International Journal of Gerontology 12 (2018) 191–195

Contents lists available at ScienceDirect

International Journal of Gerontology

journal homepage: www.ijge-online.com

Original Article

High Serum Leptin Level is Associated with Peripheral Artery Disease in Geriatric Individuals

Tse-Hao Chen [a], Chung-Jen Lee [b], Ji-Hung Wang [a, c, 1], Bang-Gee Hsu [a, d *, 1]

[a] School of Medicine, Tzu Chi University, Hualien, Taiwan, [b] Department of Nursing, Tzu Chi University of Science and Technology, Hualien, Taiwan, [c] Division of Cardiology, Buddhist Tzu Chi General Hospital, Hualien, Taiwan, [d] Division of Nephrology, Buddhist Tzu Chi General Hospital, Hualien, Taiwan

ARTICLE INFO

Article history:
Received 23 July 2017
Received in revised form
1 November 2017
Accepted 17 January 2018
Available online 21 February 2018

Keywords:
leptin,
peripheral arterial disease,
ankle-brachial index,
geriatric

SUMMARY

Background: Leptin contributes to the pathogenesis of atherosclerosis, endothelial dysfunction, and thrombosis. Peripheral arterial disease (PAD) is a common manifestation of atherosclerosis and is expected to prevail among geriatric individuals. The present study aimed to determine whether serum leptin level is associated with PAD in a geriatric group.

Methods: Blood samples were obtained from 60 participants in the study who were >65 years of age. Ankle-brachial index (ABI) values were measured using the automated oscillometric method. PAD was considered to be present if the left or right ABI values were less than 0.90, and these participants were included in the low ABI group. Serum leptin levels were measured using a commercial enzyme immunoassay kit.

Results: Of these geriatric participants, ten (16.7%) were in the low ABI group. Compared with the elderly participants in the normal ABI group, those in the low ABI group were current smokers ($p = 0.048$) and had higher serum C-reactive protein (CRP, $p = 0.018$) and leptin levels ($p = 0.005$). Multivariate logistic regression analysis of the factors significantly associated with PAD demonstrated that leptin (odds ratio: 1.078, 95% confidence interval: 1.021–1.138, $p = 0.006$) was an independent predictor of PAD. Female ($p = 0.001$), body mass index ($p = 0.008$), and a logarithmically transformed CRP (log-CRP, $p = 0.035$) were found to be associated with serum log-leptin levels among geriatric participants after multivariate forward stepwise linear regression analysis.

Conclusion: High serum leptin level is a risk marker for PAD in geriatric individuals.

1. Introduction

Peripheral artery disease (PAD), which frequently occurs in the elderly, is a condition in which arteries connecting to the extremities become narrow or occult owing to an accumulation of fatty tissue.[1] One of the known risk factors of PAD is aging.[2] Within a population above 60 years of age, the overall incidence of PAD is approximately 20%.[3] PAD also results in elevated risk of other cardiovascular diseases, such as heart attack and ischemic stroke, even in asymptomatic individuals.[4] Ankle-brachial index (ABI) is an important tool for noninvasive detection of this disease.[5] An abnormal ABI, lower than 0.9, is an indicator of PAD and can be used to assess the severity of PAD.[6]

There is evidence that an increase in plasma leptin level is associated with unfavorable outcomes in coronary artery disease and heart failure.[7,8] Leptin induced detrimental responses including vascular inflammation, vasodilatation dysfunction, and arterial stiffness in peripheral tissues have been reported in previous studies.[9,10] However, the relationship between PAD and high serum leptin level in geriatric individuals is unclear. Therefore, in the present study, we aimed to determine the relationship between serum leptin level and PAD in geriatric individuals.

* Corresponding author. Division of Nephrology, Buddhist Tzu Chi General Hospital, 707, Section 3, Chung Yang Road, Hualien, Taiwan.
E-mail address: gee.lily@msa.hinet.net (B.-G. Hsu).

[1] Ji-Hung Wang and Bang-Gee Hsu equally contributed to this study.

https://doi.org/10.1016/j.ijge.2018.02.001

2. Methods

2.1. Participants

In total, 60 participants aged >65 years were enrolled in this cross-sectional study conducted between January and December 2012 in a medical center in Hualien, Taiwan. Blood pressure (BP) of each patient was measured in the morning by trained staff using standard mercury sphygmomanometers with appropriate cuff sizes after the patient was seated for at least 10 min. Systolic and diastolic BP measurements were recorded thrice at 5-min intervals and were averaged for analysis. The patients were regarded as having hypertension if they had systolic BP $\geq$ 140 mmHg, diastolic BP $\geq$ 90 mmHg, or received any anti-hypertensive medication in the past 2 weeks. The patients were diagnosed with diabetes mellitus if their fasting plasma glucose level was $\geq$126 mg/dl or if they were administered oral hypoglycemic medication or insulin.[11] The Protection of the Human Subjects Institutional Review Board of the Tzu-Chi University and Hospital approved this study. All of the patients provided informed consent prior to participating in this study. Patients were excluded if they had acute infection, acute myocardial infarction, or pulmonary edema, took protease-activated receptor-1 antagonists or Warfarin at the time of blood sampling, or declined to provide informed consent.

