao et al., "Perilipin 5 improves hepatic lipotoxicity by inhibiting lipolysis,," *Hepatology*, vol. 61, no. 3, pp. 870–882, 2015.

[36] H. Wang, M. Bell, U. Sreenivasan et al., "Unique regulation of adipose triglyceride lipase (ATGL) by perilipin 5, a lipid droplet-associated protein," *The Journal of Biological Chemistry*, vol. 286, no. 18, pp. 15707–15715, 2011.

[37] T. Okumura, "Role of lipid droplet proteins in liver steatosis," *Journal of Physiology and Biochemistry*, vol. 67, no. 4, pp. 629–636, 2011.

[38] H. Li, Y. Song, L. J. Zhang et al., "LSDP5 enhances triglyceride storage in hepatocytes by influencing lipolysis and fatty acid β-Oxidation of lipid droplets," *PLoS One*, vol. 7, no. 6, p. e36712, 2012.

[39] R. R. Mason, R. C. Meex, A. P. Russell, B. J. Canny, and M. J. Watt, "Cellular localization and associations of the major lipolytic proteins in human skeletal muscle at rest and during exercise," *PLoS One*, vol. 9, no. 7, p. e103062, 2014.

[40] T. Fujimoto and R. G. Parton, "Not just fat: the structure and function of the lipid droplet," *Cold Spring Harbor Perspectives in Biology*, vol. 3, no. 3, p. a004838, 2011.

[41] L. A. Harris, J. R. Skinner, T. M. Shew, T. A. Pietka, N. A. Abumrad, and N. E. Wolins, "Perilipin 5-driven lipid droplet accumulation in skeletal muscle stimulates the expression of fibroblast growth factor 21," *Diabetes*, vol. 64, no. 8, pp. 2757–2768, 2015.

[42] T. R. Koves, L. M. Sparks, J. P. Kovalik et al., "PPARγ coactivator-1α contributes to exercise-induced regulation of intramuscular lipid droplet programming in mice and humans," *Journal of Lipid Research*, vol. 54, no. 2, pp. 522–534, 2013.

[43] R. R. Mason, R. Mokhtar, M. Matzaris et al., "PLIN5 deletion remodels intracellular lipid composition and causes insulin resistance in muscle," *Molecular Metabolism*, vol. 3, no. 6, pp. 652–663, 2014.

[44] C. Bindesb, O. Berg, B. Arntsen, H. I. Nebb, and K. T. Dalen, "Fatty acids regulate perilipin5 in muscle by activating PPARδ[S]," *Journal of Lipid Research*, vol. 54, no. 7, pp. 1949–1963, 2013.

[45] R. Rinnankoski-Tuikka, J. J. Hulmi, S. Torvinen et al., "Lipid droplet-associated proteins in high-fat fed mice with the effects of voluntary running and diet change," *Metabolism*, vol. 63, no. 8, pp. 1031–1040, 2014.

[46] I. M. Gjelstad, F. Haugen, H. L. Gulseth et al., "Expression of perilipins in human skeletal muscle in vitro and in vivo in relation to diet, exercise and energy balance," *Archives of Physiology and Biochemistry*, vol. 118, no. 1, pp. 22–30, 2012.

[47] S. J. Peters, I. A. Samjoo, M. C. Devries, I. Stevic, H. A. Robertshaw, and M. A. Tarnopolsky, "Perilipin family (PLIN) proteins in human skeletal muscle: the effect of sex, obesity, and endurance training," *Applied Physiology, Nutrition, and Metabolism*, vol. 37, no. 4, pp. 724–735, 2012.

[48] R. Minnaard, P. Schrauwen, G. Schaart et al., "Adipocyte differentiation-related protein and OXPAT in rat and human skeletal muscle: involvement in lipid accumulation and type 2 diabetes mellitus," *The Journal of Clinical Endocrinology & Metabolism*, vol. 94, no. 10, pp. 4077–4085, 2009.

[49] M. Bosma, R. Minnaard, L. M. Sparks et al., "The lipid droplet coat protein perilipin 5 also localizes to muscle mitochondria," *Histochemistry and Cell Biology*, vol. 137, no. 2, pp. 205–216, 2012.

[50] M. Bosma, L. M. Sparks, G. J. Hooiveld et al., "Overexpression of PLIN5 in skeletal muscle promotes oxidative gene expression and intramyocellular lipid content without compromising insulin sensitivity," *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, vol. 1831, no. 4, pp. 844–852, 2013.

[51] B. Cannon and J. Nedergaard, "Brown adipose tissue: function and physiological significance," *Physiological Reviews*, vol. 84, no. 1, pp. 277–359, 2004.

[52] H. Onken, W. Zeiske, and B. Harvey, "Effect of mucosal H^+ and chemical modification on transcellular K^+ current in frog skin," *Biochimica et Biophysica Acta*, vol. 1024, no. 1, pp. 95–102, 1990.

[53] V. K. Khor, W. J. Shen, and F. B. Kraemer, "Lipid droplet metabolism," *Current Opinion In Clinical Nutrition And Metabolic Care*, vol. 16, no. 6, pp. 632–637, 2013.

[54] L. Cui and P. Liu, "Two types of contact between lipid droplets and mitochondria," *Frontiers in Cell and Developmental Biology*, vol. 8, article 618322, 2020.

[55] K. Aquilano, P. Vigilanza, S. Baldelli, B. Pagliei, G. Rotilio, and M. R. Ciriolo, "Peroxisome Proliferator-activated Receptor γ Co-activator 1α (PGC-1α) and Sirtuin 1 (SIRT1) Reside in Mitochondria," *Journal of Biological Chemistry*, vol. 285, no. 28, pp. 21590–21599, 2010.

[56] H. Wang, U. Sreenivasan, H. Hu et al., "Perilipin 5, a lipid droplet-associated protein, provides physical and metabolic linkage to mitochondria," *Journal of Lipid Research*, vol. 52, no. 12, pp. 2159–2168, 2011.

[57] I. Y. Benador, M. Veliova, K. Mahdaviani et al., "Mitochondria bound to lipid droplets have unique bioenergetics, composition, and dynamics that support lipid droplet expansion," *Cell Metabolism*, vol. 27, no. 4, pp. 869–885, 2018.

[58] J. Ren, J. R. Sowers, and Y. Zhang, "Metabolic stress, autophagy, and cardiovascular aging: from pathophysiology to therapeutics," *Trends in Endocrinology & Metabolism*, vol. 29, no. 10, pp. 699–711, 2018.

[59] Y. Zhang, J. R. Sowers, and J. Ren, "Targeting autophagy in obesity: from pathophysiology to management," *Nature Reviews Endocrinology*, vol. 14, no. 6, pp. 356–376, 2018.

[60] P. Y. Ke, "Mitophagy in the pathogenesis of liver diseases," *Cells*, vol. 9, no. 4, p. 831, 2020.

[61] X. Zhi, W. Feng, Y. Rong, and R. Liu, "Anatomy of autophagy: from the beginning to the end," *Cellular and Molecular Life Sciences*, vol. 75, no. 5, pp. 815–831, 2018.

[62] J. J. Lemasters, "Variants of mitochondrial autophagy: Types 1 and 2 mitophagy and micromitophagy (Type 3)," *Redox Biology*, vol. 2, pp. 749–754, 2014.

[63] J. J. Lemasters, "Selective mitochondrial autophagy, or mitophagy, as a targeted defense against oxidative stress, mitochondrial dysfunction, and aging," *Rejuvenation Research*, vol. 8, no. 1, pp. 3–5, 2005.

[64] W. Xu, U. Ocak, L. Gao et al., "Selective autophagy as a therapeutic target for neurological diseases," *Cellular and Molecular Life Sciences*, vol. 78, no. 4, pp. 1369–1392, 2021.

[65] M. Onishi, K. Yamano, M. Sato, N. Matsuda, and K. Okamoto, "Molecular mechanisms and physiological functions of mitophagy," *EMBO Journal*, vol. 40, no. 3, p. e104705, 2021.

[66] T. Saito, J. Nah, S. I. Oka et al., "An alternative mitophagy pathway mediated by Rab 9 protects the heart against ischemia," *Journal of Clinical Investigation*, vol. 129, no. 2, pp. 802–819, 2019.

[67] E. Zhang, W. Cui, M. Lopresti et al., "Hepatic PLIN5 signals via SIRT1 to promote autophagy and prevent inflammation during fasting," *Journal of Lipid Rresearch*, vol. 61, no. 3, pp. 338–350, 2020.

[68] S. Y. Ma, K. S. Sun, M. Zhang et al., "Disruption of Plin 5 degradation by CMA causes lipid homeostasis imbalance in NAFLD," *Liver International*, vol. 40, no. 10, pp. 2427–2438, 2020.

[69] S. Kaushik and A. M. Cuervo, "Degradation of lipid droplet-associated proteins by chaperone-mediated autophagy facilitates lipolysis," *Nature Cell Biology*, vol. 17, no. 6, pp. 759–770, 2015.

[70] A. Asimakopoulou, E. Borkham-Kamphorst, M. Henning et al., "Lipocalin-2 (LCN2) regulates PLIN5 expression and intracellular lipid droplet formation in the liver," *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, vol. 1842, no. 10, pp. 1513–1524, 2014.

[71] X. Gao, Y. Nan, Y. Zhao et al., "Atorvastatin reduces lipid accumulation in the liver by activating protein kinase A-mediated phosphorylation of perilipin 5," *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids*, vol. 1862, no. 12, pp. 1512–1519, 2017.

[72] L. Zhou, L. Zhang, Q. Meng et al., "C/EBPα promotes transcription of the porcine _perilipin5_ gene," *Molecular and Cellular Endocrinology*, vol. 364, no. 1-2, pp. 28–35, 2012.

[73] Y. Zhu, X. Zhang, L. Zhang et al., "Perilipin5 protects against lipotoxicity and alleviates endoplasmic reticulum stress in pancreatic beta-cells," *Nutrition & Metabolism*, vol. 16, no. 1, pp. 1–14, 2019.

[74] X. Q. Han, S. Q. Xu, and J. G. Lin, "Curcumin recovers intracellular lipid droplet formation through increasing perilipin 5 gene expression in activated hepatic stellate cells in vitro," *Current Medical Science*, vol. 39, no. 5, pp. 766–777, 2019.

[75] W. Chen, B. Chang, X. Wu, L. Li, M. Sleeman, and L. Chan, "Inactivation of Plin 4 downregulates Plin5 and reduces cardiac lipid accumulation in mice," *American Journal of Physiology. Endocrinology and Metabolism*, vol. 304, no. 7, pp. E770–E779, 2013.

[76] S. Tian, P. Lei, C. Teng et al., "Targeting PLIN2/PLIN5-PPARγ: sulforaphane disturbs the maturation of lipid droplets," *Molecular Nutrition & Food Research*, vol. 63, no. 20, p. e1900183, 2019.

[77] W. Zhong, B. Fan, H. Cong, T. Wang, and J. Gu, "Oleic acid-induced perilipin 5 expression and lipid droplets formation are regulated by the PI3K/PPARα pathway in HepG2 cells," *Applied Physiology, Nutrition, and Metabolism*, vol. 44, no. 8, pp. 840–848, 2019.

[78] Y. Ke, J. Qiu, S. Ogus, W. J. Shen, F. B. Kraemer, and F. F. Chehab, "Overexpression of leptin in transgenic mice leads to decreased basal lipolysis, PKA activity, and perilipin levels,"

Biochemical and Biophysical Research Communications, vol. 312, no. 4, pp. 1165–1170, 2003.

[79] Y. B. Zhao, J. Zhao, L. J. Zhang et al., "MicroRNA-370 protects against myocardial ischemia/reperfusion injury in mice following sevoflurane anesthetic preconditioning through PLIN5-dependent PPAR signaling pathway," *Biomedicine & Pharmacotherapy*, vol. 113, p. 108697, 2019.

[80] Y. Tan, Y. Jin, Q. Wang, J. Huang, X. Wu, and Z. Ren, "Perilipin 5 protects against cellular oxidative stress by enhancing mitochondrial function in HepG2 Cells," *Cells*, vol. 8, no. 10, p. 1241, 2019.

[81] N. M. Pollak, D. Jaeger, S. Kolleritsch et al., "The Interplay of Protein Kinase A and Perilipin 5 Regulates Cardiac Lipolysis," *Journal of Biological Chemistry*, vol. 290, no. 3, pp. 1295–1306, 2015.

[82] Q. Liu, Z. Zhou, P. Liu, and S. Zhang, "Comparative proteomic study of liver lipid droplets and mitochondria in mice housed at different temperatures," *FEBS letters*, vol. 593, no. 16, pp. 2118–2138, 2019.

[83] M. Zappaterra, M. Mazzoni, P. Zambonelli, and R. Davoli, "Investigation of the _Perilipin 5_ gene expression and association study of its sequence polymorphism with meat and carcass quality traits in different pig breeds," *Animal*, vol. 12, no. 6, pp. 1135–1143, 2018.

[84] A. Yamada, K. Honma, K. Mochizuki, and T. Goda, "BRD4 regulates fructose-inducible lipid accumulation-related genes in the mouse liver," *Metabolism*, vol. 65, no. 10, pp. 1478–1488, 2016.

[85] A. Gemmink, S. Daemen, H. J. H. Kuijpers et al., "Super-resolution microscopy localizes perilipin 5 at lipid droplet- mitochondria interaction sites and at lipid droplets juxtaposing to perilipin 2," *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids*, vol. 1863, no. 11, pp. 1423–1432, 2018.

[86] C. P. Najt, S. A. Khan, T. D. Heden et al., "Lipid droplet-derived monounsaturated fatty acids traffic via PLIN5 to allosterically activate SIRT1," *Molecular cell*, vol. 77, no. 4, pp. 810–824.e8, 2020.

[87] R. E. MacPherson, R. Vandenboom, B. D. Roy, and S. J. Peters, "Skeletal muscle PLIN3 and PLIN5 are serine phosphorylated at rest and following lipolysis during adrenergic or contractile stimulation," *Physiological Reports*, vol. 1, no. 4, p. e00084, 2013.

[88] M. A. Sanders, F. Madoux, L. Mladenovic et al., "Endogenous and synthetic ABHD5 ligands regulate ABHD5-Perilipin interactions and lipolysis in fat and muscle," *Cell Metabolism*, vol. 22, no. 5, pp. 851–860, 2015.

[89] J. G. Granneman, H. P. Moore, E. P. Mottillo, Z. Zhu, and L. Zhou, "Interactions of perilipin-5 (Plin5) with adipose triglyceride lipases," *The Journal of Biological Chemistry*, vol. 286, no. 7, pp. 5126–5135, 2011.

[90] M. B. Trevino, Y. Machida, D. R. Hallinger et al., "Perilipin 5 regulates islet lipid metabolism and insulin secretion in a cAMP-dependent manner: implication of its role in the postprandial insulin secretion," *Diabetes*, vol. 64, no. 4, pp. 1299–1310, 2015.

[91] J. Feng, L. Xie, X. Yu et al., "Perilipin 5 ameliorates high-glucose-induced podocyte injury via Akt/GSK-3β/Nrf2-mediated suppression of apoptosis, oxidative stress, and inflammation," *Biochemical and Biophysical Research Communications*, vol. 544, pp. 22–30, 2021.

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Research Article

The Imbalance of Mitochondrial Homeostasis of Peripheral Blood-Derived Macrophages Mediated by MAFLD May Impair the Walking Ability of Elderly Patients with Osteopenia

Xiaojun Wang,[1,2] Xuanqi Liu,[3] Peqing He,[1] Kangwei Guan,[1] Yijing Yang,[1] Yiming Lei,[1] Jianhua Cai,[4] Wenhao Wang,[1,2] and Tao Wu[1,2]

[1]*Department of Traditional Chinese Medicine, Huadong Hospital Affiliated to Fudan University, Shanghai 200040, China*
[2]*Shanghai Key Laboratory of Clinical Geriatric Medicine, Huadong Hospital Affiliated to Fudan University, Shanghai 200040, China*
[3]*Department of Respiratory and Critical Care Medicine, Huadong Hospital Affiliated to Fudan University, Shanghai 200040, China*
[4]*Department of General Surgery, Huadong Hospital Affiliated to Fudan University, Shanghai 200040, China*

Correspondence should be addressed to Jianhua Cai; jianhuacai@139.com, Wenhao Wang; whwang88@hotmail.com, and Tao Wu; taowuh@hotmail.com

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Introduction. Many Asian cohort studies have shown that nonalcoholic fatty liver disease (NAFLD), now renamed as metabolic dysfunction-associated fatty liver disease (MAFLD), increases the risk of osteoporosis, yet the effect of MAFLD on elderly patients with osteopenia (OPe) has not been reported. *Objective*. This study aimed to explore the influence of MAFLD on the function of macrophages in patients with OPe. *Methods*. A total of 107 elderly OPe patients with or without MAFLD who visited the Huadong Hospital Affiliated to Fudan University (Shanghai, China) between January 1st, 2021, and September 30th, 2021, were evaluated for an interviewer-assisted questionnaire, as well as clinical and biological assessments. *Results*. Comparing two groups of elderly patients with the same bone mass level, we found that the six-minute walking distance ($P = 0.012$) and short physical performance battery (SPPB) score ($P = 0.0029$) of the elderly OPe patients with MAFLD are worse than those in OPe patients without MAFLD. Our results confirmed that the mitochondrial reactive oxygen species (mtROS) in peripheral blood of OPe patients with MAFLD was significantly higher than those without. We also observed the mitochondrial metabolism level of peripheral blood-derived macrophages in the included patients and peripheral blood macrophages in patients with MAFLD with more unbalanced mitochondrial dynamics of macrophages, more weakened mitochondrial respiratory capacity, and greater mitochondrial microstructure damage, when compared with the elderly patients without MAFLD. *Conclusions*. To conclude, our data revealed that MAFLD itself may aggravate the inflammatory state in elderly OPe people due to mitochondrial homeostasis imbalance of peripheral blood macrophages. Damaged monocyte-macrophages might trigger attenuation of the walking ability of OPe patients.

1. Introduction

Metabolic dysfunction-associated fatty liver disease (MAFLD), formerly known as nonalcoholic fatty liver disease (NAFLD), due to its high global prevalence, causes a huge economic burden to society [1]. Unhealthy habits such as sedentary and less active lifestyles and unhealthy dietary patterns are closely related to a high incidence rate of MAFLD [2], which can be diagnosed accurately by certain evaluating criteria like obesity, type 2 diabetes mellitus (T2DM), or metabolic disorders [3]. The prevalence of fatty liver disease (FLD) and osteoporosis is increasing in elderly people [4, 5]. Therefore, it has also been studied in various Asian cohorts reporting that FLD increases the risk of osteoporosis in the elderly [6, 7], though the association between FLD and osteoporosis remains ambiguous [8, 9].

Osteopenia (OPe) refers to the decrease in bone mass per unit volume of bone, which can manifest into osteoporosis on further aggravation. In the elderly population, fractures can occur as a primary clinical endpoint for both osteoporosis and persistent history of falls [10] due to a gradual decrease of lower limb muscle strength, which directly affects the balancing function and the walking ability [11]. Fatty liver can increase the risk of fractures in osteoporotic patients [12] and may interact through complex pathways such as inflammatory mediators, hormones, substance metabolism, and intestinal flora imbalance in conjunction with osteoporosis [8]; however, it has not been clarified yet.

The excessive triglycerides accumulation in the liver during MAFLD might lead to pro-inflammatory and anti-inflammatory factor imbalance, affecting the function of immune cells whose homeostasis is largely influenced by their metabolic activities [13]. Thus, specific metabolic adaptability should be acquired to support their diverse immune functions [14]. As an important member of innate immunity, the function of macrophages is strictly regulated by metabolic pathways and metabolic intermediates [15, 16], which sometimes get altered by innate and acquired immunity changes, known as "inflammatory aging" due to chronic low-level systemic inflammation that can affect macrophage polarization [17, 18]. This study attempts to investigate whether MAFLD will affect the function of macrophages in patients with OPe, resulting in the decrease of walking and balance ability to aggravate the risk of fracture or fall in elderly patients.

2. Methods

2.1. Data Extract and Bioinformatics Analysis. The transcription and expression profiles by an array of 28 elderly patients with osteoporosis and 73 females with low or high bone mass were downloaded from the ArrayExpress and Gene Expression Omnibus (GEO) databases, which included 28 bone tissue (E-MEXP-1618) and 73 circulating monocytes (Query DataSets for GSE56816). The disease-associated targets were based on Gene disease database (DisGeNET) (http://www.disgenet.org) and ImmPort (The Immunology Database and Analysis Portal). By using the DAVID 6.8 database [19], the Kyoto Encyclopedia of Genes and Genomes (KEGG) signal pathway enrichment of the important target was analyzed. Gene Oncology (GO) annotation was carried out using clusterProfiler, and R Package was used for comparing biological themes among gene clusters [20].

2.2. Subjects and Study Design. The study protocol was reviewed and approved by the Institutional Ethics Committee of Shanghai Huadong Hospital (2019 K111, 2021 K073). All patients included in this study signed informed consent, and they were enrolled at Huadong Hospital in Shanghai, China, from January 1st, 2021, to September 30th, 2021. In total, 107 participants completed an interviewer-assisted questionnaire and muscle strength evaluation of the research center. At the same time, their biological information was collected. Patient exclusion criteria are given in the flowchart (Supplement 1).

2.3. Biochemical Determination. Blood samples were collected from each participant in the morning after 12 h of fasting, and assayed at the Huadong Hospital Laboratory. The Chronic Kidney Disease Epidemiology Collaboration equation was used to determine glomerular filtration rate (Estimated Glomerular Filtration Rate (eGFR); mL/min/1.73 m^2) to assess renal function. In addition, triglycerides (TG), total cholesterol (TC), serum creatinine (Scr), serum uric acid (SUA), blood urea nitrogen (BUN), alanine aminotransferase (ALT), aspartate transaminase (AST), blood phosphorus and calcium (P and Ca), N-Propeptide of Type I Procollagen (P1NP), β-Crosslaps (CROSSL), parathyroid hormone (PTH), osteocalcin (OSTEOC), C reactive protein (CRP), and high- and low-density lipoprotein (HDL and LDL) levels in serum were measured.

2.4. Criteria for MAFLD and Human Liver Samples. MAFLD was diagnosed based on blood biomarkers and imaging evidence for hepatic fat deposition, besides the three other criteria, which included metabolic dysregulation, T2DM, and overweight [3]. The hepatic samples were collected from patients undergoing liver biopsies after approval from the Institutional Ethics Committee of Shanghai Huadong Hospital (2021 K073). Liver tissue samples were collected from six participants whose age was above 60 years old.

2.5. Bone Mineral Density (BMD) Measurement. According to specific instructions, we determined BMD of the total hip, femoral neck, and lumbar spine through single X-ray absorptiometry (HOLOGIC, Discovery W, USA). As per the guidelines of the World Health Organization (WHO), we considered a patient to be suffering from OPe when the BMD T-score ranged from −1.0 to −2.5 and osteoporosis when the T-score was <−2.5. Severe osteoporosis was indicated by BMD < −2.5, or brittle fractures [21].

2.6. Muscle Strength Measurement. To evaluate muscle strength, grip strength was measured by using an electronic hand dynamometer (CAMRY, MODEL: EH101, CHINA). We considered the highest value from three measurements on bilateral sides as the maximal grip strength. The five-time sit-to-stand test (FTSST) and six-minute walking distance were conducted to determine the strength of the lower extremity muscle. Typically, in the FTSST, the subjects were instructed to sit at the edge of a chair, fold their arms, and hit the chair with their buttocks in every repetition. The participants were given standardized instructions in a six-minute walking test and asked to walk "as far as possible in a 6-min period" along a 90 m course. The subjects were verbally encouraged at intervals of 30 s by standardized phrases and allowed to sit on chairs throughout the experimental period. Six minutes later, we determined the distance walked (to the nearest meter).

The short physical performance battery (SPPB) score, which consisted of 3 components, gait speed, repeated chair stands, and standing balance, is a subjective approach to determine the alteration of physical performance and balance ability among the elderly population as well as a standard measure both for research and clinical practice [22].

Additionally, an inextensible tape was used to flex the knees at 90°; then, we determined the average calf circumference at the broadest level of the bilateral calves in the relaxed and seated position.

2.7. Study Questionnaires and Clinical Assessments. The Pittsburgh Sleep Quality Index (PSQI) provided data regarding subjective sleep quality in the previous month. The nurses or assistant nurses were invited to assess the Mini Nutritional Assessment-Short Form (MNA-SF). We also determined the body weight (BW) and body height (BH) (with no shoes on), and then, the body weight (kg) was divided by the square of the body height (m^2) to determine body mass index (BMI). We collected baseline data from all subjects, including age, gender, drinking history, smoking history, medical history, and the usual amount of exercise, by distributing the self-administered questionnaires. We measured the waistline, BH, and BW during the interview, and subjects were instructed to wear light clothing and no shoes. Blood samples were collected from participants who had fasted for 8 h in the morning and analyzed at the Huadong Hospital laboratory.

2.8. Isolation of Peripheral Blood Mononuclear Cells (PBMCs) and Magnetic Bead Sorting. Blood samples were collected from 20 OPe cases with MAFLD, 20 OPe patients without MAFLD, and 20 patients with severe osteoporosis. Then, we extracted total blood by Ficoll density gradient centrifugation to obtain PBMCs. CD14+ monocytes were magnetically sorted by the MojoSort™ Human Pan Monocyte Isolation kit (Biolegend, San Diego, CA, USA).

2.9. Cell Culture and Treatment. We cultured the isolated PBMCs and human myeloid leukemia mononuclear (THP-1) cells in Dulbecco's modified eagle medium (DMEM) with 20% fetal bovine serum (FBS; Millipore, USA) at 5% CO2 and 37°C. Additionally, we cultivated human hepatocellular carcinoma (HepG2) cells in DMEM low sugar medium that contained 5% FBS. To induce overloading of free fatty acids (FFAs), we cultured cells till they reached 70~80% confluence and exposed them to a mixture of 1 mM long-chain FFAs (palmitic acid and oleic acid (PAOA)) (oleic acid: palmitic acid = 1 : 2) for 24 h [23].

2.10. Flow Cytometry (FCM) and Enzyme-Linked Immunosorbent Assay (ELISA). FCM was used to examine the cells (FACS Aria ™ II, BD Bioscience, NJ, China). The Flowjo™ 10 software was used to analyze the data. Peripheral blood macrophages were defined as CD14+ and CD16+ cells, from which M1 and M2 macrophages were identified as CD86 + HLR-DR+ or CD163 + CD206+ cells, respectively, and monocytes were derived from the patients. All antibodies were obtained from Biolegend (San Diego, CA, USA). ELISA was used to determine interleukin-6 (IL-6) and IL-8 levels in the plasma (X-Y Biotechnology, Shanghai, China).

2.11. Reverse Transcription-Polymerase Chain Reaction (RT-PCR). We used TRIzol reagent (Invitrogen, USA) to extract total cellular RNA, which was later used to prepare cDNA through reverse transcription using a kit (Vazyme Biotech, Nanjing, China). The prepared cDNA served as the template for RT-PCR that was performed using a Roche Light Cycler 96 system (Roche, Switzerland) with the 2X SYBR-Green-based qPCR reagent. The 2-$\Delta\Delta$Ct method was used to determine the relative gene expression [24], and GAPDH was used as the endogenous control. Each assay was performed three times. Supplement 3 lists the sequences of all the primers used.

2.12. Oxygen Consumption Rate (OCR) and Extracellular Acidification Rate (ECAR) Measurements. Glycolysis and mitochondrial respiration were quantified using the Seahorse XF Analyzer (Seahorse XF96, Agilent, USA). To measure OCR, we incubated cells (5 × 103/well) in 96-well XF96 plates overnight. The Seahorse Assay medium was used to substitute the original medium in the XF96 plates 1 h before measurement. We determined OCR under three conditions, which included baseline, 0.5 μM antimycin and 0.5 μM rotenone, and 0.3 μM carbonyl cyanide p-(trifluoromethoxy) phenylhydrazone (FCCP) and 1 μM oligomycin. To quantify ECAR, we injected the glycolysis inhibitor 2-deoxy-D-glucose (2-DG) to stop glycolytic acidification.

2.13. Mitochondrial Isolation and Reactive Oxygen Species (ROS), Mitochondrial Reactive Oxygen Species (mtROS) Quantification. The mitochondria were isolated from PBMCs using a Mitochondria Isolation Kit (Thermo Fisher Scientific). MitoSOX™ Red (Yeasen, China) and DCFH-DA (Beyotime, China) were used to determine mtROS and intracellular ROS contents according to certain protocols. The average fluorescence intensities of mtROS and intracellular ROS were determined using FCM (FACS Aria TM II, BD Bioscience, NJ, China). Transmission electron microscopy (TEM) (suht7700, Hitachi, Japan) was used to examine mitochondrial morphology which was used to examine mitochondrial ultrastructural damage by other researchers [25, 26].

2.14. Mitochondrial Membrane Potential (MMP) and Mitochondrial Permeability Transition Pore (MPTP) Assay. We determined MMP using the mitochondrial membrane potential detection kit (JC1, Beyotime Biotech, China) following specific protocols. Later, we tested the opening degree of the MPTP using the MPTP Detection Kit (Beyotime Biotech, China), following specific instructions (http://www.beyotime.com/index.htm). Flow cytometry was used to analyze the MMP and MPTP opened of the macrophages derived from peripheral blood [27–29].

2.15. Immunofluorescence (IF) and Nile Red Staining. The cells were fixed with paraformaldehyde (PFA) at room temperature (RT). Next, 10% BSA (Sangon Biotech, Shanghai, China) was used to block the cells, followed by incubation using primary antibodies (1 : 100, ab113748, Abcam) for 2 h at 37°C; the cells were then washed thrice with PBS. The cells were incubated with a secondary antibody (1 : 100, A-21244, Thermo Fisher Scientific) for 1 h at 37°C. Supplement 4 lists the antibodies used in this study. The instructions for the use of all antibodies can be found on the website (https://abclonal.com.cn,https://www.ptgcn.com/).

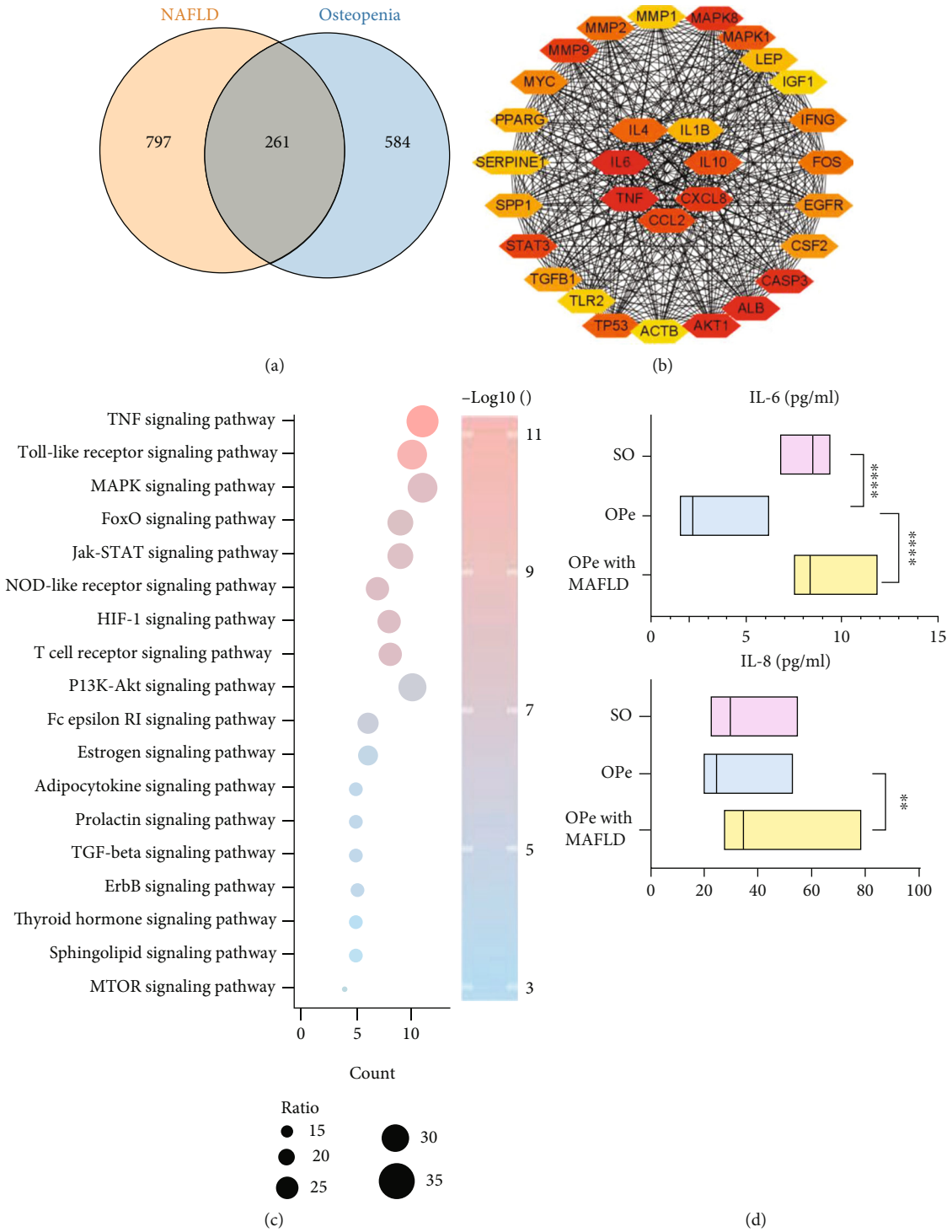
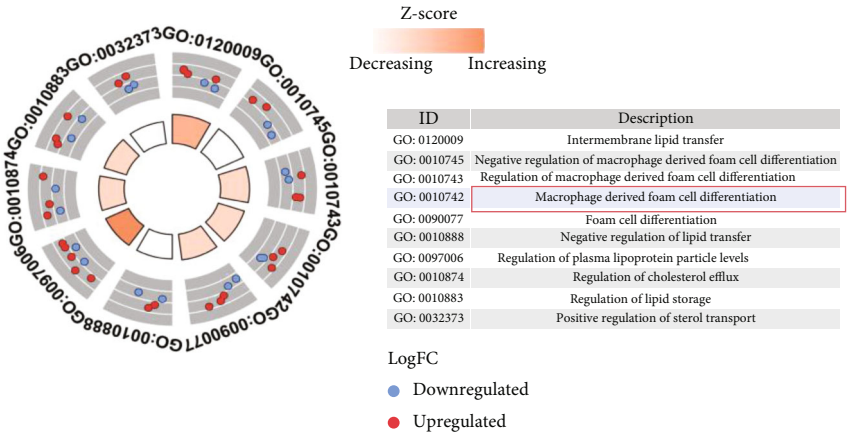
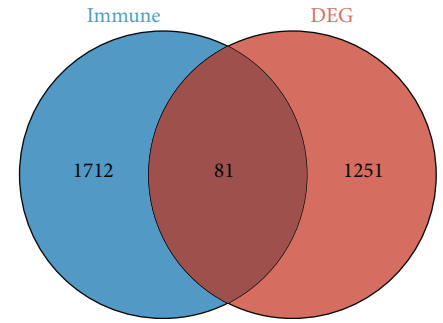


Figure 1: Potential ways of interaction between NAFLD and osteopenia. (a) Venn diagram of the overlap genes between 1058 NAFLD-related genes and 845 osteopenia-related genes from DisGeNET. (b) The hub gene was determined by the construction of PPI network. (c) Top 18 KEGG enrichment pathways of 261overlap genes. (d) The levels of IL-6 and IL-8 in plasma of the patients from SO, OPe, and OPe with MAFLD group were determined by ELISA. Bars, means SE; $*, P < 0.05$; $**$, $P < 0.01$; $***$, $****$, $P < 0.0001$. Abbreviations: NAFLD: nonalcoholic fatty liver disease; PPI: protein-protein interaction; KEGG: Kyoto Encyclopedia of Genes and Genomes; ELISA: enzyme-linked immunosorbent assay; IL-6: interleukin-6; SO: severe osteoporosis; OPe: osteopenia; MAFLD: metabolic dysfunction-associated fatty liver disease.



ID	Description
GO: 0120009	Intermembrane lipid transfer
GO: 0010745	Negative regulation of macrophage derived foam cell differentiation
GO: 0010743	Regulation of macrophage derived foam cell differentiation
GO: 0010742	Macrophage derived foam cell differentiation
GO: 0090077	Foam cell differentiation
GO: 0010888	Negative regulation of lipid transfer
GO: 0097006	Regulation of plasma lipoprotein particle levels
GO: 0010874	Regulation of cholesterol efflux
GO: 0010883	Regulation of lipid storage
GO: 0032373	Positive regulation of sterol transport

(a)



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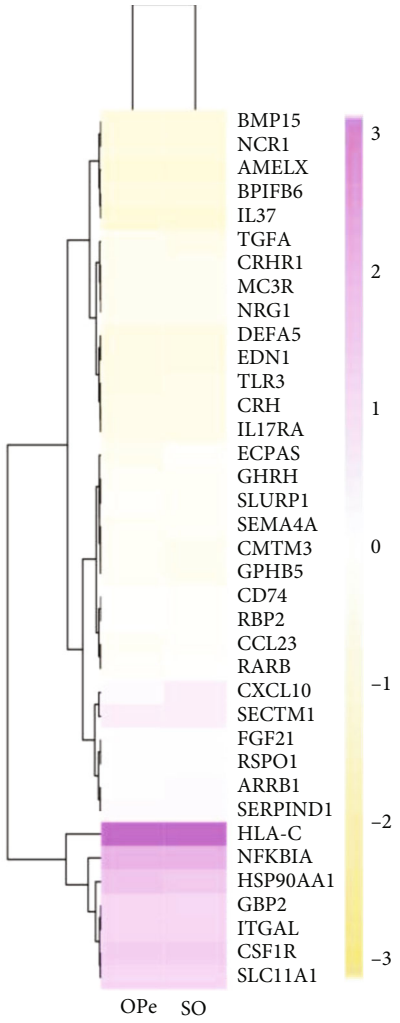

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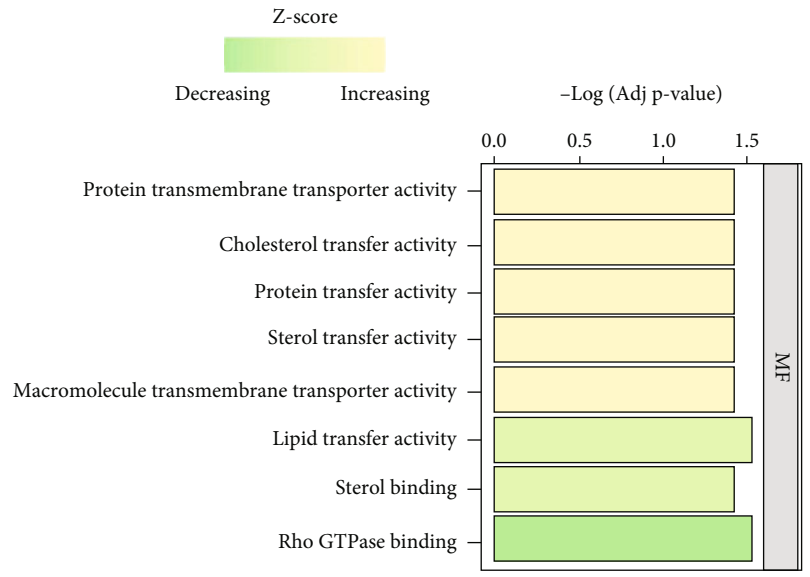


(d)

Figure 2: Continued.

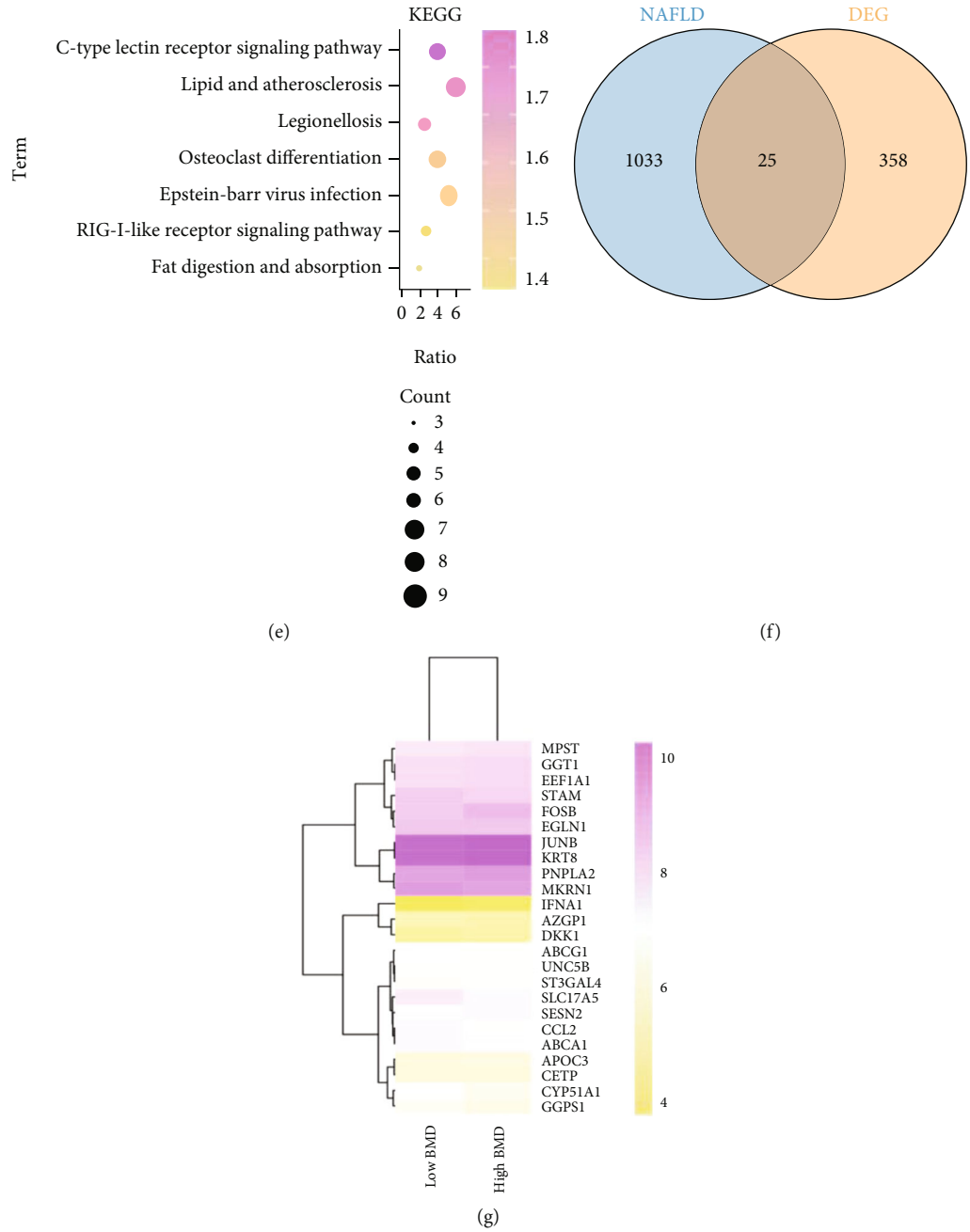
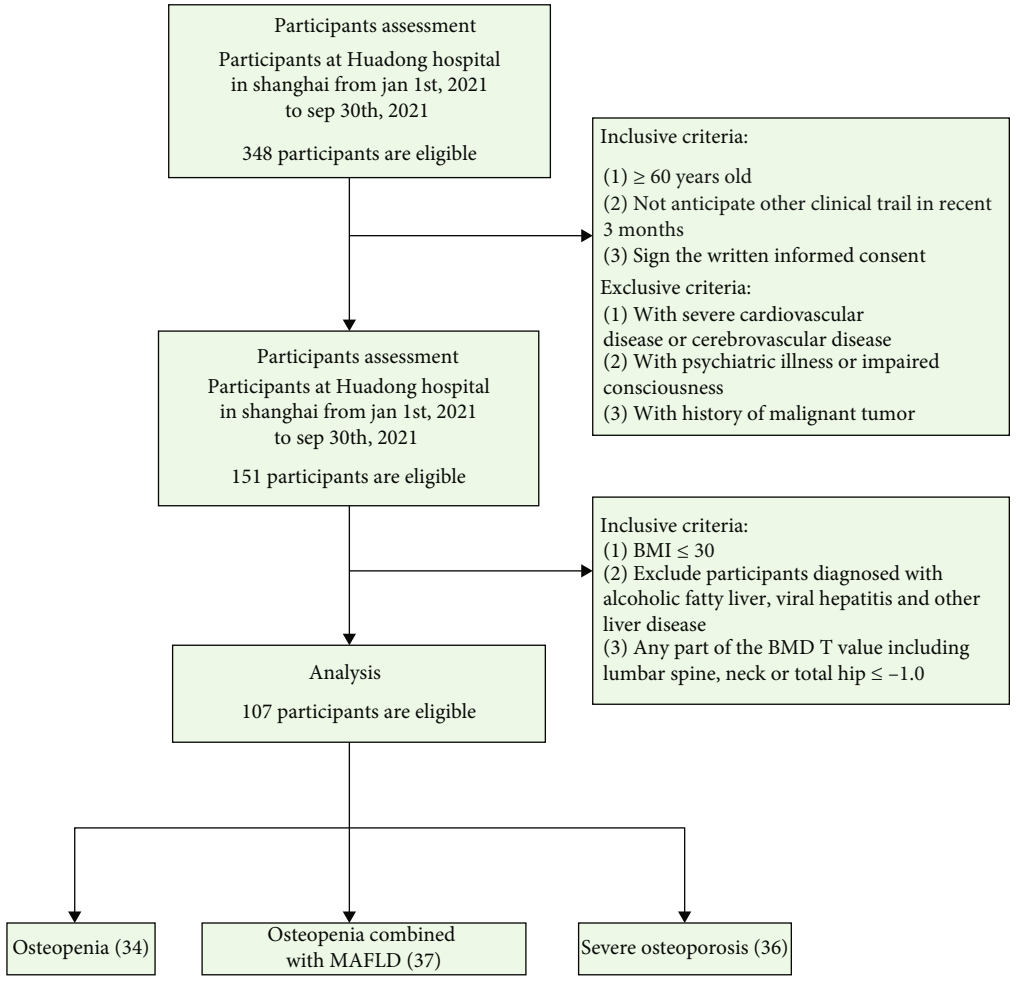