2.2. Anthropometric analysis

Participant weight was measured without shoes, while wearing light clothing to the nearest 0.5 kg, while height was measured to the nearest 0.5 cm. Body mass index (BMI) was calculated as the weight in kilograms divided by the height in meters squared.[12–14]

2.3. Biochemical investigation

After 8 h overnight fasting, blood (approximately 5 ml) of all participants was sampled at morning and were immediately centrifuged at 3000 $\times$ *g* for 10 min. Serum levels of blood urea nitrogen), creatinine, fasting glucose, total cholesterol (TCH), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total calcium, phosphorus, and C-reactive protein (CRP) were measured using an autoanalyzer (COBAS Integra 800; Roche Diagnostics, Basel, Switzerland).[12–14] Serum leptin (SPI-BIO, Montigny le Bretonneux, France) level and intact parathyroid hormone level (iPTH; Diagnostic Systems Laboratories, Webster, TX, USA) were determined using a commercially available enzyme immunoassay or enzyme-linked immunosorbent assay, respectively.[12,13]

2.4. Ankle-brachial index measurements

Using an oscillometric method, ABI values were measured using an ABI-form device (VaSera VS-1000; Fukuda Denshi Co, Ltd, Tokyo, Japan) that automatically and simultaneously measures BP in both of the arms and ankles.[14] With the participants lying in the supine position, occlusion and monitoring cuffs were placed tightly around the four extremities, an electrocardiogram was recorded, and heart sounds were measured for at least 10 min. ABI was calculated as the ratio of ankle SBP to arm SBP, and the lower value of ankle SBP was used for calculation. We repeatedly measured these parameters for both legs and calculated the mean values. PAD was diagnosed on the basis of ABI <0.9.[15] In the present study, the left or right ABI values < 0.9 were used to define the low ABI group.[14]

2.5. Statistical analysis

Data were tested for normal distribution using the Kolmogorov–Smirnov test. Normally distributed data were expressed as a mean $\pm$ standard deviation, and comparisons between the patients were performed using the Student's independent *t*-test (two-tailed). Non-normally distributed data were expressed as medians and interquartile ranges, and comparisons between the patients were performed using the Mann–Whitney *U* test (TG, fasting glucose, CRP, and leptin). Data expressed as the number of patients were analyzed by the chi-square test. Because TG, fasting glucose, CRP, and leptin not normally distributed and underwent base 10 logarithmic transformations to achieve normality. Clinical variables that correlated with serum logarithmically transformed leptin (log-leptin) levels in the elderly participants were evaluated using univariate linear regression analysis. Variables that were significantly associated with log-leptin levels in the elderly participants were tested for independency in the multivariate forward stepwise regression analysis. Receiver operating characteristic (ROC) was used to calculate the area under the curve (AUC) to identify log-leptin and log-CRP levels to predict PAD in the participants. Variables that were significantly associated with PAD were tested for independence using multivariate logistic regression analysis. Data were analyzed using SPSS for Windows (version 19.0; SPSS Inc., Chicago, IL, USA). A p value < 0.05 was considered statistically significant.

3. Results

Demographic, clinical, and biochemical characteristics of the 60 geriatric participants are presented in Table 1. Twenty-four of the participants (40.0%) had diabetes mellitus, and 47 (78.3%) had hypertension. Ten geriatric participants (16.7%) were included in the low ABI group. Those in the low ABI group had high frequency of current smoking ($p = 0.048$) and higher serum CRP ($p = 0.018$) and leptin ($p = 0.005$) levels than those in the normal ABI group. There was no statistically significant difference based on gender, co-existing diabetes or hypertension, or the use of ACEi, ARB, β-blockers, CCB, statins, fibrate, Aspirin, or Clopidogrel between the two groups.