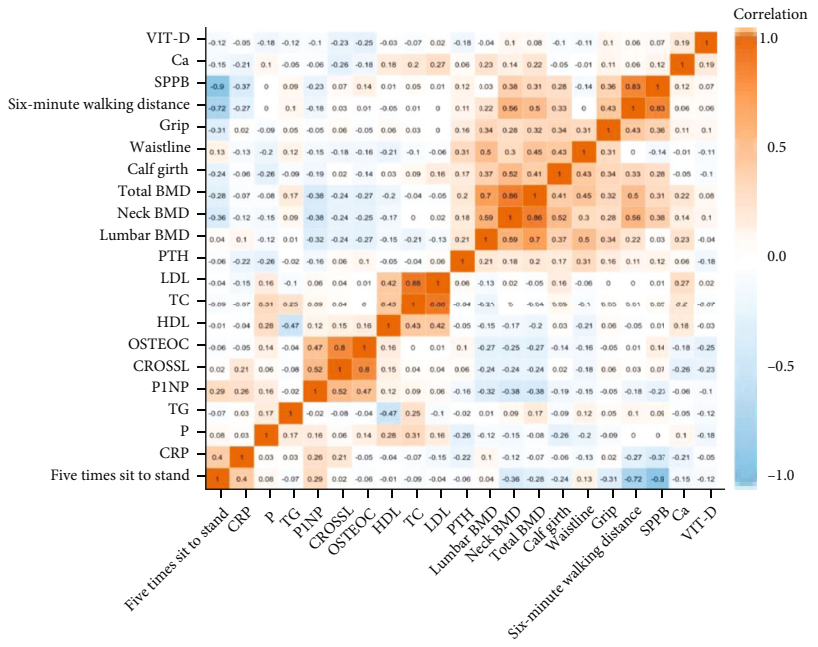
Figure 2: NAFLD and osteopenia may interact through immune pathways. (a) Top 10 GO enrichment items of DEGs between OPe and SO patients from Array Express. (b) Venn diagram of the overlap between immune-related genes and DEGs. (c) The heat map shows the expression of the overlap genes of bone biopsy between OPe and SO patients. (d and e) GO enrichment analysis and KEGG pathway analysis of these 81 overlap genes. (f) Venn plot indicated the overlap between NAFLD-related genes and DEGs. DEGs are derived from the monocytes samples of low and high BMD subjects in GEO database. (g) The heat map indicates the expression level of genes between low and high BMD samples. Abbreviations: SO: severe osteoporosis; OPe: osteopenia; DEG: differentially expressed genes; GO: Gene Oncology; DEGs: differentially expressed genes; KEGG: Kyoto Encyclopedia of Genes and Genomes; BMD: Bone Mineral Density; NAFLD: nonalcoholic fatty liver disease.

Nile Red (Sangon Biotech, Shanghai, China) (1 mM) was used to observe the intracellular lipid droplets. We mounted the cells with DAPI (abs9235, Absin) using a laser confocal cell culture dish (Thermo Fisher Scientific, NY, USA). A confocal imaging system (LSM 780) (Carl Zeiss, Jena, Germany) was used to take images.

2.16. Western Blotting (WB) Assay. We extracted total cellular or hepatic tissue proteins for the WB assay using the RIPA lysis buffer. Then, the bicinchoninic acid (BCA) Protein Assay Kit (Beyotime, China) was used to determine the protein content. Supplement 4 lists the antibodies used in this study. The instructions for the use of all antibodies



(a)



(b)

Figure 3: Continued.

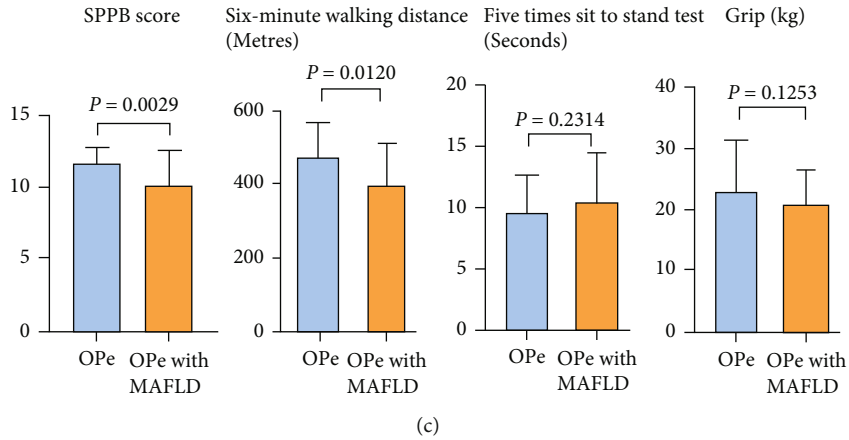


Figure 3: Clinical indicators of all included patients. (a) Flowchart shows the process of subject enrollment and subgroups (OPe grouping =34 subjects, OPe with MAFLD grouping =37 subjects, SO grouping =36 subjects). (b) The correlation matrix of various clinical indicators is presented. (c) Comparison of limb muscle strength in patients between OPe group and OPe with MAFLD group. Abbreviations: SO: severe osteoporosis; OPe: osteopenia; MAFLD: metabolic dysfunction-associated fatty liver disease; SPPB: Short Physical Performance Battery; BMD: bone mineral density; TG: triglycerides; TC: total cholesterol; HDL: high-density lipoprotein; LDL: low-density lipoprotein; P: blood phosphorus; Ca: blood calcium; P1NP: N-Propeptide of Type I Procollagen; CROSSL: β-Crosslaps; PTH: parathyroid hormone; OSTEOC: osteocalcin; CRP: C reactive protein.

can be found on the website (https://abclonal.com.cn,https://www.ptgcn.com/).

2.17. Oil-Red O Staining and Immunohistochemistry (IHC). We used the Oil-Red O staining kit (Abcam, USA) for Oil-Red O staining as per specific protocols. The sections were also incubated with the silent mating type information regulation 1 (SIRT1) primary antibody (1 : 100, Abcam). Each tissue section was mixed with the reaction enhancer (Record Biological, Shanghai, China). The sections were then incubated with the enhanced enzyme-conjugated goat anti-rabbit IgG polymer (Record Biological, Shanghai, China). Images were captured using a light microscope (Nikon ECLIPSE 80i, Nikon, Japan).

2.18. Transfection with Small Interfering RNA (siRNA). According to specific protocols, the siRNA was transfected into CD14+ PBMCs using the Lipofectamine RNAiMax reagent (100 nM, Thermo Fisher Scientific, USA). The siRNA sequence for SIRT1 was $5'$ – GGCTGGTGATCGCAGATTT – $3'$ (RiboBio, Guangzhou, China).

2.19. Statistical Analysis and Art Work. Differentially expressed genes are displayed in a heat map in R. All statistics were performed using Prism (version 8.0.2 for Mac; GraphPad Software, San Diego, CA). The use of 1-way ANOVA established statistical comparisons between the different groups. All values are presented as the mean ± SD P values were specified as follows: $*P < 0.05$; $**P < 0.01$; $** * P < 0.005$; $*** *P < 0.0001$.

3. Results

3.1. Potential Ways of Interaction between Fatty Liver and OPe. DisGeNET screened out a total of 1058 NAFLD genes and 845 OPe genes which later led to an overlap of 261 targets between NAFLD and OPe by the VENN map (Figure 1(a)). After the construction of the protein-protein interaction (PPI) network, key modules and pivotal hub genes were determined using the STRING and Cytoscape software. This finding suggested that tumor necrosis factor-α (TNF-α), IL-8, IL-6, IL-4, IL-1β, and chemokine (C-C motif) ligand 2 (CCL2) were important pathogenic targets (Figure 1(b)). After that, KEGG pathway annotation showed enrichment of overlapping genes and identification of a total of 111 enriched pathways along with 18 enriched signaling pathways with the highest p-adjust values. These pathways included the TNF signal pathway, Toll-like receptor signal pathway, Nod-like receptor signal pathway, hypoxia-inducible factor 1 (HIF-1) signal pathway, and T cell receptor signal pathway (Figure 1(c)). In order to test this hypothesis, the patient's peripheral blood was extracted, and the expression of IL-6 and IL-8 was detected in the elderly OPe patients' plasma with MAFLD, which was remarkably elevated when compared to OPe patients without MAFLD (Figure 1(d)).

The ArrayExpress database extracted one dataset, E-MEXP-1618, which was subsequently made a cohort. The elderly patients over 60 years old were selected as per the received BMD data and divided into the OPe group and the severe osteoporosis (SO) group based on the osteoporosis diagnostic criteria. A total of 13 OPe and 15 SO group samples were analyzed for differentially expressed genes (DEGs) and were subsequently subjected to GO annotations to identify the potential biological functions, as well as differential genes enrichment pathways such as intermembrane lipid transfer and macrophage-derived foam cell differentiation (Figure 2(a)). Additionally, 81 overlapped genes were obtained from 1332 and 1793 genes acquired from DEGs and the Immunology Database and Analysis Portal (ImmPort), respectively (Figure 2(b)), followed by differential gene expression and heat map analysis to determine the gene

Table 1: Baseline characteristics and clinical features.

	OPe	OPe with MAFLD	SO	*P*-value
Gender	12/22	12/25	3/33	0.016
Age	67.88 ± 5.05	67.32 ± 6.15	70.69 ± 7.54	0.070
BMI	24.42 ± 2.05	25.42 ± 2.29	22.28 ± 2.40	0.648
Smoke	26/4	32/5	29/7	0.724
Alcohol	25/5	32/5	30/6	0.914
Hypertension	18/11	22/15	26/10	0.490
Diabetes	28/1	31/6	30/6	0.207
Exercise (yes or no)	9/17	23/11	18/13	0.035
MNA	12.66 ± 1.60	12.86 ± 1.78	10.89 ± 2.27	0.116
PSQI	6.55 ± 4.95	8.03 ± 4.56	9.69 ± 5.23	0.733
Calf girth	33.44 ± 2.87	33.73 ± 3.67	30.47 ± 3.01	0.293
Waist line	84.03 ± 8.12	87.38 ± 8.10	80.47 ± 7.95	0.991
FTSST	9.59 ± 3.04	10.62 ± 3.91	14.62 ± 7.70	<0.001
Six-minute walking	470.73 ± 98.44	405.51 ± 107.34	326.41 ± 112.62	0.755
SPPB	11.56 ± 1.24	10.06 ± 2.50	8.06 ± 3.05	<0.001
Grip	22.08 ± 7.55	19.68 ± 5.25	17.73 ± 5.03	0.034
Ca	2.29 ± 0.11	2.29 ± 0.087	2.25 ± 0.08	0.309
P	1.11 ± 0.13	1.11 ± 0.16	1.14 ± 0.15	0.542
P1NP/CROSSL	0.10 ± 0.03	0.11 ± 0.06	0.14 ± 0.17	<0.001
CROSSL	500.71 ± 227.88	499.88 ± 248.11	610.19 ± 322.61	0.146
PTH	43.09 ± 15.21	47.43 ± 13.42	42.51 ± 17.57	0.338
VITAMIN D	21.90 ± 9.60	17.82 ± 5.91	16.86 ± 7.29	0.036
OSTEOC	19.01 ± 7.68	17.15 ± 5.91	19.58 ± 6.50	0.360
P1NP	46.44 ± 14.25	46.48 ± 19.58	60.93 ± 34.67	<0.001
Lumbar BMD	0.90 ± 0.15	0.92 ± 0.16	0.74 ± 0.15	0.889
Neck BMD	0.67 ± 0.08	0.65 ± 0.08	0.47 ± 0.19	<0.001
Total BMD	0.81 ± 0.10	0.82 ± 0.10	0.61 ± 0.12	0.711
ALT	16.56 ± 7.63	20.52 ± 11.13	15.43 ± 8.85	0.096
AST	18.77 ± 4.88	20.79 ± 7.77	18.18 ± 5.65	0.023
TC	4.76 ± 0.98	4.74 ± 0.99	4.48 ± 0.90	0.824
TG	1.37 ± 0.41	2.09 ± 1.38	1.53 ± 0.94	<0.001
LDL	2.87 ± 0.90	2.71 ± 0.85	2.52 ± 0.80	0.849
HDL	1.48 ± 0.28	1.37 ± 0.38	1.48 ± 0.29	0.209
BUN	5.58 ± 1.34	5.30 ± 1.28	5.67 ± 1.41	0.840
Scr	70.64 ± 14.72	69.87 ± 15.62	68.71 ± 18.38	0.010
SUA	311.55 ± 63.42	338.03 ± 60.47	276.41 ± 76.99	0.324
eGFR	84.12 ± 12.23	83.89 ± 11.12	83.86 ± 16.93	0.037
Lumbar-t value	−1.18 ± 1.08	-1.05 ± 1.24	-2.51 ± 1.28	0.564
Neck of femur-t value	−1.64 ± 0.59	−1.87 ± 0.66	−3.24 ± 0.87	0.064
Total-t value	−1.38 ± 0.75	−1.23 ± 0.82	−2.91 ± 1.04	0.153

Abbreviations: OPe: osteopenia; OPe with MAFLD: osteopenia combined with MAFLD; SO: severe osteoporosis; BMI: body mass index; FTSST: five times sit to stand test; PSQI: Pittsburgh Sleep Quality Index; MNA-SF: Mini Nutritional Assessment-Short Form; SPPB: Short Physical Performance Battery; BMD: bone mineral density; eGFR: Estimated Glomerular Filtration Rate; TG: triglycerides; TC: total cholesterol; Scr: serum creatinine; SUA: serum uric acid; BUN: blood urea nitrogen; HDL: high-density lipoprotein; LDL: low-density lipoprotein; ALT: alanine aminotransferase; AST: aspartate transaminase; P: blood phosphorus; Ca: blood calcium; P1NP: N-Propeptide of Type I Procollagen; CROSSL: β-Crosslaps; PTH: parathyroid hormone; OSTEOC: osteocalcin. The *P* value in red means that the *P* value <0.05 and is considered to be significantly important.

Table 2: Multiple logistic regression analysis of muscle strength related indexes in elderly patients with osteopenia (including osteoporosis) with or without MAFLD.

	OR	95% CI	*P*-value
Exercise	1.64	0.20 – 13.67	0.648
FTSST	1.44	–0.96 – 2.16	0.082
Six-minute walking	1.02	1.00 – 1.04	0.024
Grip	1.19	0.94 – 1.51	0.140
P1NP/CROSSL	714.54	8.90e – 10 – 5.74e + 14	0.638
VITMIN D	1.10	0.98 – 1.23	0.122
P1NP	1.03	0.97 – 1.09	0.380
AST	0.96	0.82 – 1.12	0.590
TG	0.13	0.02 – 1.09	0.059
Scr	0.88	0.75 – 1.03	0.121
eGFR	0.93	0.78 – 1.10	0.375

Abbreviations: OPe: osteopenia; OPe with MAFLD: osteopenia combined with MAFLD; SO: severe osteoporosis; FTSST: five times sit to stand test; eGFR: Estimated Glomerular Filtration Rate; Scr: serum creatinine; TG: triglycerides; AST: aspartate transaminase; P1NP: N-Propeptide of Type I Procollagen; CROSSL: β-Crosslaps. The *P* value in red means that the *P* value <0.05 and is considered to be significantly important.

variation and patterning in bone biopsy between the OPe and SO patients (Figure 2(c)). A subsequent GO and KEGG pathway enrichment analysis on 81 overlapped genes revealed that they were primarily enriched in the lipid metabolism-related pathways (Figures 2(d) and 2(e)). Furthermore, the expression profiling of 73 circulating monocytes by array from 73 pre and postmenopausal females with low or high bone mineral density was downloaded from the GEO database, including 32 low and 41 high BMD group samples, which were analyzed for DEGs, resulting in 25 and 1058 overlapped genes from DEGs and NAFLD from DisGeNET (CUI number: C0400966), respectively (Figure 2(f)). The heat map analysis was also used to ascertain the gene variation in monocytes between the low and high BMD group patients (Figure 2(g)).

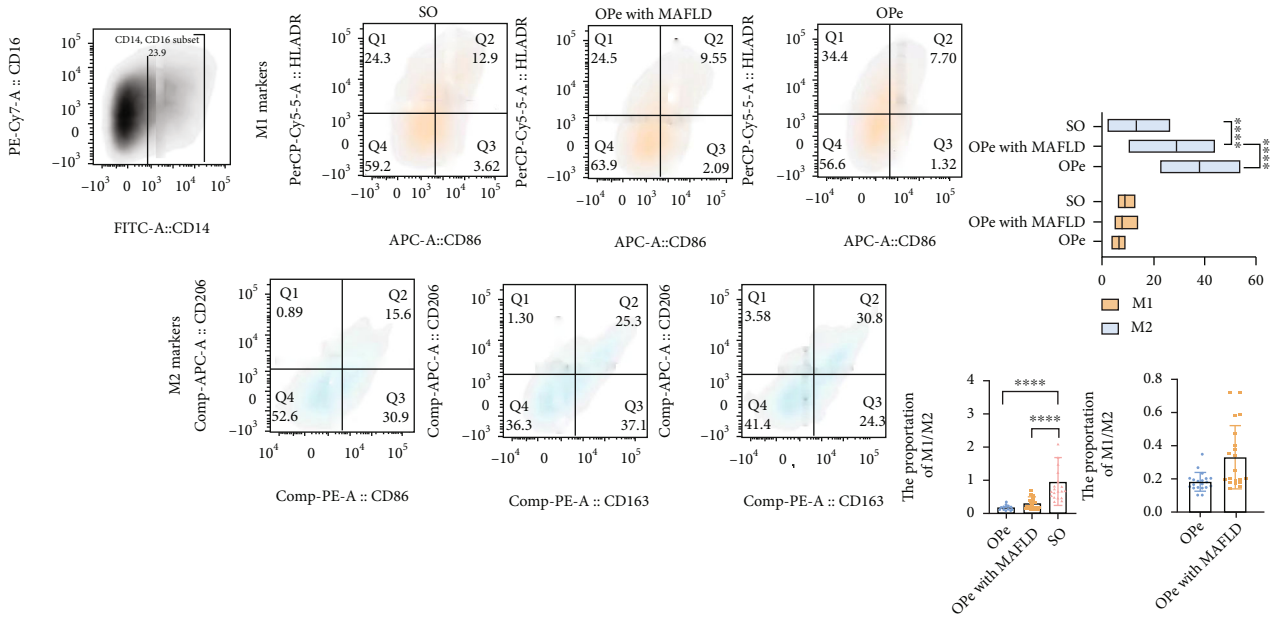
3.2. Clinical Features, Including the Indicators Related to Muscle Strength and Exercise Performance, Differed between OPe Patients with or without MAFLD. A flowchart of subject recruitment is shown in Figure 3(a). A total of 107 participants were used in a subsequent analysis, consisting of Group 1: OPe, including osteoporosis patients without MAFLD; Group 2: OPe, including osteoporosis with MAFLD; Group 3: severe osteoporosis without MAFLD (patients with a history of fracture). There was no statistically significant difference in BMI index, blood glucose, comorbidity, smoking and drinking history, sleeping, and nutritional status between Groups 1, 2, and 3 (Table 1).

In order to further observe the effect of MAFLD on muscle strength of elderly patients with OPe, we analyzed the limb muscle strength of Group 1 and Group 2, which was measured by following parameters such as the upper extremities strength was represented by the grip. In contrast, the lower limbs strength was defined by the FTSST and six-minute walking distance, along with the evaluation of lower limb balance by SPPB score. We found that the six-minute walking distance ($P = 0.012$) and SPPB score ($P = 0.0029$) of the elderly OPe patients with MAFLD are worse than those in OPe patients without MAFLD, which suggested that the walking ability and balance ability of the elderly patients with MAFLD are worse (Table 1, Figure 3(c)). Furthermore, after adjusting the confounders, it was observed that the six-minute walking distance reduction was still markedly associated with the prevalence of MAFLD among patients with OPe or osteoporosis ($P = 0.024$) (Table 2).

3.3. The Proportion of Peripheral Blood-Derived Macrophages in OPe Patients with or without MAFLD. In order to investigate the potential role of peripheral blood-derived macrophages in elderly OPe patients with or without MAFLD and SO patients without MAFLD, we first evaluated the levels of macrophages in the peripheral blood of patients using flow cytometric analysis using the gating strategies of macrophage M1-like and M2-like as follows: M1-like ($CD14^+CD16^+CD86^+HLA\text{-}DR^+$) and M2-like ($CD14^{+-}CD16^+CD163^+CD206^+$) (Supplement 1). Our study results demonstrated that the absolute cell counts of M2-like macrophages in PBMCs were significantly increased while M1/M2% was decreased in PBMCs of OPe patients when compared with those with MAFLD and SO patients (Figure 4(a)). Further, an analysis of the correlation between M1/M2% and BMD (neck BMD, lumbar BMD, and total BMD) in all patients revealed that M1/M2% was associated with neck BMD and total BMD, both in OPe with MAFLD group and the SO group patients, thereby suggesting the importance of M1/M2% in the pathogenesis and the progression of OPe in the elderly population (Figure 4(b)). The negative results of the correlation between M1/M2% and BMD are recorded in Supplement 2.

In order to verify the above-mentioned results, a few in vitro experiments were carried out. Firstly, the fatty liver cell model was successfully established by inducing HepG2 cells in vitro with a mixture of FFAs like palmitic and oleic acid (Figure 4(c)), followed by extricating the CD14+ PBMCs extracted from peripheral blood of OPe patients and inducing them to differentiate into M0 macrophages with the fatty liver cell supernatant. The flow cytometry results revealed that the decrease in M2% and the increase in M1/M2% proportion suggested that the intervention of fatty liver cell supernatant can promote differentiation of M0 macrophages into M1-like macrophages (Figure 4(d)). The WB results also revealed that the protein expression levels of toll-like receptor 4 (TLR4) and myeloid differentiation factor 88 (MyD88), a class of important protein molecules involved in innate immunity, increased significantly following the intervention in both THP-1 cells and CD14+ PBMCs (Figure 4(e)).

3.4. MAFLD Impairs CD14+ Mononuclear Cellular Aerobic Respiration and Mitochondrial Homeostasis in Elderly OPe Patients. WB results displayed that the BCL2-associated X protein (BAX) and Cytochrome C (CYCS) genes expression levels increased with THP-1 and PBMCs extracted from OPe patients after the HepG2 supernatant PAOA



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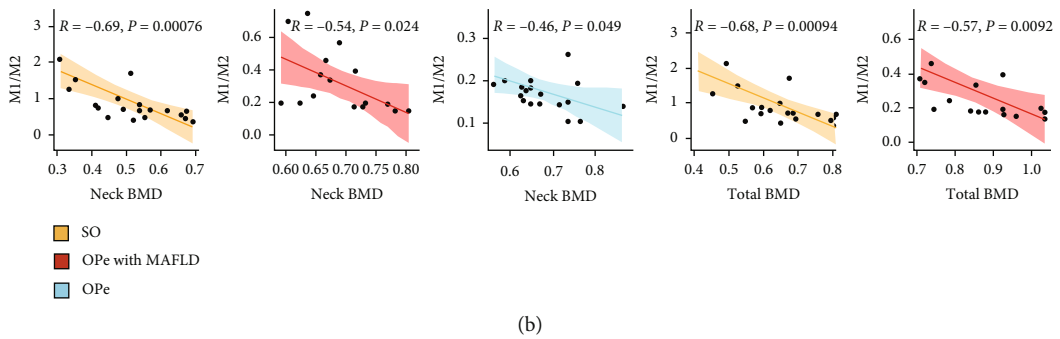

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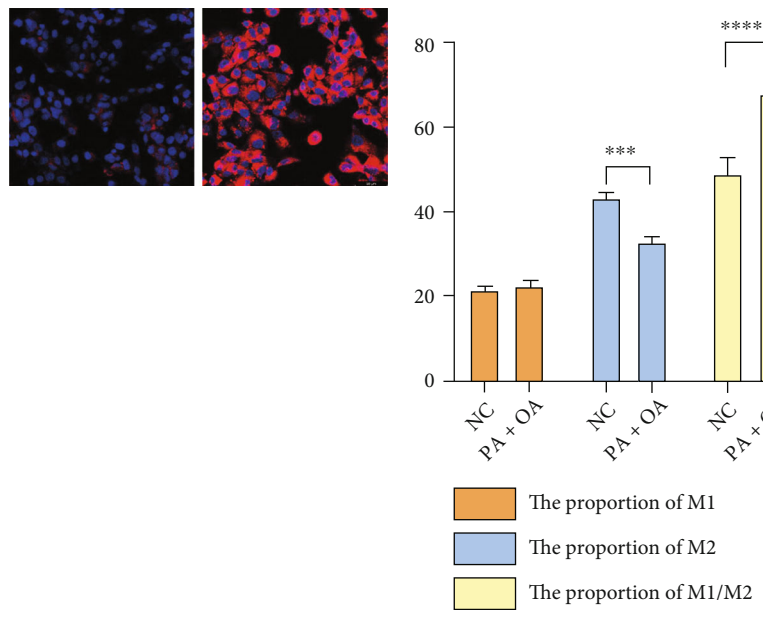

(c) (d)

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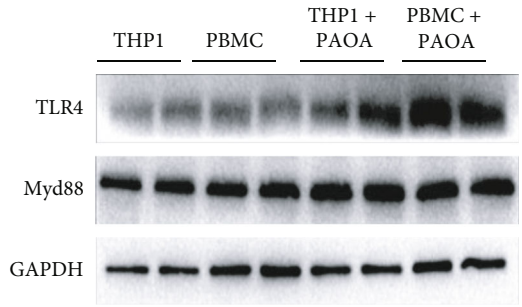


(e)

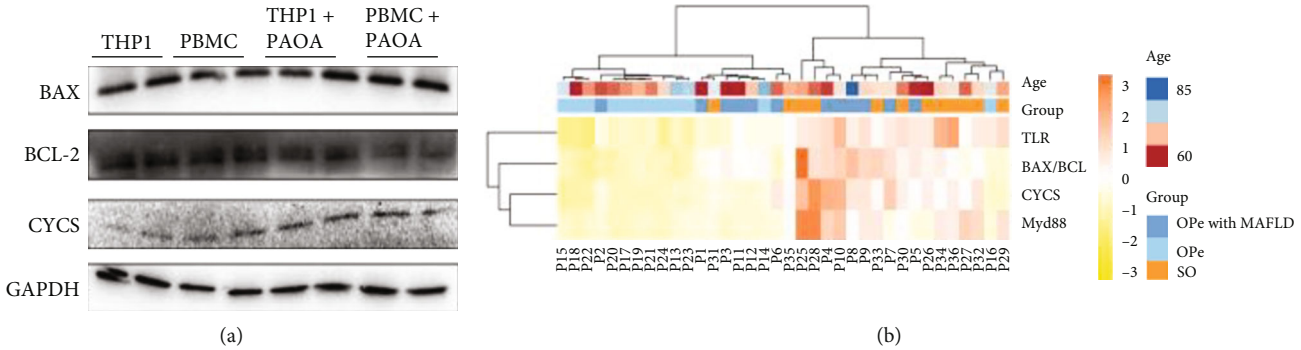
Figure 4: The proportion of peripheral blood-derived macrophages differed between OPe and OPe with MAFLD patients. (a) FCA is performed to compare the M1-like and M2-like monocytes in PBMCs among SO, OPe with MAFLD, and OPe patients. (b) Linear regression analysis showing the relationship between M1-like/M2-like monocytes ratio and bone density-related parameters (neck BMD and total BMD). (c) The validation of fatty liver cell model based on HepG2 cells by Nile red. (d) The proportion of M1 and M2 and M1-M2 ratio is detected by FCA after inducing M0 by conditional media and the supernatant of HepG2 fatty liver model. (e) The protein level of TLR4 and Myd88 in THP-1 induced by PMA and CD14 + momocytes in human peripheral blood are detected by WB. Bars, means SE; $*$, $P < 0.05$; $**$, $P < 0.01$; $***$, $P < 0.005$, $****$, $P < 0.0001$. Abbreviations: FCA: flow cytometric analysis; PBMC: peripheral blood monocytes cell; SO: severe osteoporosis; OPe: osteopenia; MAFLD: metabolic dysfunction-associated fatty liver disease; BMD: bone mineral density; THP-1: human myeloid leukemia mononuclear cells; HepG2: human hepatocellular carcinoma cells; TLR4: toll-like receptor 4; PMA: phorbol 12-myristate 13-acetate; PA + OA: palmitic acid and oleic acid.

intervention, while the expression of B-cell lymphoma-2 (BCL-2) was decreased (Figure 5(a)). Furthermore, the heat map analysis revealed that the relative BAX/BCL-2, CYCS, TLR4, and MyD88 mRNA expressions in PBMCs from OPe patients with MAFLD group and SO group patients were elevated as compared to OPe patients (Figure 5(b)), which were further confirmed by IF staining that observed CYCS upregulation in THP-1 cells after the intervention (Figure 5(c)).

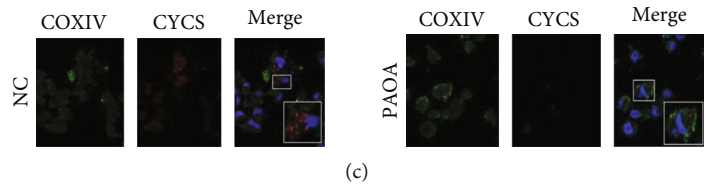
Owing to the fact that mitochondria, as highly dynamic organelles, can adjust their morphology depending on the energy demand and metabolic conditions in the majority of cells, the CD14+ PBMCs bioenergetics analysis used the Seahorse XF Extracellular Flux Analyzer, which simultaneously quantifies two energetic pathways-glycolysis denoted by ECAR and oxidative phosphorylation measured by OCR, suggesting that CD14+ PBMCs from OPe patients with MAFLD group consumed oxygen at a lower basal level and produced less adenosine 5-triphosphate (ATP), when compared to patients in OPe and SO groups, and showed the least OCR increase in response to FCCP, resulting in a significant decrease in maximum respiratory capacity (Figure 5(d)) followed by the analysis of the mitochondria's state in CD14 + monocytes by TEM that indicated severe mitochondrial dysfunction developed in the CD14+ monocytes in MAFLD patients along with swelling up of mitochondrial cristae (Figure 5(e)). The oxidative stress was assessed by intracellular ROS quantification, especially mtROS (Figure 5(f)), exhibiting that the mtROS level in CD14+ PBMCs in OPe patients with the MAFLD group was the highest among all.

Mitochondrial function was also assessed by the MMP and MPTP using flow cytometry detection analysis, which revealed that the mitochondrial membrane potential of PBMCs in OPe patients with MAFLD was lower while the membrane permeability was higher than the OPe group without MAFLD (Figures 6(a)–6(c)). The protein and gene expressions of fission proteins like dynamin-related protein 1 (DRP1), mitochondrial fission 1 protein (FIS1), mitochondrial fission factor (MFF), a mitochondrial dynamic fusion protein, and mitochondrial elongation factor 1 (MIEF1) were evaluated by RT-PCR and WB. The data revealed decreased DRP1 and MIEF1 levels, with a concurrent increase in MFF and FIS1 levels in CD14+ PBMCs in OPe patients with MAFLD and SO patients compared to the OPe group (Figures 6(d) and 6(e)). A subsequent WB test on extracted mitochondria from CD14+ PBMCs from all groups revealed that, while there was no difference in FIS1 expression in PBMC mitochondria in both OPe and OPe with MAFLD patients, DRP1 and mitofusin 2 (MFN2) expression levels decreased significantly (Figure 6(f)).

3.5. SIRT1 Defects Accentuate Impaired Mitochondrial Monocytes in the Elderly Osteopenia Patients. The SIRT1 expression level in OPe patients with MAFLD and without MAFLD was evaluated by IHC and WB to investigate the role of SIRT1 (Figure 7(a)). It was observed that SIRT1 expression level was reduced in liver tissue from MAFLD patients (Figure 7(b)). An RT-PCR assessment of mRNA expression profile in PBMCs obtained from all included patients showed that patients in OPe with MAFLD and SO group were clustered into one group according to the system cluster and hierarchical cluster analysis. The relative SIRT1 mRNA expression in PBMCs from OPe patients was significantly higher than that in OPe with MAFLD group and SO group (Figure 7(c)). The heat map was also utilized to exhibit the associations between the SIRT1 expression and various clinical indices such as BMD, limb muscle strength assessment, waistline, calf girth in three group patients, which suggested that the SIRT1 level was highly correlated with neck BMD ($r = 0.5$), total BMD ($r = 0.5$) and six-minute walking distance ($r = 0.68$) (Figure 7(d)).



(a) (b)



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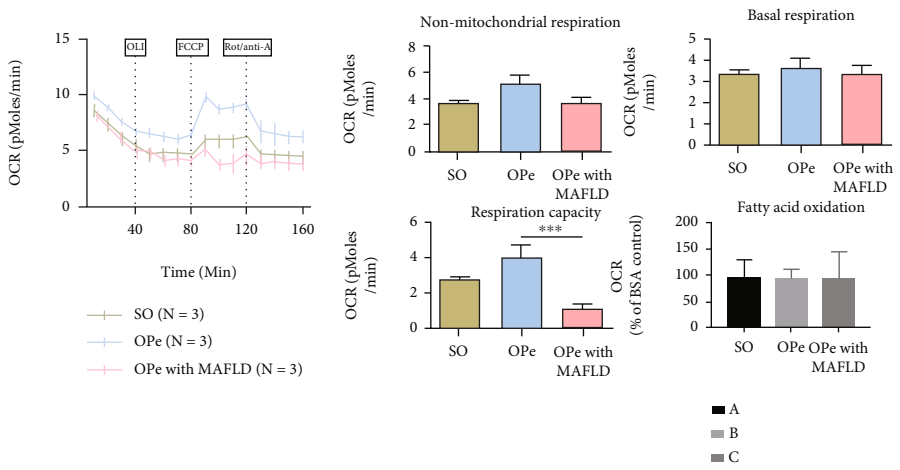

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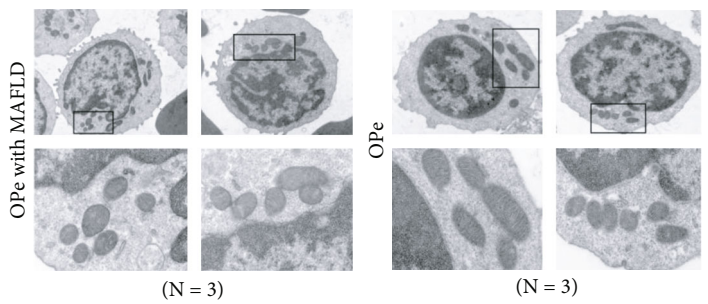

(e)

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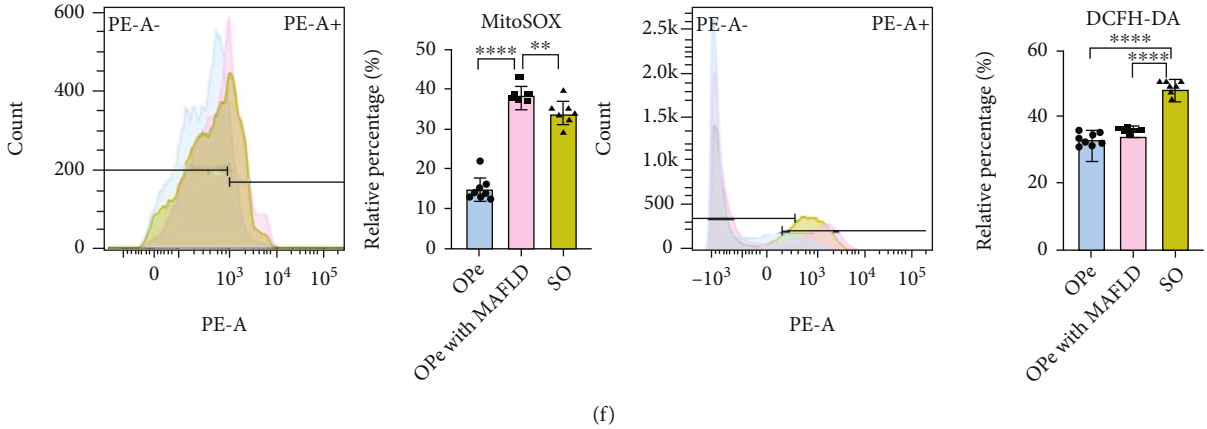


(f)

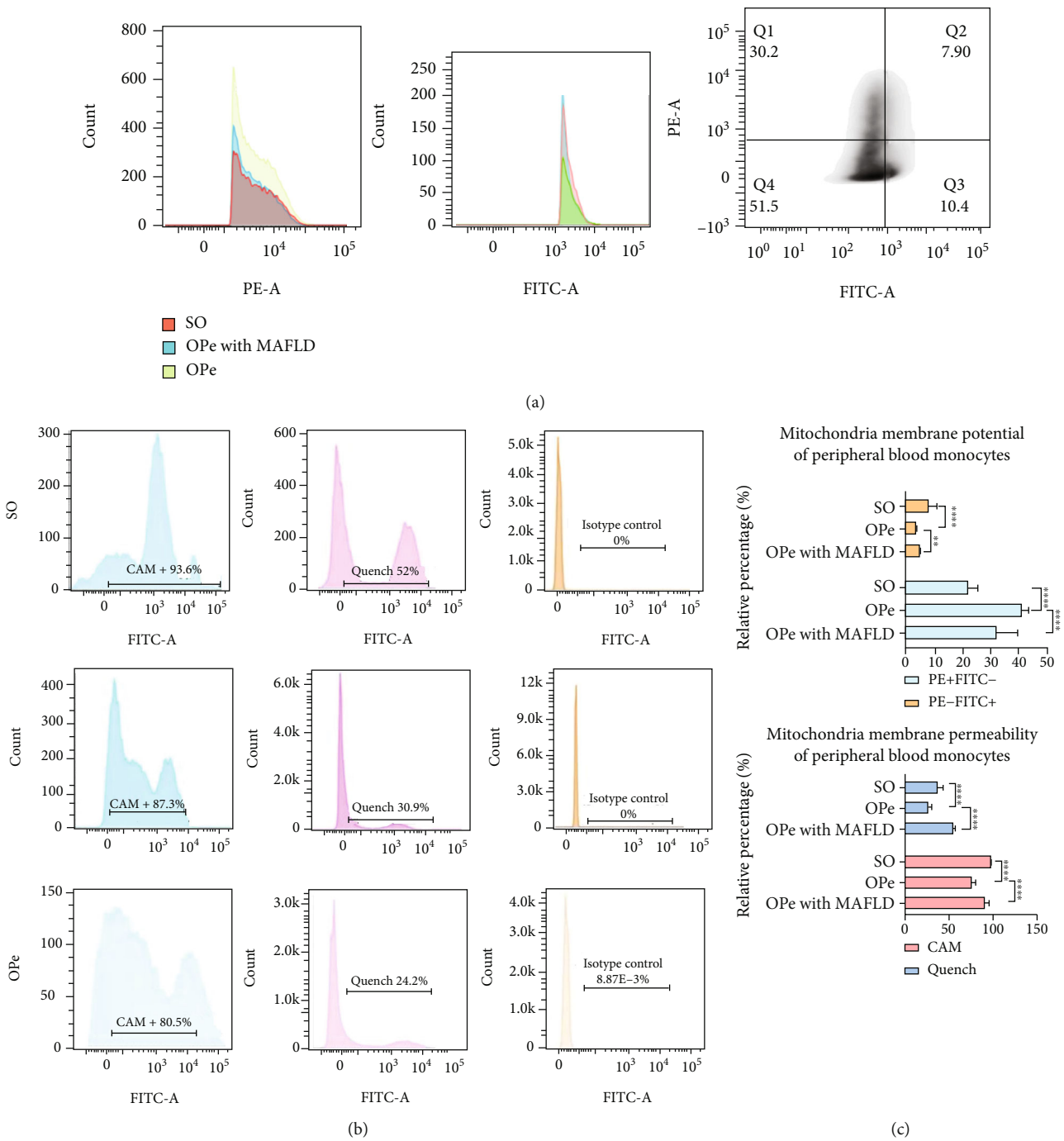
Figure 5: MAFLD impaired aerobic respiration and mitochondrial morphology of CD14+ monocytes in peripheral blood of patients with osteopenia. (a) The protein level of BAX, BLC2, and CYCS in Thp-1 induced by PMA and CD14 + momocytes in human peripheral blood is detected by WB. (b) In the heat map, the relative mRNA level of TLR4, BAX/BCL-2, CYCS, and Myd88 in PBMCs from OPe, OPe with MAFLD, and SO patients is determined by RT-PCR. (c) Representative image from immunofluorescence staining assay of COXIV and CYCS in THP-1 induced by PMA and the supernatant of HepG2 fatty liver model. COXIV (green), CYCS (red). (d) The OCR and ECAR in CD14 + PBMCs from the patients among OPe, OPe with MAFLD, and SO are detected by Seahorse XF. The bar plots indicate the quantitation of non-mitochondrial respiration, basal respiration, respiration capacity, and fatty acid oxidation among OPe, OPe with MAFLD, and SO. (e) The mitochondrial microstructure and morphology of CD14+ PBMCs from OPe and OPe with MAFLD patients are observed via transmission electron microscopy. (f) The level of mitochondrial ROS and intracellular ROS is assessed by FCA among OPe, OPe with MAFLD, and SO. The quantitative results showed right. Bars, means SE; $*$, $P < 0.05$; $**$, $P < 0.01$; $***$, $P < 0.005$; $****$, $P < 0.0001$. Abbreviations: TLR4: toll-like receptor 4; MyD88: myeloid differentiation factor 88; BCL-2: B-cell lymphoma-2; BAX: BCL2-associated X protein; CYCS: Cytochrome C; COXIV: Cytochrome c oxidase subunit IV; ROS: reactive oxygen species; PBMC: peripheral blood monocytes cell; SO: severe osteoporosis; OPe: osteopenia; OPe with MAFLD: osteopenia with metabolic dysfunction-associated fatty liver disease.

To investigate the role of SIRT1 in macrophage mitochondrial function further, CD14+ PBMCs extracted from OPe patients were treated with the supernatant from HepG2 cells treated in vitro with PAOA. Meanwhile, a decreased SIRT1 expression in CD14+ PBMCs by siRNA was used before the stimulation of PAOA, which observed that the DRP1, MIEF1, MFN1, MFN2 (mitochondrial fusion proteins), and BCL-2 (mitochondrial anti-apoptotic protein) levels were decreased after the intervention, whereas the MFF, FIS1 (mitochondrial fission proteins) along with CYCS and BAX (mitochondrial apoptosis-related proteins) levels were upregulated in WB results (Figure 7(e)) and were further substantiated by IF that DRP1 downregulation and CYCS upregulation after the intervention that might be due to downregulation of SIRT1 expression by siRNA (Figure 7(f)). Moreover, TLR4 and MyD88 protein levels also increased after intervention, thereby indicating that SIRT1 expression is crucial for modulating the mitochondrial function of peripheral blood-derived macrophages in OPe patients (Figure 7(e)).

4. Discussion

Metabolic diseases have always been thought to be a significant risk factor for osteoporosis, whereas fatty liver disease is usually associated with metabolic dysfunction and is a major predisposing factor in obesity and diabetes, leading to chronic inflammation [30, 31]. Our study results revealed weaker walking ability in OPe patients with MAFLD than those without MAFLD. Several studies have proposed that pro-inflammatory cytokines secreted after macrophage polarization might contribute to skeletal muscle aging [32]. Oxidative stress is an important mechanism of osteoporosis. Free radicals affect the differentiation, function, and apoptosis of osteoblasts and osteoclasts by regulating signal pathways or inducing inflammatory reactions. Antioxidants can effectively prevent and treat osteoporosis [33]. Initial research, led by Narayan Avadhani of the University of Pennsylvania, concluded that when mitochondrial function is affected, it will not only affect energy production, but also trigger a stress signal that induces excessive production of osteoclasts. In the future, they will study how to prevent osteoporosis by protecting mitochondrial function [34]. Furthermore, recent studies indicate that mitochondrial regulators/nutrients from natural products can remedy mitochondrial dysfunction mediated by MAFLD [35, 36]. Meanwhile, a number of researches nowadays are developing innovative drugs for the prevention and treatment of fatty liver diseases based on mitochondrial dysfunction [37]. The role of mitochondria as the metabolic center of cells in regulating macrophage function has been gradually revealed [38]. This study attempts to clarify whether MAFLD reduces the walking ability of patients by affecting the mitochondrial function of peripheral blood-derived macrophages in the elderly OPe patients.