The univariable and multivariate linear analysis of the log-leptin levels in the elderly participants are presented in Table 2. The elderly females ($r = 0.428$, $p = 0.001$), BMI ($r = 0.295$, $p = 0.022$), DBP ($r = 0.261$, $p = 0.044$), TCH ($r = 0256$, $p = 0.048$), and log-CRP ($r = 0.313$, $p = 0.015$) positively correlated with, while height ($r = -0.263$, $p = 0.042$) negatively correlated with the serum log-leptin levels in the elderly participants. After a multivariate forward stepwise linear regression analysis of the factors (gender, height, BMI, DBP, TCH, and log-CRP) that were significantly associated with fasting serum log-leptin levels, it was demonstrated that the females ($\beta = 0.384$, $p = 0.001$), BMI ($\beta = 0.302$, $p = 0.008$) and log-CRP ($\beta = 0.241$, $p = 0.035$) were positively associated with serum log-leptin levels among the elderly participants.

Then a ROC curve analysis was applied to estimate the optimal level of log-leptin and log-CRP predicting the PAD of the elderly participants (Fig. 1). The AUC for log-leptin was 0.782 (95% confidence interval (CI): 0.657–0.878, $p = 0.001$), and AUC for log-CRP was 0.739 (95% CI: 0.609–0.844, $p = 0.004$), respectively.

An adjustment of the factors significantly associated with PAD (diabetes mellitus, hypertension, smoking, gender, age, TCH, TG, HDL-C, LDL-C, CRP and leptin) in a multivariate logistic regression analysis demonstrated that each increase of 1 ng/ml in the serum leptin level (odds ratio: 1.0782, 95% CI: 1.021–1.138, $p = 0.006$) and current smoking (odds ratio: 22.460, 95% CI: 1.428–353.336,

Table 1
Clinical variables of the 60 geriatric participants in the normal or low ankle brachial index group.

Characteristic	All participants (n = 60)	Normal ABI group (n = 50)	Low ABI group (n = 10)	p value
Age (years)	72.65 ± 5.49	72.08 ± 5.01	75.50 ± 7.06	0.072
Height (cm)	160.25 ± 7.50	160.72 ± 7.26	157.90 ± 8.65	0.282
Body weight (kg)	64.65 ± 10.36	64.54 ± 10.17	65.20 ± 11.83	0.856
Body mass index (kg/m^2)	25.13 ± 3.27	24.95 ± 3.29	26.01 ± 3.18	0.352
Left ankle-brachial index	1.05 ± 0.11	1.09 ± 0.08	0.90 ± 0.12	<0.001*
Right ankle-brachial index	1.03 ± 0.12	1.07 ± 0.07	0.83 ± 0.12	<0.001*
Systolic blood pressure (mmHg)	129.12 ± 16.18	127.60 ± 15.08	136.70 ± 20.05	0.105
Diastolic blood pressure (mmHg)	70.47 ± 8.95	70.58 ± 9.24	69.90 ± 7.70	0.828
Total cholesterol (mg/dl)	167.97 ± 36.25	164.50 ± 36.19	185.30 ± 32.87	0.098
Triglyceride (mg/dl)	122.00 (84.50–159.75)	116.50 (81.75–181.00)	128.50 (113.75–153.00)	0.487
HDL-C (mg/dl)	46.73 ± 12.67	46.34 ± 11.72	48.70 ± 17.32	0.595
LDL-C (mg/dl)	97.05 ± 30.23	94.04 ± 30.59	112.10 ± 24.34	0.085
Fasting glucose (mg/dl)	107.00 (95.25–156.00)	105.50 (93.75–142.75)	138.50 (103.00–188.75)	0.110
Blood urea nitrogen (mg/dl)	17.88 ± 6.19	17.52 ± 5.53	19.70 ± 8.96	0.314
Creatinine (mg/dl)	1.18 ± 0.34	1.15 ± 0.30	1.32 ± 0.48	0.156
Total calcium (mg/dl)	9.15 ± 0.34	9.13 ± 0.34	9.24 ± 0.30	0.332
Phosphorus (mg/dl)	3.40 ± 0.45	3.43 ± 0.46	3.26 ± 0.36	0.280
Intact parathyroid hormone (pg/ml)	52.12 ± 27.25	50.95 ± 25.44	57.96 ± 36.07	0.462
CRP (mg/dl)	0.20 (0.14–0.26)	0.19 (0.14–0.23)	0.26 (0.20–0.78)	0.018*
Leptin (ng/ml)	9.47 (3.70–27.92)	7.93 (3.51–19.61)	45.67 (7.63–85.17)	0.005*
Female, n (%)	17 (28.3)	13 (26.0)	4 (40.0)	0.370
Smoking, n (%)	7 (11.7)	4 (8.0)	3 (30.0)	0.048*
Diabetes, n (%)	24 (40.0)	19 (38.0)	5 (50.0)	0.480
Hypertension, n (%)	47 (78.3)	39 (78.0)	8 (80.0)	0.889
ACE inhibitor use, n (%)	17 (28.3)	16 (32.0)	1 (10.0)	0.159
ARB use, n (%)	24 (40.0)	19 (38.0)	5 (50.0)	0.480
β-blocker use, n (%)	29 (48.3)	23 (46.0)	6 (60.0)	0.419
CCB use, n (%)	21 (35.0)	19 (38.0)	2 (20.0)	0.276
Statin use, n (%)	30 (50.0)	25 (50.0)	5 (50.0)	1.000
Fibrate use, n (%)	11 (18.3)	7 (14.0)	4 (40.0)	0.052
Aspirin, n (%)	34 (56.7)	30 (60.0)	4 (40.0)	0.244
Clopidogrel, n (%)	15 (25.0)	14 (28.0)	1 (10.0)	0.230