Fragility fracture is a complete fracture caused by a spontaneous or slight external force, which is the most serious consequence of osteoporosis [39]. In our study, all participants were divided into three groups: OPe (including osteoporosis) patients with MAFLD (OPe with MAFLD), OPe



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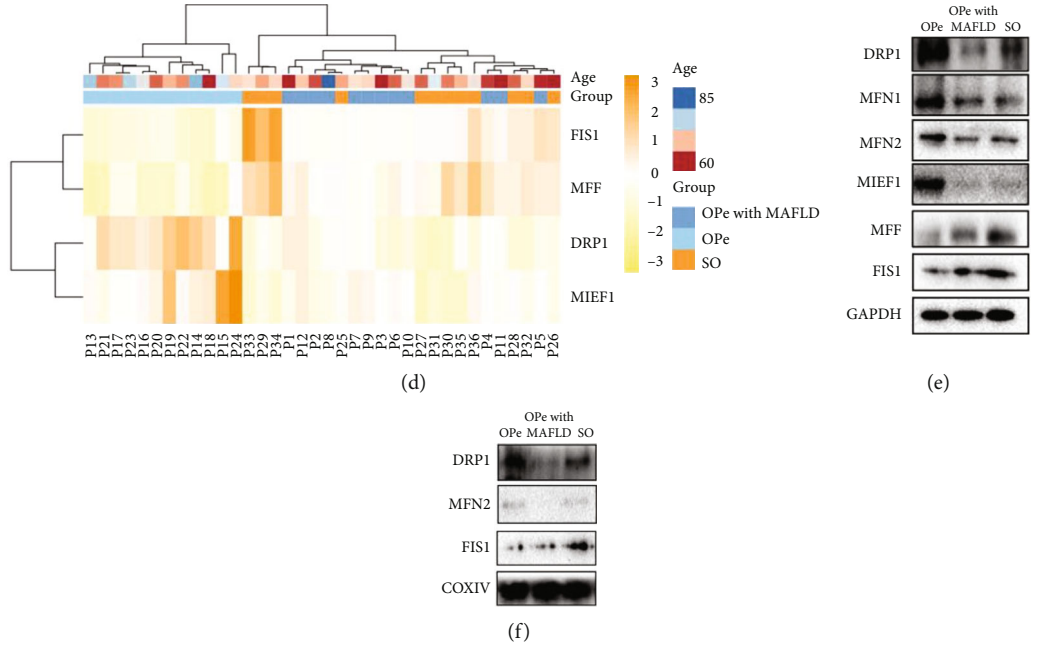
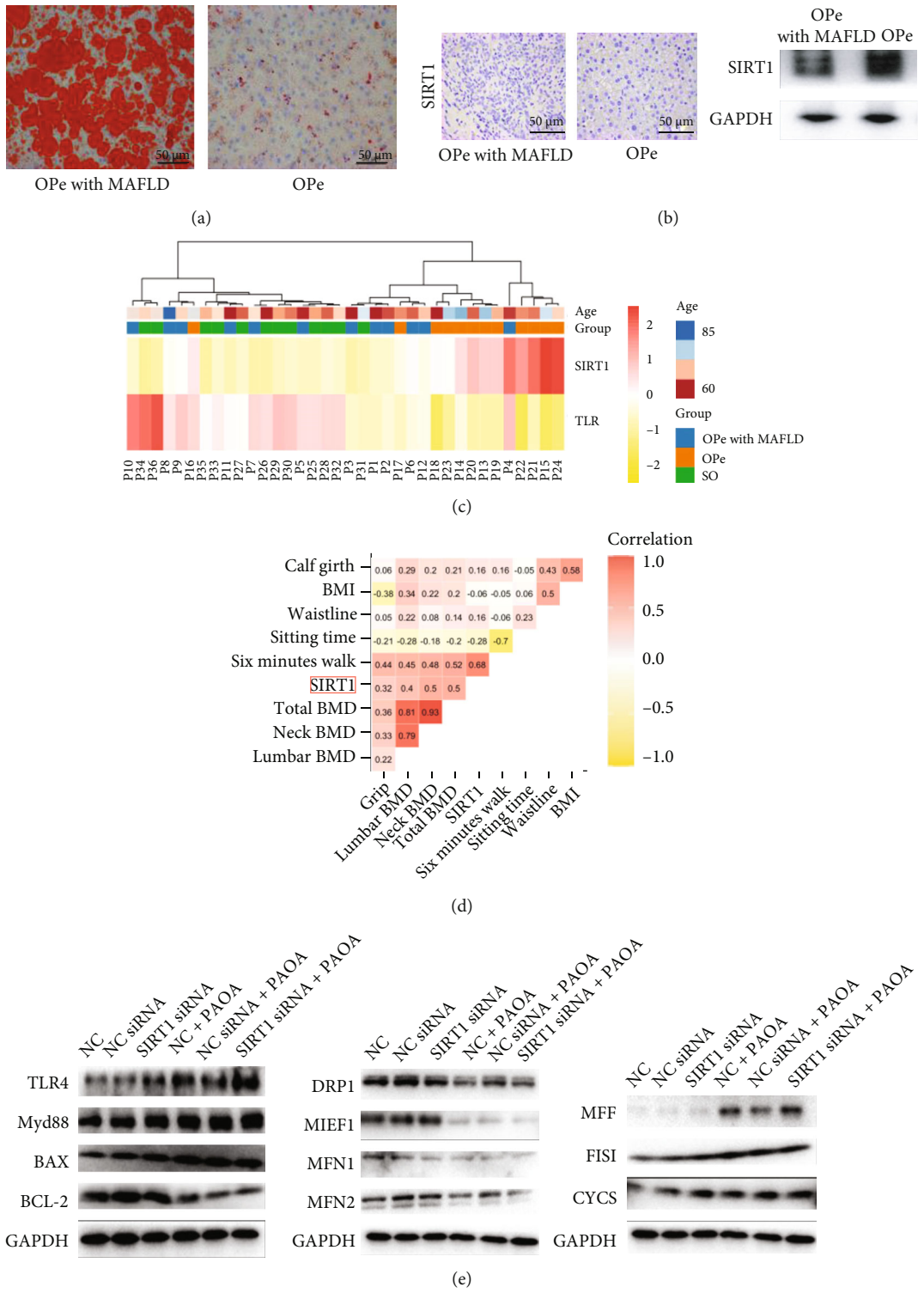


Figure 6: MAFLD impairs the function of CD14 + mononuclear mitochondria in peripheral blood of osteopenia patients. (a, b) MMP and MPTP of PBMCs from the patients among OPe, OPe with MAFLD, and SO based on FCA. (c) The quantitative results of MMP and MPTP are, respectively, presented in bar plots. (d) In the heat map, the relative mRNA level of FIS1, MFF, DRP1, and MIEF1 in PBMCs from the patients among OPe, OPe with MAFLD, and SO group is determined by RT-PCR. (e) The protein level of mitochondrial fusion and division-related protein in the PBMCs from patients among OPe, OPe with MAFLD, and SO group detected by WB. (f) The protein level of mitochondrial fusion and division-related protein in the mitochondrial of PBMCs from patients among OPe, OPe with MAFLD, and SO group detected by WB. Bars, means SE; $*$, $P < 0.05$; $**$, $P < 0.01$; $***$, $P < 0.005$; $****$, $P < 0.0001$. Abbreviations: MMP: mitochondrial membrane potential; MPTP: mitochondrial permeability transition pore; PBMC: peripheral blood monocytes cell; SO: severe osteoporosis; OPe: osteopenia; OPe with MAFLD: osteopenia with metabolic dysfunction-associated fatty liver disease; DRP1: dynamin-related protein 1; FIS1: mitochondrial fission 1 protein; MFF: mitochondrial fission factor; MIEF1: mitochondrial elongation factor 1; MFN1: mitofusin 1; MFN2: mitofusin 2; COXIV: Cytochrome c oxidase subunit IV.

patients (including osteoporosis) without MAFLD, and SO patients without MAFLD who experienced fragility fractures. Activation of the monocyte-macrophage system is an important feature of chronic inflammation, which was found by the flow cytometry that the M1/M2 ratio in PBMCs in SO patients was the highest among the three groups. Further analysis of the OPe patients with and without MAFLD denoted that the M1/M2 ratio in the MAFLD group was higher than that in the group without MAFLD, although it was not statistically significant. TLR4 is mainly expressed in cells involved in host defense function, such as monocyte-macrophages, dendritic cells, and lymphocytes, which mediate chronic inflammation [12], along with an essential adapter protein, MyD88, which is crucial for all TLRs except TLR3 in the innate immunity system [40]; our results displayed that the TLR4 and MyD88 expressions in PBMCs obtained from MAFLD patients were significantly higher than those without MAFLD.

The cells of the innate immune system, including macrophages and antigen-presenting cells, play a vital role in providing host resistance to infection and promoting inflammatory response [41], which were also corroborated by various studies that the mitochondrial morphological changes were involved in the regulation of cellular metabolism, which may indirectly affect the activation and response of immune cells [42, 43] along with the presence of mtROS, produced by electron transport chain (ETC) can trigger innate immune signals or cause immune cell damage in accordance with the measure and timing of their production [44]. Chronic inflammation results in the release of a substantial number of cellular mtROS into the blood, thereby interfering with their functions and disrupting intercellular communication [45]. Although ROS levels were comparable in patients with and without MAFLD, mtROS levels in peripheral blood of OPe patients with MAFLD were significantly higher than those without MAFLD. It was also evident that JC-1, mitochondrial permeability test, TEM, and WB tests also stated that peripheral blood macrophages in OPe patients with MAFLD possessed more damaged mitochondria than other groups.

Mitochondrial oxidative phosphorylation (OXPHOS) provides sufficient energy to perform all cellular tasks through aerobic metabolism, which converts energy substrate into energy stored in the ATP. Although OXPHOS carries out electron transfer in the mitochondrial membrane respiratory chain to produce ATP [46], inflammatory macrophages sometimes enhance glycolytic metabolism and inhibit mitochondrial OXPHOS [47]. The present study employed seahorse XF Analyzer to detect oxidative phosphorylation and glycolysis of $CD14^+$ PBMCs obtained from



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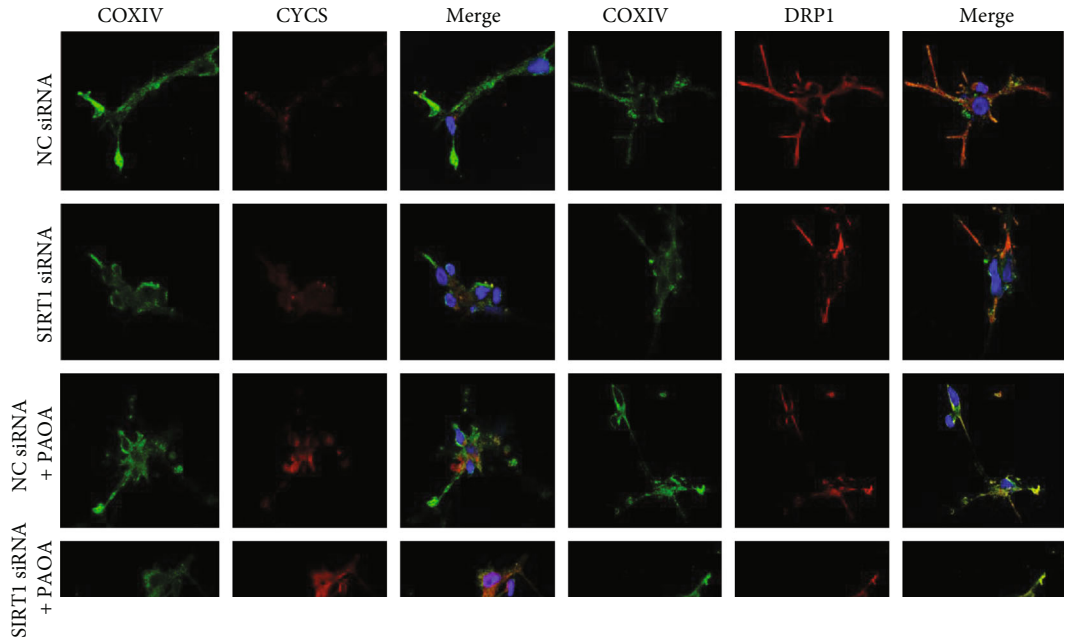

(f)

Figure 7: SIRT1 defects accentuated impaired monocyte mitochondria in osteopenia patients. (a) The accumulation of lipid droplets of liver from OPe and OPe with MAFLD patients is assessed by Red Oil O staining. (b) Immunohistochemical detection of SIRT1 in the liver from OPe and OPe with MAFLD patients. The protein level of SIRT1 in liver tissue is assessed by WB. (c) The relative mRNA level of SIRT1 and TLR4 in PBMCs from the patients among OPe, OPe with MAFLD, and SO group is detected by RT-PCR. (d) The correlation matrix shows the relationship between the expression of SIRT1 and clinical indicators of patients. (e) The protein level of mitochondrial apoptosis, fusion and division-related protein is detected using WB. (f) Representative image for immunofluorescence staining shows the expression and distribution of COXIV and CYCS in PBMCs intervened by various conditioned medium. Abbreviations: SIRT1: the silent mating type information regulation 1; TLR4: toll-like receptor 4; MyD88: myeloid differentiation factor 88; BCL-2: B-cell lymphoma-2; BAX: BCL2-associated X protein; CYCS: Cytochrome C; DRP1: dynamin-related protein 1; FIS1: mitochondrial fission 1 protein; MFF: mitochondrial fission factor; MIEF1: mitochondrial elongation factor 1; MFN1: mitofusin 1; MFN2: mitofusin 2; COXIV: Cytochrome c oxidase subunit IV; PAOA: palmitic acid and oleic acid; BMI: body mass index; BMD: bone mineral density; SO: severe osteoporosis; OPe: osteopenia; OPe with MAFLD: osteopenia with metabolic dysfunction-associated fatty liver disease.

all three groups and suggested that the monocyte respiratory capacity in patients with OPe with MAFLD is the lowest among all, even when compared to SO patients.

Mitochondria are complex dynamic organelles that perform many functions related to cell metabolism and homogeneous stability [48] and bear a close association with the process of aging [49] that might reduce mitochondrial integrity as well as dysfunction of the fusion-fission cycle, resulting in the accumulation of a large number of abnormal mitochondria, leading to an increase in oxidative stress and resulting in defective autophagy [50]. As the center of cell energy metabolism, the mitochondrial shape constantly changes through different fusion and fission cycles to adapt to the varied energy needs of the different environments [51]. Mitochondrial key proteins that induce fusion and fission and dynamic proteins play critical regulatory roles in the process of constitutive fission and fusion reactions, which maintain steady-state mitochondrial morphology [52]. The present study assessed the relative mRNA expression and protein level of genes related to mitochondrial fusion-fission cycle in PBMCs of all the three group patients by RT-PCR and WB, and proved that the expression levels of mitochondrial fusion and division-related proteins was abnormal in PBMCs obtained from patients with MAFLD and SO patients when compared with patients without MAFLD.

As a regulator of various cellular and body processes, including metabolism, immune response, and aging, SIRT1 remains the most studied member of this class of proteins engaging in gene regulation [53]. SIRT1, a NAD-dependent histone deacetylase, plays a vital role in hepatic steatosis and inflammation [54], along with active participation in other cellular events like metabolism, inflammatory response, cell aging, and apoptosis through a variety of signaling pathways. In our study, it was reflected that the SIRT1expression was lesser in the liver tissue of OPe patients with MAFLD than patients without MAFLD. In order to further clarify the role of SIRT1, we observed the effects of SIRT1 siRNA on PBMCs obtained from OPe patients on cell signaling and the mitochondrial function of cells by WB and IF, which suggested that SIRT1 knockdown aggravated the mitochondrial damage of induced monocytes in vitro, which might be related to the TLR4 signal activation.

"Inflammatory aging" is the most common manifestation of abnormal intercellular communication. Age-related dysfunction and immune system decline can stimulate a

large number of immune cells to produce numerous inflammatory factors and cause chronic inflammation, potentially accelerating the aging process. Our study revealed that OPe patients with MAFLD had increased levels of plasma pro-inflammatory factors like IL-6 and IL-8 and higher M1/M2% as compared to the OPe patients without MAFLD. To conclude, our results suggest that MAFLD itself may aggravate the inflammatory state of the elderly OPe patients, which may be related to the mitochondrial homeostasis imbalance in peripheral blood-derived macrophages that might lead to the decreased walking ability of patients.

Abbreviations

MAFLD: Metabolic dysfunction-associated fatty liver disease
NAFLD: Nonalcoholic fatty liver disease
FLD: Fatty liver disease
OPe: Osteopenia
SO: Severe osteoporosis
T2DM: Type 2 diabetes mellitus
BMD: Bone mineral density
WHO: World Health Organization
PSQI: Pittsburgh sleep quality index
MNA-SF: Mini nutritional assessment-short form
SPPB: Short physical performance battery
FTSST: Five times sit to stand test
BW: Body weight
BH: Body height
BMI: Body mass index
eGFR: Estimated Glomerular Filtration Rate
TG: Triglycerides
TC: Total cholesterol
Scr: Serum creatinine
SUA: Serum uric acid
BUN: Blood urea nitrogen
HDL: High-density lipoprotein
LDL: Low-density lipoprotein
ALT: Alanine aminotransferase
AST: Aspartate transaminase
P: Blood phosphorus
Ca: Blood calcium
P1NP: N-Propeptide of Type I Procollagen
CROSSL: β-Crosslaps
PTH: Parathyroid hormone
OSTEOC: Osteocalcin
CRP: C reactive protein
KEGG: Kyoto Encyclopedia of Genes and Genomes
GEO: Gene Expression Omnibus
GO: Gene Oncology
DisGeNET: Gene disease database
PPI: Protein-protein interaction
DEGs: Differentially expressed genes
ImmPort: The Immunology Database and Analysis Portal
THP-1: Human myeloid leukemia mononuclear cells
PBMC: Peripheral blood mononuclear cells
HepG2: Human hepatocellular carcinoma cells
DMEM: Dulbecco's modified eagle medium
FBS: Fetal bovine serum
RT: Room temperature
BSA: Bovine serum albumin
PBS: Phosphate buffer saline
BCA: Bicinchoninic acid
FFAs: Free fatty acids
PFA: Paraformaldehyde
PAOA: Palmitic acid and oleic acid
FCCP: Carbonyl cyanide p-(trifluoromethoxy)phenylhydrazone
2-DG: 2-deoxy-D-glucose
TEM: Transmission electron microscopy
FCM: Flow cytometry
ROS: Reactive oxygen species
mtROS: Mitochondrial reactive oxygen species
MPTP: Mitochondrial permeability transition pore
MMP: Mitochondrial membrane potential
ECAR: Extracellular acidification rate
OCR: Oxygen consumption rate
WB: Western blotting
IHC: Immunohistochemistry
IF: Immunofluorescence
ELISA: Enzyme-linked immunosorbent assay
IL-6: Interleukin-6
TNF-α: Tumor necrosis factor-α
CCL2: Chemokine (C-C motif) ligand 2
HIF-1: Hypoxia-inducible factor 1
SIRT1: The silent mating type information regulation 1
TLR4: Toll-like receptor 4
MyD88: Myeloid differentiation factor 88
BCL-2: B-cell lymphoma-2
BAX: BCL2-associated X protein
CYCS: Cytochrome C
DRP1: Dynamin-related protein 1
FIS1: Mitochondrial fission 1 protein
MFF: Mitochondrial fission factor
MIEF1: Mitochondrial elongation factor 1
MFN1: Mitofusin 1
MFN2: Mitofusin 2
COXIV: Cytochrome c oxidase subunit IV
siRNA: Small interfering RNA
ETC: Electron transport chain
OXPHOS: Oxidative phosphorylation
ATP: Adenosine 5-triphosphate.

Data Availability

The data used to support the findings of this study are available from the corresponding authors upon request.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Contributions

Xiaojun Wang and Xuanqi Liu contributed equally to this study.

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Supplementary Materials

Supplement 1. The process of Macrophage flowchart. Supplement 2. Negative results of correlation between M1/M2% and bone mineral density. Supplement 3. The sequences of all the primers used. Supplement 4. All IF and WB antibodies used in this study. *(Supplementary Materials)*

References

[1] G. Shiha, M. Korenjak, W. Eskridge et al., "Redefining fatty liver disease: an international patient perspective," *The Lancet Gastroenterology & Hepatology*, vol. 6, no. 1, pp. 73–79, 2021.

[2] A. Mantovani and L. Valenti, "A call to action for fatty liver disease," *Liver International*, vol. 41, no. 6, pp. 1182–1185, 2021.

[3] Y. Nan, J. An, J. Bao et al., "The Chinese Society of Hepatology position statement on the redefinition of fatty liver disease," *Journal of Hepatology*, vol. 75, no. 2, pp. 454–461, 2021.

[4] Z. Gu, Y. Bi, F. Yuan et al., "Polymorphisms are associated with metabolic dysfunction-associated fatty liver disease (MAFLD) susceptibility in the older Chinese Han population," *Clinical Interventions in Aging*, vol. 15, pp. 1333–1341, 2020.

[5] J. A. Morris, J. P. Kemp, S. E. Youlten et al., "An atlas of genetic influences on osteoporosis in humans and mice," *Nature Genetics*, vol. 51, no. 2, pp. 258–266, 2019.

[6] K. Mikami, T. Endo, N. Sawada et al., "Association of bone metabolism with fatty liver disease in the elderly in Japan: a community-based study," *Internal Medicine*, vol. 59, no. 10, pp. 1247–1256, 2020.

[7] S. H. Loosen, C. Roderburg, M. Demir et al., "Non-alcoholic fatty liver disease (NAFLD) is associated with an increased incidence of osteoporosis and bone fractures," *Zeitschrift für Gastroenterologie*, 2021.

[8] S. Ciardullo, E. Muraca, F. Zerbini, G. Manzoni, and G. Perseghin, "NAFLD and liver fibrosis are not associated with reduced femoral bone mineral density in the general US population," *The Journal of Clinical Endocrinology and Metabolism*, vol. 106, no. 8, pp. e2856–e2865, 2021.

[9] N. Guanabens and A. Pares, "Osteoporosis in chronic liver disease," *Liver International*, vol. 38, no. 5, pp. 776–785, 2018.

[10] J. P. van den Bergh, T. A. van Geel, and P. P. Geusens, "Osteoporosis, frailty and fracture: implications for case finding and therapy," *Nature Reviews Rheumatology*, vol. 8, no. 3, pp. 163–172, 2012.

[11] D. Bassi-Dibai, A. V. Dibai-Filho, L. P. Carvalho et al., "Obesity, but not metabolic control, is associated with muscle strength and endurance in diabetic older adults," *Physiotherapy Research International*, vol. 25, no. 1, article e1808, 2020.

[12] A. Hasan, N. Akhter, A. Al-Roub et al., "TNF-α in combination with palmitate enhances IL-8 production via the MyD88- independent TLR4 signaling pathway: potential relevance to metabolic inflammation," *International Journal of Molecular Sciences*, vol. 20, no. 17, p. 4112, 2019.

[13] C. J. Li, Y. Xiao, Y. C. Sun et al., "Senescent immune cells release grancalcin to promote skeletal aging," *Cell Metabolism*, vol. 33, no. 10, pp. 1957–1973, 2021.

[14] G. Zhang and J. Liu, "Targeting senescent immune cells to rejuvenate the aging skeleton," *Cell Metabolism*, vol. 33, no. 10, pp. 1903–1905, 2021.

[15] N. Nandagopal, A. Jambhekar, and G. Lahav, "Preparing macrophages for the future," *Science*, vol. 372, no. 6548, pp. 1263-1264, 2021.

[16] J. Baardman, S. G. S. Verberk, S. van der Velden et al., "Macrophage ATP citrate lyase deficiency stabilizes atherosclerotic plaques," *Nature Communications*, vol. 11, no. 1, p. 6296, 2020.

[17] A. Santoro, E. Bientinesi, and D. Monti, "Immunosenescence and inflammaging in the aging process: age-related diseases or longevity?," *Ageing Research Reviews*, vol. 71, article ???, 2021.

[18] J. A. G. Hamilton, M. Y. Lee, R. Hunter et al., "Interleukin-37 improves T-cell-mediated immunity and chimeric antigen receptor T-cell therapy in aged backgrounds," *Aging Cell*, vol. 20, no. 2, article e13309, 2021.

[19] Y. Deng, Q. Li, M. Li, T. Han, G. Li, and Q. Liu, "Network pharmacology identifies the mechanisms of Sang-Xing-Zhi-Ke-Fang against pharyngitis," *Evidence-based Complementary and Alternative Medicine*, vol. 2020, Article ID 2421916, 2020.

[20] G. Yu, L. G. Wang, Y. Han, and Q. Y. He, "clusterProfiler: an R package for comparing biological themes among gene clusters," *OMICS*, vol. 16, no. 5, pp. 284–287, 2012.

[21] J. A. Kanis and J. A. Kanis, "Assessment of fracture risk and its application to screening for postmenopausal osteoporosis: synopsis of a WHO report," *Osteoporosis International*, vol. 4, no. 6, pp. 368–381, 1994.

[22] J. M. Guralnik, L. Ferrucci, C. F. Pieper et al., "Lower extremity function and subsequent disability: consistency across studies, predictive models, and value of gait speed alone compared with the short physical performance battery," *The Journals of Gerontology*, vol. 55, no. 4, pp. M221–M231, 2000.

[23] D. Liu, P. Zhang, J. Zhou et al., "TNFAIP3 interacting protein 3 overexpression suppresses nonalcoholic steatohepatitis by blocking TAK1 activation," *Cell Metabolism*, vol. 31, no. 4, pp. 726–740.e8, 2020.

[24] K. J. Livak and T. D. Schmittgen, "Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method," *Methods*, vol. 25, no. 4, pp. 402–408, 2001.

[25] S. Ranjbarvaziri, K. B. Kooiker, M. Ellenberger et al., "Altered cardiac energetics and mitochondrial dysfunction in hypertrophic cardiomyopathy," *Circulation*, vol. 144, no. 21, pp. 1714–1731, 2021.

[26] L. Y. Yang, J. L. Gao, T. Gao et al., "Toxicity of polyhydroxylated fullerene to mitochondria," *Journal of Hazardous Materials*, vol. 301, pp. 119–126, 2016.

[27] L. Troiano, R. Ferraresi, E. Lugli et al., "Multiparametric analysis of cells with different mitochondrial membrane potential during apoptosis by polychromatic flow cytometry," *Nature Protocols*, vol. 2, no. 11, pp. 2719–2727, 2007.

[28] X. L. Wang, L. Wang, F. L. Lin, S. S. Li, T. X. Lin, and R. W. Jiang, "Protective effect of penetratin analogue-tagged SOD1 on cisplatin-induced nephrotoxicity through inhibiting oxidative stress and JNK/p38 MAPK signaling pathway," *Oxidative Medicine and Cellular Longevity*, vol. 2021, Article ID 5526053, 2021.

[29] X. Zhou, L. Yong, Y. Huang et al., "The protective effects of distal ischemic treatment on apoptosis and mitochondrial permeability in the hippocampus after cardiopulmonary resuscitation," *Journal of Cellular Physiology*, vol. 233, no. 9, pp. 6902–6910, 2018.

[30] H. Charles-Messance, K. A. J. Mitchelson, C. E. De Marco, F. J. Sheedy, and H. M. Roche, "Regulating metabolic inflammation by nutritional modulation," *The Journal of Allergy and Clinical Immunology*, vol. 146, no. 4, pp. 706–720, 2020.

[31] J. W. Guo, X. Liu, T. T. Zhang et al., "Hepatocyte TMEM16A deletion retards NAFLD progression by ameliorating hepatic glucose metabolic disorder," *Advanced Science*, vol. 7, no. 10, p. 1903657, 2020.

[32] Y. Y. Chen, T. W. Kao, Y. L. Chiu, T. C. Peng, H. F. Yang, and W. L. Chen, "Association between interleukin-12 and sarcopenia," *Journal of Inflammation Research*, vol. 14, pp. 2019–2029, 2021.

[33] R. Yang, J. Zhang, J. Li et al., "Inhibition of Nrf2 degradation alleviates age-related osteoporosis induced by 1,25-Dihydroxyvitamin D deficiency," *Free Radical Biology & Medicine*, vol. 178, pp. 246–261, 2022.

[34] R. Angireddy, H. R. Kazmi, S. Srinivasan et al., "Cytochrome c oxidase dysfunction enhances phagocytic function and osteoclast formation in macrophages," *The FASEB Journal*, vol. 33, no. 8, pp. 9167–9181, 2019.

[35] K. Qiu, Q. Zhao, J. Wang, G. H. Qi, S. G. Wu, and H. J. Zhang, "Effects of pyrroloquinoline quinone on lipid metabolism and anti-oxidative capacity in a high-fat-diet metabolic dysfunction-associated fatty liver disease Chick model," *International Journal of Molecular Sciences*, vol. 22, no. 3, p. 1458, 2021.

[36] S. Lim, J. W. Kim, and G. Targher, "Links between metabolic syndrome and metabolic dysfunction-associated fatty liver disease," *Trends in Endocrinology and Metabolism*, vol. 32, no. 7, pp. 500–514, 2021.

[37] T. Shi, L. Yu, R. Zhuang et al., "Regulation of mitochondrial function by natural products for the treatment of metabolic associated fatty liver disease," *Canadian Journal of Gastroenterology & Hepatology*, vol. 2021, article 5527315, pp. 1–9, 2021.

[38] M. Z. Nassef, J. E. Hanke, and K. Hiller, "Mitochondrial metabolism in macrophages," *American Journal of Physiology. Cell Physiology*, vol. 321, no. 6, pp. C1070–C1081, 2021.

[39] Y. Chen, M. Yang, W. Huang et al., "Mitochondrial metabolic reprogramming by CD36 signaling drives macrophage inflammatory responses," *Circulation Research*, vol. 125, no. 12, pp. 1087–1102, 2019.

[40] G. F. Sonnenberg and M. R. Hepworth, "Functional interactions between innate lymphoid cells and adaptive immunity," *Nature Reviews. Immunology*, vol. 19, no. 10, pp. 599–613, 2019.

[41] H. Lee, R. J. Fenster, S. S. Pineda et al., "Cell type-specific transcriptomics reveals that mutant huntingtin leads to mitochondrial RNA release and neuronal innate immune activation," *Neuron*, vol. 107, no. 5, pp. 891–908.e8, 2020.

[42] V. Tiku, M. W. Tan, and I. Dikic, "Mitochondrial functions in infection and immunity:," *Trends in Cell Biology*, vol. 30, no. 9, p. 748, 2020.

[43] F. Correa-da-Silva, J. A. S. Pereira, C. F. de Aguiar, and P. M. M. de Moraes-Vieira, "Mitoimmunity—when mitochondria dictates macrophage function," *Cell Biology International*, vol. 42, no. 6, pp. 651–655, 2018.

[44] N. Arulkumaran, S. J. Pollen, R. Tidswell et al., "Selective mitochondrial antioxidant MitoTEMPO reduces renal dysfunction and systemic inflammation in experimental sepsis in rats," *British Journal of Anaesthesia*, vol. 127, no. 4, pp. 577–586, 2021.

[45] C. J. Hall, L. E. Sanderson, L. M. Lawrence et al., "Blocking fatty acid-fueled mROS production within macrophages alleviates acute gouty inflammation," *The Journal of Clinical Investigation*, vol. 128, no. 5, pp. 1752–1771, 2018.

[46] J. Song, N. Pfanner, and T. Becker, "Assembling the mitochondrial ATP synthase," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 115, no. 12, pp. 2850–2852, 2018.

[47] H. G. Sprenger and T. Langer, "The good and the bad of mitochondrial breakups," *Trends in Cell Biology*, vol. 29, no. 11, pp. 888–900, 2019.

[48] R. N. Jadeja, P. M. Martin, and W. Chen, "Mitochondrial oxidative stress and energy metabolism: impact on aging and longevity," *Oxidative Medicine and Cellular Longevity*, vol. 2021, Article ID 9789086, 3 pages, 2021.

[49] H. Li, J. Slone, and T. Huang, "The role of mitochondrial-related nuclear genes in age-related common disease," *Mitochondrion*, vol. 53, pp. 38–47, 2020.

[50] D. Sebastian, M. Palacin, and A. Zorzano, "Mitochondrial dynamics: coupling mitochondrial fitness with healthy aging," *Trends in Molecular Medicine*, vol. 23, no. 3, pp. 201–215, 2017.

[51] D. C. Chan, "Mitochondrial dynamics and its involvement in disease," *Annual Review of Pathology*, vol. 15, pp. 235–259, 2020.

[52] M. Packer, "Longevity genes, cardiac ageing, and the pathogenesis of cardiomyopathy: implications for understanding the effects of current and future treatments for heart failure," *European Heart Journal*, vol. 41, no. 39, pp. 3856–3861, 2020.

[53] H. Amano, A. Chaudhury, C. Rodriguez-Aguayo et al., "Telomere dysfunction induces sirtuin repression that drives telomere-dependent disease," *Cell Metabolism*, vol. 29, no. 6, pp. 1274–1290.e9, 2019, e 1279.

[54] C. Xu, L. Wang, P. Fozouni et al., "SIRT1 is downregulated by autophagy in senescence and ageing," *Nature Cell Biology*, vol. 22, no. 10, pp. 1170–1179, 2020.

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Review Article

Recent Progress of Chronic Stress in the Development of Atherosclerosis

Shang Gao,[1,2] **Xiang Wang**,[2,3] **Ling-bing Meng**,[2,3] **Yuan-meng Zhang**,[4] **Yue Luo**,[5] **Tao Gong**,[6] **De-ping Liu**,[1,2] **Zuo-guan Chen**,[1] **and Yong-jun Li**[1]

[1]*Department of Vascular Surgery, Beijing Hospital, National Center of Gerontology, Institute of Geriatric Medicine, Chinese Academy of Medical Sciences, No. 1 DaHua Road, Dong Dan, Beijing 100730, China*
[2]*Graduate School of Peking Union Medical College and Chinese Academy of Medical Sciences, No. 9 Dongdansantiao, Dongcheng District, Beijing 100730, China*
[3]*Department of Cardiology, Beijing Hospital, National Center of Gerontology, Institute of Geriatric Medicine, Chinese Academy of Medical Sciences, No. 1 DaHua Road, Dong Dan, Beijing 100730, China*
[4]*Department of Internal Medicine, The Third Medical Centre of Chinese PLA General Hospital, The Training Site for Postgraduate of Jinzhou Medical University, Beijing, China*
[5]*Department of Respiratory, The First Affiliated Hospital of Jinzhou Medical University, Jinzhou, Liaoning 121001, China*
[6]*Department of Neurology, Beijing Hospital, National Center of Gerontology, Institute of Geriatric Medicine, Chinese Academy of Medical Sciences, No. 1 DaHua Road, Dong Dan, Beijing 100730, China*

Correspondence should be addressed to Tao Gong; mac0852@163.com, De-ping Liu; lliudeping@263.net, Zuo-guan Chen; 918804034@qq.com, and Yong-jun Li; liyongjun4679@qq.com

Shang Gao, Xiang Wang, and Ling-bing Meng contributed equally to this work.

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With the development of the times, cardiovascular diseases have become the biggest cause of death in the global aging society, causing a serious social burden. Atherosclerosis is a chronic inflammatory disease, which can occur in large and medium-sized blood vessels in the whole body. It takes atherosclerotic plaque as the typical pathological change and endothelial injury as the core pathophysiological mechanism. It is the pathological basis of coronary heart disease, peripheral artery disease, cerebrovascular disease, and other diseases. Recent studies have shown that chronic stress plays an important role in the occurrence and development of atherosclerosis, endothelial injury, lipid metabolism, and chronic inflammation. This process involves a large number of molecular targets. It is usually the cause of atherosclerotic cardiovascular and cerebrovascular diseases. If chronic stress factors exist for a long time, patients have genetic susceptibility, and the combination of environmental factors triggers the pathogenesis, which may eventually lead to complete blockage of the blood vessels, unstable rupture of plaques, and serious adverse cardiovascular events. This paper reviews the role of chronic stress in the occurrence and development of atherosclerosis, focusing on the pathophysiological mechanism.

1. Introduction

With the development of economy, the total prevalence of cardiovascular disease in the world is increasing year by year. At present, the total number of cardiovascular diseases exceeds 587 million. In 2019 alone, 18.6 million people died and 34.4 million were disabled, with an upward trend year by year [1]. Among them, atherosclerosis is the pathological basis of a variety of cardiovascular and cerebrovascular diseases, which is easy to appear in various diameter arteries, common in the coronary artery, aorta, carotid artery, etc. [2]. It is generally believed that atherosclerosis originates from various stimulating factors, including mechanical factors, LDL particle deposition, toxins, and viruses, which lead

to the destruction of endothelial cells on the arterial intima. The damaged endothelial cells secrete cytokines and growth factors, attract monocytes gathering, migrate to endothelial cells, and transform into macrophages, These macrophages swallow cholesterol-rich oxidized low-density lipoprotein (ox LDL) through TLRs to form foam cells [3]. The persistence of proinflammatory factors leads to the accumulation of more macrophages, mast cells, and activated T cells and B cells into foam cells, showing a lipid streak [4]. At the same time, growth factor activates smooth muscle cells in the arterial membrane, enters the intima and secretes extracellular matrix, makes the endothelium thickening, fibrosis, and hardening, and absorbs lipids through LPL receptors to form foam cells [5, 6]. The two types of foam cells are progressed and eventually become atherosclerotic.

Chronic stress refers to the nonspecific pathophysiological response caused by the change of the body's steady state under the long-term stimulation of various adverse factors in vivo and abroad. Generally speaking, it is the emotional experience of people under the pressure that they are difficult to adapt [7, 8]. The source of this stress is often psychological stress. It is generally believed that there will be feelings of tension, depression, and sadness under psychological stress; the sources of this stress are often four categories: work, family, finance, and major life events. A large cohort study of more than 10000 people found that one or more levels of psychological stress in patients with myocardial infarction were significantly elevated [9]. For the animal model of chronic stress, the commonly used chronic stressors include fasting, closed environment, long-term forced swimming, or electrical stimulation. Chronic stress is one of the promoting factors of many peripheral vascular diseases. Studies have shown that chronic stress can cause a variety of cardiovascular diseases, such as dysfunction of vascular smooth muscle cells, even leading to rupture of aortic aneurysm [10, 11]. The most important thing is that chronic stress can cause the occurrence and development of atherosclerosis. Studies have shown that chronic stress is an independent risk factor for carotid atherosclerosis in Mexican women [12]. For the rat model of atherosclerotic intimal hyperplasia, most carotid arteries are blocked by atherosclerotic lesions after two weeks under chronic stress [13]. The possible pathophysiological mechanism of promoting the progression of atherosclerosis involves many aspects. Chronic stress reduces the activity of hypothalamic pituitary adrenal axis, leads to the decline of anti-inflammatory ability, stimulates the sympathetic adrenal medulla, and increases the content of blood catecholamine. Catecholamine binds to the β-adrenal receptor on the surface of macrophages, stimulates macrophages to produce more cytokine catecholamine, induces the expression of related inflammatory cytokines, and promotes the progress of inflammation [14–16]. Chronic stress can also promote oxidative stress and vascular sensitivity by increasing blood triglycerides and low-density lipoprotein. It can also reduce the content of NO synthase and NO, which can produce contractile effect on the aortic vessels and promote the development of atherosclerosis [17, 18]. Chronic stress can also lead to changes in plaque stability and poor prognosis of atherosclerosis.

The purpose of this paper is to comprehensively review the effects of chronic stress on the occurrence and development of atherosclerosis, pay special attention to the pathophysiological mechanism of chronic stress in the occurrence and development of atherosclerosis, and especially explore how chronic stress accelerates the progress of atherosclerosis from the aspects of chronic inflammation, hemodynamics, lipid metabolism, adipose tissue interaction, and the progress of atherosclerotic plaque, so as to provide some ideas for clinical intervention and basic research (Figure 1).

1.1. Inflammation: The Core Cause Induced by Chronic Stress. It has been elucidated that atherosclerosis is essentially a chronic inflammatory disease. Inflammation plays a role in all stages of atherosclerosis, including accumulation of foam cells, formation of fatty streaks and fibrous plaques, rupture of acute plaques, and formation of thrombus [19–23], eventually leading to atherosclerosis and thrombotic complications. A large number of studies have confirmed that for chronic stress, it is currently considered that it may lead to chronic low-grade inflammation through a variety of ways and is related to atherosclerosis. Inflammation is even further developed by activating platelets and endothelial dysfunction, which is reviewed in detail in the part of endothelial dysfunction.

Clinical and animal experiments have shown that for inflammatory factors, long-term chronic stress can increase the concentration of blood cortisol through HPA axis on the one hand and change the steady-state balance of autonomic nervous system and increase the content of catecholamine by stimulating sympathetic adrenal system and reducing the vagus nerve tension on the other hand. Both cause the decrease of anti-inflammatory ability; the continuous progress of inflammation; the increase of the concentration of inflammatory cytokines, serum IL-6, and TNF; the increase of the expression of IL-6 and TNF in the liver and spleen; and the increase of CRP can also cause the change of inflammatory cytokines [24–26]. In addition, norepinephrine (NE) and neuropeptide Y (NPY) released by activated sympathetic activity can also promote the phosphorylation of mitogen-activated protein kinase (MAPK) or the release of high-mobility group protein B1 (HMGB1), thus inducing systemic inflammation and accelerating the development of cardiovascular diseases [27]. In addition, chronic stress can also enhance the activity of dipeptidyl peptidase-4 (DPP4) in plasma and reduce the concentration of plasma glucagon-like peptide (GLP-1) and adiponectin (APN), so as to promote the development of inflammation [28, 29]. However, it is still unclear whether it is possible to reduce the promoting effect of chronic stress on atherosclerosis by targeted inhibition of cellular inflammatory factors. For inflammatory cells, chronic stress can cause bone marrow cells to enter a highly reactive inflammatory state, cause leukocyte proliferation, and increase the number of circulating inflammatory monocytes [30, 31]. In addition, inflammatory cells and inflammatory cytokines are not isolated from each other. The activated sympathetic adrenal system can increase the number of immune response cells expressing

M receptor and inflammatory cytokines [32, 33] and produce a large number of cytokines. After HPA axis changes caused by chronic stress, TLR4/NF-κB pathway activates proinflammatory cytokines such as MCP-1 and IL-1$\alpha$and IL-6 and, at the same time, leads to the increase of intimal macrophage/monocyte ratio [17, 34], so that under the interaction of inflammatory factors and cells, endothelial homeostasis changes and inflammation further progresses and forms a vicious circle [35]. Interestingly, recent studies have also demonstrated that TLR4/NF-κB pathway also downregulates HMGB1 protein-mediated PPARγ/LXRα. The expression of ABCA1 pathway reduces the antiatherosclerotic effect of ATP binding cassette transporter 1 (ABCA-1) [36]. Therefore, the progression of atherosclerotic inflammation caused by chronic stress is a complex system, and the specific mechanism needs to be further studied.

2. Results and Discussion

2.1. Dyslipidemia: An Important Risk Factor of Chronic Stress Promoting the Progression of AS. Dyslipidemia is the first recognized independent risk factor for intima and media thickening of atherosclerosis [4]. Higher levels of serum low-density lipoprotein and total cholesterol can induce atherosclerotic precipitation, while low-density lipoprotein oxidation modified product (ox LDL) can be recognized and ingested by monocyte macrophage TLR and finally form lipid plaque [37]. However, simple dyslipidemia cannot fully explain the progress of atherosclerosis. Some studies have conducted large-scale clinical trials with statins that can reduce low-density lipoprotein, and cardiovascular events have been significantly reduced. However, even with intensive statin therapy, the ability to prevent cardiovascular events is still limited to 30% to 40% of treated patients [38], indicating that hyperlipidemia is not the only cause of atherosclerosis. Therefore, chronic stress comes into our sight. It has been reported that it can induce hyperlipidemia and lipid oxidation, cause lipid deposition to form plaque, may also lead to hypercoagulable state of arterial thrombosis, accelerate the progress of atherosclerosis, and produce adverse results [39, 40].On the one hand, it has been reported that in the control study of stressed mice and ordinary mice, high concentrations of serum total cholesterol, triglycerides, low-density lipoprotein, and very low-density lipoprotein can increase the atherosclerosis index of the chronic stress group, while the change of mice in the control group is not obvious [37]. In turn, chronic stress will change the blood lipid profile. In the study of hyperlipidemia rabbit model, with the extension of chronic stress exposure, the circulating concentrations of cholesterol, LDL, VLDL, and TG will significantly increase with time, while high-density lipoprotein will remain unchanged or decrease, and the atherosclerosis index will increase [18, 40, 41].

Hyperlipidemia and chronic stress interact to form a vicious circle, which together leads to the progress of atherosclerosis. Some researchers stimulated mice with chronic mild unpredictable stress (CMS), which also proved that CMS can increase the plasma concentration of corticosterone and lipids, increase the atherosclerosis index, and lead to the impairment of thoracic aortic function [42]. In addition, some studies stimulated atherosclerotic mice with cold stress, and the blood lipid of stressed rats was significantly higher than that of the control mice. Pathologically, it was found that cardiac oxidative stress was aggravated, macrophage infiltration and proinflammatory gene expression were found in the left ventricle and visceral adipose tissue, and the incidence of cardiac-related adverse events was further increased [43]. From the perspective of mechanism, chronic stress for more than 4 weeks can cause adrenal cortical stress hyperplasia; increase GC synthase, citrate synthase, and ketoglutarate dehydrogenase; increase glucocorticoid; promote ATP synthesis and energy metabolism [40, 44]; appear insulin resistant; promote hepatic triglyceride synthesis; and delay the binding and degradation of LDL by hepatocytes. Finally, it promotes circulating hyperlipidemia, which will continue after the removal of chronic stressors [45–47]. Chronic stress can also induce adrenoceptor desensitization and receptor downregulation in adipocytes, resulting in reduced catecholamine-induced lipolysis capacity and lipid accumulation [48]. In addition, it has been studied that the fatty acids released by lipolysis of adipose tissue under chronic stress can be used as substrates for cholesterol synthesis, causing the increase of blood cholesterol and aggravating the progress of atherosclerosis [49]. In addition, chronic stress beyond the threshold will stimulate the sympathetic nerve to directly upregulate the expression of neuropeptide Y or indirectly upregulate the expression of neuropeptide Y and its receptor Y2R by increasing glucocorticoid, resulting in abnormal lipid metabolism [50]. It can also regulate ABCG1 gene by upregulating TLR4, mediating inflammation and intracellular lipid accumulation are also necessary ways for macrophages to transform into foam cells [51–53]. In addition, the expression of aortic matrix metalloproteinase -9 (MMP-9) and MMP-2 gene will also increase, reduce the expression of adiponectin in preadipocytes, promote LDL-induced monocyte uptake of lipids, and promote the formation of foam cells [28].

2.2. NO: The Core Molecule Causing Endothelial Dysfunction Under Chronic Stress. Normal endothelium maintains vascular tension and structure by regulating the balance between vasodilators (such as NO and prostacyclin) and vasoconstrictors (such as endothelin-1 and norepinephrine) [54].The result of endothelial dysfunction is to cause the progress of atherosclerosis, hypertension, and other changes. Among them, NO is an important vasodilator molecule, which cooperates with other endothelial-derived factors to participate in endothelium-dependent relaxation [55, 56]. NO is produced by the precursor L-arginine, which is affected by NO synthase, and at least three functional forms of NO synthase (endothelial (eNOS), neuronal (nNOS), and inducible (iNOS)) are known [57]. In terms of function, NO is related to various endothelial functions, including regulating vascular tension, platelet aggregation, and vascular smooth muscle cell proliferation [58].

The response of vascular endothelium to chronic stress is the adaptation to its harmful effects. This adaptation is NO dependent [59, 60]. In early chronic stress, chronic stress

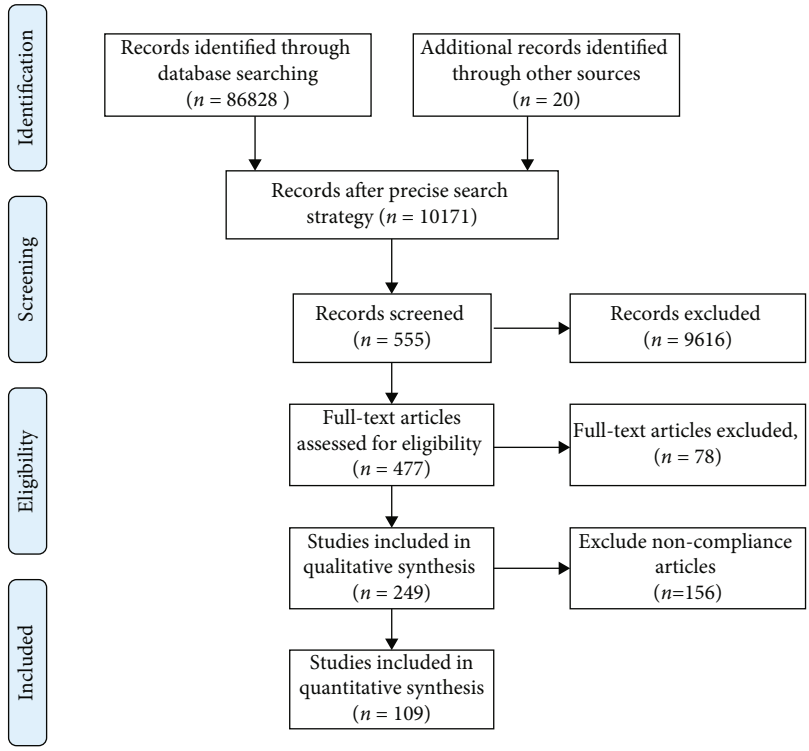

Figure 1: Flow diagram showing the procedure of searching the references in the databases.

hormone reduces endothelial injury by stimulating the release of ET-1 and maintaining a high level of NO [61]. In the study of different types of chronic stress mouse models, it was found that the level of NOx increased significantly and the time-dependent iNOS activity increased [57]. This increased activity of NO system will weaken the vasoconstrictive effect of catecholamine and ANGII [62, 63] and platelet aggregation caused by increased sympathetic activity and resist the vascular system disorder caused by chronic stress [64, 65].It was also confirmed in another study. Early chronic stress will have vascular relaxation changes and reach the peak eight weeks after the administration of chronic stressor and decrease twelve weeks. However, the analysis of blood components found that this is related to the reduction of relaxation components independent of NO, and NO will not decrease in the early stage [28]. At the same time, early chronic stress can also improve the response of endothelium to NO, weaken the vasoconstriction caused by calcium ion, and play a certain role in vasodilation [66].

However, long-term chronic stress may lead to endothelial dysfunction, vascular remodeling, and systolic hypertension through vascular oxidative stress. The decrease of endothelium-dependent relaxation was observed in this process, which may be related to the decrease of endothelial NO synthase activity and the decrease of NO bioavailability [67, 68]. In terms of mechanism, excessive ROS is produced under chronic stress, which changes the balance of oxidants and antioxidants and leads to the development of various pathological states, dysfunction of intracellular mitochondria, interruption of energy pathway, and induction of apoptosis [69]. More importantly, it causes the reduction of NO production and disorder of vasoconstriction and relaxation and induces MMP-2 and MMP-9 to decompose fiber caps containing collagen, elastin, and proteoglycan. The removal of ROS can reduce blood pressure, which can also explain the harm of chronic stress [69–72], and these injuries are controlled by the differential regulation of NO [73]. Studies have shown that *Salvia miltiorrhiza* can restore endothelial function to a certain extent by increasing the amount of NO and the level of eNOS [72]. Similarly, the role of chronic stress may also involve NO-dependent endothelial dysfunction [28]. Studies have shown that there is a compensatory vasodilation mechanism in chronic stress mice with impaired NO bioavailability. This mechanism may be related to hydrogen peroxide as a compensatory dilation metabolite, which ensures vascular reactivity to a certain extent [68]. Similarly, after long-term chronic stress, the effect of related hormones can no longer be antagonized by vasodilators such as NO. For example, glucocorticoid and proinflammatory cytokines, norepinephrine, and endothelin-1 may aggravate endothelial dysfunction by reducing eNOS expression, increasing eNOS inactivation, and promoting NO degradation and antagonism of NO-induced vasodilation [74]. On the other hand, the elevation of aldosterone and sodium and water retention of glucocorticoid can hardly get NO against [75–77]. Therefore, many factors work together to cause hemodynamic decompensation after long-term chronic stress.

2.3. Adipose Tissue: Correlates under Chronic Stress. Obesity is associated with chronic stress and atherosclerosis. Chronic

stress can cause excessive fat accumulation to a certain extent [43]. Studies have shown that obesity can increase the incidence rate of cardiovascular and cerebrovascular diseases. Obesity may cause inflammation and atherosclerosis by secreting a large number of adipokines and proinflammatory cytokines [78, 79]. But interestingly, through big data analysis, it can be found that there is no nonlinear relationship between the degree of obesity and atherosclerosis. Studies have shown that when CPC is used as an index of endogenous vascular proliferation to study this paradox, the number of good outcomes of high regenerative capacity (i.e., high CPC count) in obese people is more [80]. On the other hand, the degree of visceral obesity and BMI index is not linear [81]. Chronic stress promotes the accumulation of visceral fat. Therefore, there are some deficiencies in using BMI as a link between obesity, cardiovascular and cerebrovascular adverse events and chronic stress. Moreover, the expansion of aorta, slowing down the shear force of blood flow and playing the role of endothelial protection is also one of the reasons for the obesity paradox [82].

At present, chronic stress is more closely related to abdominal obesity. One view is that excessive glucocorticoid secretion caused by chronic stress will affect fat distribution and promote the selective accumulation of visceral fat [78], accompanied by a series of metabolic disorders, including dyslipidemia, impaired glucose tolerance and insulin resistance, and unstable or elevated blood pressure [83–86]. In addition, these factors are harmful to arteries and promote the development of atherosclerosis. However, the current research suggests that chronic stress has little to do with aggravating the inflammatory response of abdominal obesity and may increase the secretion of proinflammatory cytokines to a certain extent [87, 88].Some research evidence suggests that peripheral neuropeptide Y induced by chronic stress may play an important role [89]. It may also promote fat accumulation through a variety of stress behavior reactions, resulting in stronger cardiac sympathetic tension after obesity, exacerbate abnormal heart rate and metabolism, and increase the risk of cardiovascular disease [90]. Interestingly, the simultaneous occurrence of chronic stress and obesity is not necessarily a vicious circle. Some studies on mice have shown that high-fat diet can alleviate the anxiety caused by chronic stress and improve the activity intensity of anxious animals [91]. At the same time, the high-fat diet under chronic stress may also reduce the level of corticosterone and reduce the incidence of obesity to a certain extent [91, 92], but some studies have shown that various types of delicious food can also increase body weight [93].Therefore, understanding the lipid metabolism under stress is of great significance to study the relationship between chronic stress and atherosclerosis. In addition, a special type of adipose tissue, perivascular adipose tissue, plays an important role in the maintenance of vascular function. It secretes a large number of paracrine signal molecules, which affect the function of vascular wall through direct diffusion, trophoblast, or catheter [94]. However, chronic stress causes perivascular adipose tissue to become an inflammatory phenotype, which is characterized by changes in the spectrum of adipokines, cytokines, and chemokines, resulting in activation of arterial oxidative stress, reduction of NO bioavailability, reduction of EDD, and increase of aortic stiffness. From the perspective of mechanism, it may be related to the overactivation of sympathetic nervous system and the increase of aldosterone production [95, 96].

2.4. Plaque Progression: The Culprit of Adverse Events Caused by Chronic Stress. The latest research shows that chronic stress can not only cause the progression of atherosclerotic plaque but also accelerate the change of plaque instability. On the one hand, after 12 weeks of mild chronic stress exposure, the area of main atherosclerotic plaque in the ApoE-/-mice doubled compared with the unexposed mice [97]. On the other hand, in many studies on coronary artery, ascending aorta and abdominal aorta, histopathology shows that in the animal model of atherosclerosis, chronic stress can cause acute thrombosis and plaque instability. It is characterized by accelerated apoptosis, thinning of fiber cap, lipid deposition, increased macrophages and neovascularization, and increased degree of perivascular fibrosis, but the reduction of smooth muscle cells and intimal mediators such as type I collagen and elastic fibers especially significantly promotes the degeneration of the inner side of the plaque, which generally aggravates the inflammatory phenotype of atherosclerosis and makes the plaque easy to fall off from the vascular wall. Large-scale clinical cohort studies have shown that there is a causal relationship between mental changes caused by chronic stress and the progression of atherosclerosis and the decrease of plaque stability in people with coronary heart disease [98–101]. The reason can be found that chronic stress can aggravate the level of inflammation and oxidative stress through inflammatory cytokines, oxidized low-density lipoprotein, mechanical damage caused by elevated blood pressure and enhanced HPA axis function, resulting in the imbalance of vascular smooth muscle cell proliferation and apoptosis, and reduce the stability of plaque [98, 102]. Some studies used a multisystem 18F-FDG-PET/CT imaging. The results show that long-term elevated stress-related neurobiological activities will promote leukocyte production and inflammatory progression and then increase the plaque load of ARI and noncalcified coronary artery, resulting in reduced plaque stability [103]. Some studies have studied the proapoptotic effect of chronic stress at the molecular level. Chronic stress can increase the activity of DPP4 and decrease the expression of GLP-1 and cause the progression of plaque inflammation and aggravation of oxidative stress. At the same time, DPP4 inhibitor has certain therapeutic significance on endothelial injury and vascular aging, while exenatide, a GLP-1 analogue, decreased the expression of MMP-9 and MMP-2 genes in (ApoE-/-) mice. Stimulation of adiponectin expression in preadipocytes inhibited the formation of monocyte-derived foam cells induced by LDL, thereby slowing plaque progression [28, 29, 104]. High levels of cortisol induced by chronic stress can induce low levels of miRNA 25, increase proapoptotic proteins, induce apoptosis of smooth muscle cells, and reduce plaque stability. This effect is significantly related to the inhibition of targeting moap1 and P70S6K pathways [105]. In addition, chronic stress can promote the expression

of cysteinyl cathepsin S (Cat-S), directly affect TLR2/4, cause the progression of inflammation and oxidative stress, proliferate vascular smooth muscle cells, lead to neointimal hyperplasia, and reduce plaque stability [106]. The absence of cysteinyl cathepsin K (Cat-K) prevents the development of experimental neointimal hyperplasia by weakening the excessive effect of inflammation, the production of oxidative stress, and the proliferation of VSMC, which has a synergistic effect with Cat-S [107]. It has been reported that chronic stress induces rapid intimal hyperplasia in angioplasty injured rats (i.e., animal model of intimal injury) through neuropeptide Y (NPY), which may be related to intimal hyperplasia and plaque progression of atherosclerotic nature [108]. The unstable progression of atherosclerotic plaque is also related to the immune environment. It has been reported that chronic stress can significantly affect the local immune environment of mouse aorta, cause the accumulation of inflammatory cells in plaque, and reduce its stability [109].

3. Conclusion and Prospect

With the change of life rhythm, the impact of chronic stress on human health has attracted more and more attention. We reviewed the effects of chronic stress on the occurrence and development of atherosclerosis, focusing on the pathophysiological mechanisms, including chronic inflammation, hemodynamic changes, lipid metabolism changes, adipose tissue interaction, plaque progression, and so on. The related changes will eventually lead to abnormal vascular structure and atherosclerotic cardiovascular disease. But due to a perfect self-regulation mechanism in the body, acute internal environment disorder has a relatively weak impact on program genes. However, under the condition of chronic stress, abnormal gene expression can be induced continuously. Since chronic stressors cannot be removed, gene-induced changes in abnormal cell function are irreversible. At present, it has been confirmed that atherosclerosis is a pathological state in which the apoptosis of endothelial cells is excessive and apoptosis of smooth muscle cells is insufficient. The abnormal expression of these cells is closely related to the disturbance of internal environment and endocrine function under chronic stress. The anti-inflammatory effect of statins is based on lipid regulation to reduce the decline of inflammatory factors caused by chronic stress in the body. Chronic diseases such as diabetes and hypertension can be used as chronic stressors to increase the corresponding inflammatory factors and promote the formation of atherosclerosis. Drugs (including betas, angiotensin-converting enzyme inhibitors, angiotensin receptor antagonists, β-blockers, and antiplatelet drugs) are essential in the treatment of these diseases and control the presence of corresponding chronic stress factors. In future studies, we will pay more attention to the influence mechanism of chronic stress on atherosclerosis, and it is a novel insight to develop targeted drugs for the prevention and treatment of atherosclerosis against chronic stress in the future.

Abbreviations

ABCA-1: ATP-binding cassette transporter 1
ADAMTS7: A disintegrin and metalloproteinase with thrombospondin motifs 7
ANGPTL3: Angiopoietin-like protein
Apo A-I: Apolipoprotein A-I
Apo E: Apolipoprotein E
APN: Adiponectin
AVP: Vasopressin
ox LDL: Oxidized low-density lipoprotein
Cat-K: Cathepsin K
CRH: Corticosteroid-releasing hormone
CRP: C-reactive protein
CPC: Circulating progenitor cells
CS: Citrate synthase
DPP4: Dipeptidyl peptidase 4
GLP-1: Glucagon-like peptide-1
HMGB1: High-mobility group B-1
HPA: Hypothalamic pituitary adrenal axis
ICAM-1: Intercellular adhesion molecule-1
IL-1: Interleukin-1
IL-6: Interleukin-6
LPL: Lipoprteinlipase
LXR α: Recombinant liver X receptor alpha
MAPKs: Mitogen-activated protein kinases
MCP-1: Monocyte chemoattractant protein-1
MMP-9: Matrix metalloproteinase-9
NE: Noradrenaline
NF-κB: Nuclear factor kappa-B
NPY: Nerve peptide Y
OGDC: Oxoglutarate dehydrogenase complex
PPAR-γ: Peroxisome proliferator-activated receptors-γ
RAAS: Renin-angiotensin-aldosterone system
TNF: Tumor necrosis factor
TLR4: Toll-like receptor 4
VSMC: Vascular smooth muscle cell.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Ethical Approval

Not applicable.

Consent

Not applicable.

Conflicts of Interest

The authors declare that they have no competing interests.

Authors' Contributions

Shang Gao, Xiang Wang, Ling-bing Meng were contributed to the paper equally, and they are the co-first authors. Shang Gao, Xiang Wang and Ling-bing Meng were major

contributors in writing and were involved in critically revising the manuscript for important intellectual content. Tao Gong and De-ping Liu made substantial contributions to the research conception, and Yongjun Li and Zuoguan Chen designed the draft of the research process. Xiang Wang, Yue Luo, and Yuan-meng Zhang were major contributors in submitting the manuscript, and they gave the technical support in the methods. All authors read and approved the final manuscript. Shang Gao, Xiang Wang, and Ling-bing Meng contributed to the paper equally.

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References

[1] G. A. Roth, G. A. Mensah, C. O. Johnson et al., "Global burden of cardiovascular diseases and risk factors, 1990-2019: update from the GBD 2019 study," *Journal of the American College of Cardiology*, vol. 76, no. 25, pp. 2982–3021, 2020.

[2] K. Kobiyama and K. Ley, "Atherosclerosis," *Circulation Research*, vol. 123, no. 10, pp. 1118–1120, 2018.

[3] A. Pirillo, G. D. Norata, and A. L. Catapano, "LOX-1, OxLDL, and atherosclerosis," *Mediators of Inflammation*, vol. 2013, 152712 pages, 2013.

[4] M. Fioranelli, A. G. Bottaccioli, F. Bottaccioli, M. Bianchi, M. Rovesti, and M. G. Roccia, "Stress and inflammation in coronary artery disease: a review psychoneuroendocrineimmunology-based," *Frontiers in Immunology*, vol. 9, p. 2031, 2018.

[5] M. R. Bennett, S. Sinha, and G. K. Owens, "Vascular smooth muscle cells in atherosclerosis," *Circulation Research*, vol. 118, no. 4, pp. 692–702, 2016.

[6] D. Gomez and G. K. Owens, "Smooth muscle cell phenotypic switching in atherosclerosis," *Cardiovascular Research*, vol. 95, no. 2, pp. 156–164, 2012.

[7] K. Sharif, A. Watad, L. Coplan et al., "The role of stress in the mosaic of autoimmunity: an overlooked association," *Autoimmunity Reviews*, vol. 17, no. 10, pp. 967–983, 2018.

[8] H. Hong, M. Ji, and D. Lai, "Chronic stress effects on tumor: pathway and mechanism," *Frontiers in Oncology*, vol. 11, p. 738252, 2021.

[9] A. Rosengren, S. Hawken, S. Ôunpuu et al., "Association of psychosocial risk factors with risk of acute myocardial infarction in 11 119 cases and 13 648 controls from 52 countries (the INTERHEART study): case-control study," *The Lancet*, vol. 364, no. 9438, pp. 953–962, 2004.

[10] J. H. Pyun, B. Y. Ahn, T. A. Vuong et al., "Inducible Prmt1 ablation in adult vascular smooth muscle leads to contractile dysfunction and aortic dissection," *Experimental & Molecular Medicine*, vol. 53, no. 10, pp. 1569–1579, 2021.

[11] Y. S. Kim, S. P. Joo, D. J. Song, T. K. Lee, and T. S. Kim, "Correlation between high hair cortisol level and intracranial aneurysm rupture," *Medicine*, vol. 100, no. 22, 2021.

[12] J. Ortega-Montiel, C. Posadas-Romero, W. Ocampo-Arcos et al., "Self-perceived stress is associated with adiposity and atherosclerosis. The GEA Study," *BMC Public Health*, vol. 15, no. 1, p. 780, 2015.

[13] L. Li, A. C. Jönsson-Rylander, K. Abe, and Z. Zukowska, "Chronic stress induces rapid occlusion of angioplasty-injured rat carotid artery by activating neuropeptide Y and its Y1 receptors," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 25, no. 10, pp. 2075–2080, 2005.

[14] E. L. Garland, A. W. Hanley, A. K. Baker, and M. O. Howard, "Biobehavioral mechanisms of mindfulness as a treatment for chronic stress: an RDoC perspective," *Chronic Stress*, vol. 1, 2017.

[15] C. D. Camell, J. Sander, O. Spadaro et al., "Inflammasome-driven catecholamine catabolism in macrophages blunts lipolysis during ageing," *Nature*, vol. 550, no. 7674, pp. 119–123, 2017.

[16] T. Zhang, Y. Chen, H. Liu, Z. Zhou, Y. Zhai, and J. Yang, "Chronic unpredictable stress accelerates atherosclerosis through promoting inflammation in apolipoprotein E knockout mice," *Thrombosis Research*, vol. 126, no. 5, pp. 386–392, 2010.

[17] Y. L. Tang, J. H. Jiang, S. Wang et al., "TLR4/NF-κB signaling contributes to chronic unpredictable mild stress-induced atherosclerosis in ApoE-/- mice," *PLoS One*, vol. 10, no. 4, 2015.

[18] V. J. Neves, M. J. C. S. Moura, M. L. Tamascia et al., "Proatherosclerotic effects of chronic stress in male rats: altered phenylephrine sensitivity and nitric oxide synthase activity of aorta and circulating lipids," *Stress*, vol. 12, no. 4, pp. 320–327, 2009.

[19] G. R. Geovanini and P. Libby, "Atherosclerosis and inflammation: overview and updates," *Clinical Science (London, England)*, vol. 132, no. 12, pp. 1243–1252, 2018.

[20] D. Wolf and K. Ley, "Immunity and inflammation in atherosclerosis," *Circulation Research*, vol. 124, no. 2, pp. 315–327, 2019.

[21] P. K. Shah, "Inflammation, infection and atherosclerosis," *Trends in Cardiovascular Medicine*, vol. 29, no. 8, pp. 468–472, 2019.

[22] M. Bäck et al., "Inflammation and its resolution in atherosclerosis: mediators and therapeutic opportunities," *Nature Reviews. Cardiology*, vol. 16, no. 7, pp. 389–406, 2019.

[23] N. Ruparelia and R. Choudhury, "Inflammation and atherosclerosis: what is on the horizon?," *Heart*, vol. 106, no. 1, pp. 80–85, 2020.

[24] W. E. Haley, D. L. Roth, G. Howard, and M. M. Safford, "Caregiving strain and estimated risk for stroke and coronary heart disease among spouse caregivers: differential effects by race and sex," *Stroke*, vol. 41, no. 2, pp. 331–336, 2010.

[25] E. S. Miller, C. G. Apple, K. B. Kannan et al., "Chronic stress induces persistent low-grade inflammation," *American Journal of Surgery*, vol. 218, no. 4, pp. 677–683, 2019.

[26] I. J. Elenkov, "Neurohormonal-cytokine interactions: implications for inflammation, common human diseases and well-being," *Neurochemistry International*, vol. 52, no. 1-2, pp. 40–51, 2008.

[27] Y. Z. Liu, Y. X. Wang, and C. L. Jiang, "Inflammation: the common pathway of stress-related diseases," *Frontiers in Human Neuroscience*, vol. 11, p. 316, 2017.

[28] G. Yang, Y. Lei, A. Inoue et al., "Exenatide mitigated diet-induced vascular aging and atherosclerotic plaque growth in

ApoE-deficient mice under chronic stress," *Atherosclerosis*, vol. 264, pp. 1–10, 2017.

[29] Y. Lei, G. Yang, L. Hu et al., "Increased dipeptidyl peptidase-4 accelerates diet-related vascular aging and atherosclerosis in ApoE-deficient mice under chronic stress," *International Journal of Cardiology*, vol. 243, pp. 413–420, 2017.

[30] T. J. Barrett, E. M. Corr, C. van Solingen et al., "Chronic stress primes innate immune responses in mice and humans," *Cell Reports*, vol. 36, no. 10, 2021.

[31] T. Heidt, H. B. Sager, G. Courties et al., "Chronic variable stress activates hematopoietic stem cells," *Nature Medicine*, vol. 20, no. 7, pp. 754–758, 2014.

[32] J. M. Huston and K. J. Tracey, "The pulse of inflammation: heart rate variability, the cholinergic anti-inflammatory pathway and implications for therapy," *Journal of Internal Medicine*, vol. 269, no. 1, pp. 45–53, 2011.

[33] A. Halaris, "Inflammation-associated co-morbidity between depression and cardiovascular disease," *Current Topics in Behavioral Neurosciences*, vol. 31, pp. 45–70, 2016.

[34] A. Haj-Mirzaian, K. Ramezanzadeh, S. Shariatzadeh et al., "Role of hypothalamic-pituitary adrenal-axis, toll-like receptors, and macrophage polarization in pre-atherosclerotic changes induced by social isolation stress in mice," *Scientific Reports*, vol. 11, no. 1, p. 19091, 2021.

[35] J. Hinterdobler, H. Schunkert, T. Kessler, and H. B. Sager, "Impact of acute and chronic psychosocial stress on vascular inflammation," *Antioxidants & Redox Signaling*, vol. 35, no. 18, pp. 1531–1550, 2021.

[36] H. F. Gu, N. Li, Z. Q. Xu et al., "Chronic unpredictable mild stress promotes atherosclerosis via HMGB1/TLR4-mediated downregulation of PPARγ/LXRα/ABCA1 in ApoE(-/-) mice," *Frontiers in Physiology*, vol. 10, p. 165, 2019.

[37] L. B. Meng, R. Qi, L. Xu et al., "The more critical murderer of atherosclerosis than lipid metabolism: chronic stress," *Lipids in Health and Disease*, vol. 17, no. 1, p. 143, 2018.

[38] R. H. Nelson, "Hyperlipidemia as a risk factor for cardiovascular disease," *Primary Care*, vol. 40, no. 1, pp. 195–211, 2013.

[39] P. H. Black and L. D. Garbutt, "Stress, inflammation and cardiovascular disease," *Journal of Psychosomatic Research*, vol. 52, no. 1, pp. 1–23, 2002.

[40] M. Devaki, R. Nirupama, and H. N. Yajurvedi, "Chronic stress-induced oxidative damage and hyperlipidemia are accompanied by atherosclerotic development in rats," *Stress*, vol. 16, no. 2, pp. 233–243, 2013.

[41] L. Xiangdong, L. Yuanwu, Z. Hua, R. Liming, L. Qiuyan, and L. Ning, "Animal models for the atherosclerosis research: a review," *Protein & Cell*, vol. 2, no. 3, pp. 189–201, 2011.

[42] V. J. Neves, M. J. C. S. Moura, B. S. Almeida et al., "Chronic stress, but not hypercaloric diet, impairs vascular function in rats," *Stress*, vol. 15, no. 2, pp. 138–148, 2012.

[43] K. Nagasawa, N. Matsuura, Y. Takeshita et al., "Attenuation of cold stress-induced exacerbation of cardiac and adipose tissue pathology and metabolic disorders in a rat model of metabolic syndrome by the glucocorticoid receptor antagonist RU486," *Nutrition & Diabetes*, vol. 6, no. 4, 2016.

[44] L. B. Meng, G. F. Hu, M. J. Shan et al., "Citrate synthase and OGDH as potential biomarkers of atherosclerosis under chronic stress," *Oxidative Medicine and Cellular Longevity*, vol. 2021, Article ID 9957908, 35 pages, 2021.

[45] I. Kyrou and C. Tsigos, "Stress hormones: physiological stress and regulation of metabolism," *Current Opinion in Pharmacology*, vol. 9, no. 6, pp. 787–793, 2009.

[46] P. H. Black, "Stress and the inflammatory response: a review of neurogenic inflammation," *Brain, Behavior, and Immunity*, vol. 16, no. 6, pp. 622–653, 2002.

[47] D. N. Brindley, B. S. McCann, R. Niaura, C. M. Stoney, and E. C. Suarez, "Stress and lipoprotein metabolism: modulators and mechanisms," *Metabolism*, vol. 42, no. 9, pp. 3–15, 1993.

[48] P. H. Black, "The inflammatory response is an integral part of the stress response: implications for atherosclerosis, insulin resistance, type II diabetes and metabolic syndrome X," *Brain, Behavior, and Immunity*, vol. 17, no. 5, pp. 350–364, 2003.

[49] P. V. Luoma, "Gene activation regresses atherosclerosis, promotes health, and enhances longevity," *Lipids in Health and Disease*, vol. 9, no. 1, 2010.

[50] L. E. Kuo, M. Czarnecka, J. B. Kitlinska, J. U. Tilan, R. Kvetňanský, and Z. Zukowska, "Chronic stress, combined with a high-fat/high-sugar diet, shifts sympathetic signaling toward neuropeptide Y and leads to obesity and the metabolic syndrome," *Annals of the New York Academy of Sciences*, vol. 1148, no. 1, pp. 232–237, 2008.

[51] X. Cao, L. Zhang, C. Chen et al., "The critical role of ABCG1 and PPARγ/LXRα signaling in TLR4 mediates inflammatory responses and lipid accumulation in vascular smooth muscle cells," *Cell and Tissue Research*, vol. 368, no. 1, pp. 145–157, 2017.

[52] K. W. Howell, X. Meng, D. A. Fullerton, C. Jin, T. B. Reece, and J. C. Cleveland Jr., "Toll-like receptor 4 mediates oxidized LDL-induced macrophage differentiation to foam cells," *The Journal of Surgical Research*, vol. 171, no. 1, pp. e27–e31, 2011.

[53] K. Yang, X. J. Zhang, L. J. Cao et al., "Toll-like receptor 4 mediates inflammatory cytokine secretion in smooth muscle cells induced by oxidized low-density lipoprotein," *PLoS One*, vol. 9, no. 4, 2014.

[54] Y. Higashi, K. Noma, M. Yoshizumi, and Y. Kihara, "Endothelial function and oxidative stress in cardiovascular diseases," *Circulation Journal*, vol. 73, no. 3, pp. 411–418, 2009.

[55] E. H. Tang and P. M. Vanhoutte, "Endothelial dysfunction: a strategic target in the treatment of hypertension?," *Pflügers Archiv*, vol. 459, no. 6, pp. 995–1004, 2010.

[56] G. Kojda and D. Harrison, "Interactions between NO and reactive oxygen species: pathophysiological importance in atherosclerosis, hypertension, diabetes and heart failure," *Cardiovascular Research*, vol. 43, no. 3, pp. 562–571, 1999.

[57] T. Thakur, K. Gulati, N. Rai, and A. Ray, "Experimental studies on possible regulatory role of nitric oxide on the differential effects of chronic predictable and unpredictable stress on adaptive immune responses," *International Immunopharmacology*, vol. 50, pp. 236–242, 2017.

[58] B. Tejovathi, M. Suchitra, V. Suresh et al., "Association of lipid peroxidation with endothelial dysfunction in patients with overt hypothyroidism," *Experimental and Clinical Endocrinology & Diabetes*, vol. 121, no. 5, pp. 306–309, 2013.

[59] T. M. Spruill, "Chronic psychosocial stress and hypertension," *Current Hypertension Reports*, vol. 12, no. 1, pp. 10–16, 2010.

[60] T. Bruder-Nascimento, D. H. Campos, A. C. Cicogna, and S. Cordellini, "Chronic stress improves NO- and Ca2+ flux-

dependent vascular function: a pharmacological study," *Arquivos Brasileiros de Cardiologia*, vol. 104, no. 3, pp. 226–233, 2015.

[61] T. Nickel, A. Deutschmann, H. Hanssen, C. Summo, and U. Wilbert-Lampen, "Modification of endothelial biology by acute and chronic stress hormones," *Microvascular Research*, vol. 78, no. 3, pp. 364–369, 2009.

[62] C. Wang, Z. Luo, G. Carter et al., "NRF2 prevents hypertension, increased ADMA, microvascular oxidative stress, and dysfunction in mice with two weeks of ANG II infusion," *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, vol. 314, no. 3, pp. R399–r406, 2018.

[63] W. W. Holbein, M. B. Blackburn, M. A. Andrade, and G. M. Toney, "Burst patterning of hypothalamic paraventricular nucleus-driven sympathetic nerve activity in Angiotensin II-salt hypertension," *American Journal of Physiology. Heart and Circulatory Physiology*, vol. 314, no. 3, 2017.

[64] S. Cordellini, R. Novo, and U. Lanza Júnior, "Exposure to stress: Differential vascular adaptive response in spontaneously hypertensive and Wistar rats: Role of nitric oxide, and prehypertensive and hypertensive states," *Life Sciences*, vol. 79, no. 7, pp. 646–653, 2006.

[65] J. C. Leza, E. Salas, G. Sawicki, J. C. Russell, and M. W. Radomski, "The effects of stress on homeostasis in JCR-LA-cp rats: the role of nitric oxide," *The Journal of Pharmacology and Experimental Therapeutics*, vol. 286, no. 3, pp. 1397–1403, 1998.

[66] R. Imperatore, L. Palomba, and L. Cristino, "Role of orexin-a in hypertension and obesity," *Current Hypertension Reports*, vol. 19, no. 4, 2017.

[67] E. Schulz, T. Gori, and T. Münzel, "Oxidative stress and endothelial dysfunction in hypertension," *Hypertension Research*, vol. 34, no. 6, pp. 665–673, 2011.

[68] A. C. d'Audiffret, S. J. Frisbee, P. A. Stapleton, A. G. Goodwill, E. Isingrini, and J. C. Frisbee, "Depressive behavior and vascular dysfunction: a link between clinical depression and vascular disease?," *Journal of Applied Physiology*, vol. 108, no. 5, pp. 1041–1051, 1985.

[69] A. Zafir and N. Banu, "Induction of oxidative stress by restraint stress and corticosterone treatments in rats," *Indian Journal of Biochemistry & Biophysics*, vol. 46, no. 1, pp. 53–58, 2009.

[70] D. G. Harrison and M. C. Gongora, "Oxidative stress and hypertension," *The Medical Clinics of North America*, vol. 93, no. 3, pp. 621–635, 2009.

[71] D. H. Endemann and E. L. Schiffrin, "Endothelial dysfunction," *Journal of the American Society of Nephrology*, vol. 15, no. 8, pp. 1983–1992, 2004.

[72] H. J. Yang, K. Y. Kim, P. Kang, H. S. Lee, and G. H. Seol, "Effects of Salvia sclarea on chronic immobilization stress induced endothelial dysfunction in rats," *BMC Complementary and Alternative Medicine*, vol. 14, no. 1, 2014.

[73] K. Gulati, A. Chakraborti, and A. Ray, "Differential role of nitric oxide (NO) in acute and chronic stress induced neurobehavioral modulation and oxidative injury in rats," *Pharmacology, Biochemistry, and Behavior*, vol. 92, no. 2, pp. 272–276, 2009.

[74] N. Toda and M. Nakanishi-Toda, "How mental stress affects endothelial function," *Pflügers Archiv*, vol. 462, no. 6, pp. 779–794, 2011.

[75] C. K. Ewart, G. J. Elder, R. S. Jorgensen, and S. T. Fitzgerald, "The role of agonistic striving in the association between cortisol and high blood pressure," *Psychosomatic Medicine*, vol. 79, no. 4, pp. 416–425, 2017.

[76] F. J. Mocayar Marón, L. Ferder, F. D. Saraví, and W. Manucha, "Hypertension linked to allostatic load: from psychosocial stress to inflammation and mitochondrial dysfunction," *Stress*, vol. 22, no. 2, pp. 169–181, 2019.

[77] S. Cohen, D. Janicki-Deverts, W. J. Doyle et al., "Chronic stress, glucocorticoid receptor resistance, inflammation, and disease risk," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 16, pp. 5995–5999, 2012.

[78] F. Molica, S. Morel, B. R. Kwak, F. Rohner-Jeanrenaud, and S. Steffens, "Adipokines at the crossroad between obesity and cardiovascular disease," *Thrombosis and Haemostasis*, vol. 113, no. 3, pp. 553–566, 2015.

[79] L. Liberale, A. Bonaventura, A. Vecchiè et al., "The role of adipocytokines in coronary atherosclerosis," *Current Atherosclerosis Reports*, vol. 19, no. 2, p. 10, 2017.

[80] A. Mehta, Q. Meng, X. Li et al., "Vascular regenerative capacity and the obesity paradox in coronary artery disease," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 41, no. 6, pp. 2097–2108, 2021.

[81] R. F. Barth, L. Maximilian Buja, L. Cao, and S. V. Brodsky, "An obesity paradox: increased body mass index is associated with decreased aortic atherosclerosis," *Current Hypertension Reports*, vol. 19, no. 7, p. 55, 2017.

[82] A. E. Davis, A. J. Lewandowski, C. J. Holloway et al., "Observational study of regional aortic size referenced to body size: production of a cardiovascular magnetic resonance nomogram," *Journal of Cardiovascular Magnetic Resonance*, vol. 16, no. 1, 2014.

[83] M. L. Palumbo, A. Prochnik, M. R. Wald, and A. M. Genaro, "Chronic stress and glucocorticoid receptor resistance in asthma," *Clinical Therapeutics*, vol. 42, no. 6, pp. 993–1006, 2020.

[84] S. Vyas, A. J. Rodrigues, J. M. Silva et al., "Chronic stress and glucocorticoids: from neuronal plasticity to neurodegeneration," *Neural Plasticity*, vol. 2016, Article ID 6391686, 15 pages, 2016.

[85] S. Chiba, T. Numakawa, M. Ninomiya, M. C. Richards, C. Wakabayashi, and H. Kunugi, "Chronic restraint stress causes anxiety- and depression-like behaviors, downregulates glucocorticoid receptor expression, and attenuates glutamate release induced by brain-derived neurotrophic factor in the prefrontal cortex," *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, vol. 39, no. 1, pp. 112–119, 2012.

[86] M. J. Horchar and E. S. Wohleb, "Glucocorticoid receptor antagonism prevents microglia-mediated neuronal remodeling and behavioral despair following chronic unpredictable stress," *Brain, Behavior, and Immunity*, vol. 81, pp. 329–340, 2019.

[87] T. T. Lewis, H. M. Kravitz, I. Janssen, and L. H. Powell, "Self-reported experiences of discrimination and visceral fat in middle-aged African-American and Caucasian women," *American Journal of Epidemiology*, vol. 173, no. 11, pp. 1223–1231, 2011.

[88] E. Delker, B. AlYami, L. C. Gallo, J. M. Ruiz, M. Szklo, and M. A. Allison, "Chronic stress burden, visceral adipose tissue, and adiposity-related inflammation: the multi-ethnic study of

atherosclerosis," *Psychosomatic Medicine*, vol. 83, no. 8, pp. 834–842, 2021.

[89] K. Aschbacher, S. Kornfeld, M. Picard et al., "Chronic stress increases vulnerability to diet-related abdominal fat, oxidative stress, and metabolic risk," *Psychoneuroendocrinology*, vol. 46, pp. 14–22, 2014.

[90] B. B. Simas, E. A. Nunes, C. C. Crestani, and G. F. Speretta, "Cardiovascular and metabolic consequences of the association between chronic stress and high-fat diet in rats," *Stress*, vol. 21, no. 3, pp. 247–256, 2018.

[91] C. Oliveira, C. M. . Oliveira, I. C. de Macedo et al., "Hypercaloric diet modulates effects of chronic stress: a behavioral and biometric study on rats," *Stress*, vol. 18, no. 5, pp. 514–523, 2015.

[92] T. Bruder-Nascimento, D. H. S. Campos, C. Alves, S. Thomaz, A. C. Cicogna, and S. Cordellini, "Effects of chronic stress and high-fat diet on metabolic and nutritional parameters in Wistar rats," *Arquivos Brasileiros de Endocrinologia e Metabologia*, vol. 57, no. 8, pp. 642–649, 2013.

[93] N. Zeeni, C. Daher, G. Fromentin, D. Tome, N. Darcel, and C. Chaumontet, "A cafeteria diet modifies the response to chronic variable stress in rats," *Stress*, vol. 16, no. 2, pp. 211–219, 2013.

[94] E. DeVallance, K. W. Branyan, K. Lemaster et al., "Aortic dysfunction in metabolic syndrome mediated by perivascular adipose tissue TNFα- and NOX2-dependent pathway," *Experimental Physiology*, vol. 103, no. 4, pp. 590–603, 2018.

[95] S. N. Saxton, S. B. Withers, and A. M. Heagerty, "Emerging roles of sympathetic nerves and inflammation in perivascular adipose tissue," *Cardiovascular Drugs and Therapy*, vol. 33, no. 2, pp. 245–259, 2019.

[96] E. R. DeVallance, K. W. Branyan, I. M. Olfert et al., "Chronic stress induced perivascular adipose tissue impairment of aortic function and the therapeutic effect of exercise," *Experimental Physiology*, vol. 106, no. 6, pp. 1343–1358, 2021.

[97] M. Kumari, C. Grahame-Clarke, N. Shanks, M. Marmot, S. Lightman, and P. Vallance, "Chronic stress accelerates atherosclerosis in the apolipoprotein E deficient mouse," *Stress*, vol. 6, no. 4, pp. 297–299, 2003.

[98] L. B. Meng, M. J. Shan, Z. M. Yu et al., "Chronic stress: a crucial promoter of cell apoptosis in atherosclerosis," *The Journal of International Medical Research*, vol. 48, no. 1, p. 300060518814606, 2020.

[99] Z. M. Yu, X. T. Deng, R. M. Qi, L. Y. Xiao, C. Q. Yang, and T. Gong, "Mechanism of chronic stress-induced reduced atherosclerotic medial area and increased plaque instability in rabbit models of chronic stress," *Chinese Medical Journal*, vol. 131, no. 2, pp. 161–170, 2018.

[100] C. Giannarelli, D. T. Rodriguez, M. U. Zafar et al., "Susceptibility to chronic social stress increases plaque progression, vulnerability and platelet activation," *Thrombosis and Haemostasis*, vol. 117, no. 4, pp. 816–818, 2017.

[101] L. Roth, M. Rombouts, D. M. Schrijvers et al., "Chronic intermittent mental stress promotes atherosclerotic plaque vulnerability, myocardial infarction and sudden death in mice," *Atherosclerosis*, vol. 242, no. 1, pp. 288–294, 2015.

[102] P. Wang, T. Y. Xu, Y. F. Guan et al., "Vascular smooth muscle cell apoptosis is an early trigger for hypothyroid atherosclerosis," *Cardiovascular Research*, vol. 102, no. 3, pp. 448–459, 2014.

[103] M. T. Osborne, S. Abohashem, H. Zureigat, T. A. Abbasi, and A. Tawakol, "Multimodality molecular imaging: gaining insights into the mechanisms linking chronic stress to cardiovascular disease," *Journal of Nuclear Cardiology*, vol. 28, no. 3, pp. 955–966, 2021.

[104] X. W. Cheng, M. Narisawa, E. Jin, C. Yu, W. Xu, and L. Piao, "Dose rectification of an imbalance between DPP4 and GLP-1 ameliorates chronic stress-related vascular aging and atherosclerosis?," *Clinical and Experimental Pharmacology & Physiology*, vol. 45, no. 5, pp. 467–470, 2018.

[105] B. Zhang, G. Zhang, T. Wei et al., "MicroRNA-25 protects smooth muscle cells against corticosterone-induced apoptosis," *Oxidative Medicine and Cellular Longevity*, vol. 2019, Article ID 2691514, 10 pages, 2019.

[106] H. Wang, X. Meng, L. Piao et al., "Cathepsin S deficiency mitigated chronic stress-related neointimal hyperplasia in mice," *Journal of the American Heart Association*, vol. 8, no. 14, 2019.

[107] X. Meng, L. Piao, H. Wang et al., "Deficiency of cysteinyl cathepsin K suppresses the development of experimental intimal hyperplasia in response to chronic stress," *Journal of Hypertension*, vol. 38, no. 8, pp. 1514–1524, 2020.

[108] F. S. Dhabhar and K. Viswanathan, "Short-term stress experienced at time of immunization induces a long-lasting increase in immunologic memory," *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, vol. 289, no. 3, pp. R738–R744, 2005.

[109] M. C. Marcondes, V. Zhukov, H. Bradlow et al., "Effects of chronic mental stress and atherogenic diet on the immune inflammatory environment in mouse aorta," *Brain, Behavior, and Immunity*, vol. 25, no. 8, pp. 1649–1657, 2011.