Values for continuous variables are shown as mean ± standard deviation after analysis using the Student's *t*-test; variables not normally distributed are shown as the median and interquartile ranges after analysis using the Mann–Whitney *U* test; values are presented as number (%) and analysis after analysis using the chi-square test.
ABI, ankle brachial index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CRP, C-reactive protein; ACE, angiotensin-converting enzyme; ARB, angiotensin-receptor blocker; CCB, calcium-channel blocker.
*$p < 0.05$ was considered statistically significant.

p = 0.027) were the independent predictors of PAD in the elderly participants (Table 3).

4. Discussion

The results of this study showed that the fasting leptin level was higher in the elderly adults in the low ABI group than in those in the normal ABI group, together with current smoking as independent predictors for the development of PAD.

A total of 6979 patients aged 70 years or older or aged 50 through 69 years with a history of cigarette smoking or diabetes throughout the United States demonstrated a 13% prevalence rate of PAD.[16] Another study also demonstrated a significantly increased prevalence of 23.5% in patients with PAD that were compared between 2000 and 2010, and a prevalence that increased more dramatically in the elderly was also reported.[17] Our study showed a PAD prevalence rate of 16.7% in an elderly population and also that current smoking is associated with PAD in elderly adults.

Diabetes mellitus and hypertension were perceived as the risk factors of PAD.[18,19] Our study demonstrated a prevalence of 50% of PAD in elderly adults with diabetes mellitus in this population similar to a previous epidemiologic study.[18] There was an 80% hypertension prevalence in the PAD elderly adults in our study. However, the prevalence of diabetes mellitus or hypertension did not manifest a statistical significance when comparing between the low ABI group and the normal ABI group in our study. Since PAD is recognized as an inflammation in peripheral tissue.[20] In a cross-sectional study surveyed among 5874 adults without any other underlying disease, it was demonstrated that an elevated CRP was associated with PAD.[21] Moreover, CRP was also reported to be a predictor of PAD mortality.[22] Our study showed a positive correlation between CRP and PAD.

Leptin stimulates vascular inflammation, causes oxidative stress, and vascular smooth muscle hypertrophy that may contribute to a pathogenesis of type 2 diabetes mellitus, hypertension, atherosclerosis, and coronary heart disease.[23] Hyperleptinemia was reported to be positively correlated to total peripheral resistance and inversely related to arterial compliance.[9] Elevated plasma leptin is known to be a key factor in arterial stiffness.[24] Leptin also induces the excretion of vasoconstrictor in endothelial cells, such as endothelin-1, which could also contribute to PAD.[25] In our study, hyperleptinemia was strongly related to PAD in an elderly population. After a multivariate logistic regression analysis, it was noted that the serum leptin level also predicts the diagnosis of PAD in elderly adults.

Testosterone and a lean body mass increase in males while they age. As a result, body fat mass as well as leptin decreases after puberty in males. On the other hand, a fat body mass and leptin elevate in females while they age.[26] A previous study demonstrated an abrupt elevation of serum leptin when BMI exceeded the level of 24.6 kg/m^2 and also demonstrated that when BMI increased to 30 kg/m^2, a decrease in the leptin receptor occurred.[27] A former study showed that a primary hepatocyte was stimulated by leptin and eventually increased the CRP secretion.[28] In addition, Calabró et al. found that an elevation of leptin was also associated with an increase of the CRP level in human artery coronary endothelial

Table 2
Correlation between serum leptin levels and clinical variables among the 60 geriatric participants.

Variable	Log-Leptin (ng/ml)			
	Univariate		Multivariate	
	r	p value	Beta	p value
Female	0.428	0.001*	0.384	0.001*
Smoking	−0.075	0.571	–	–
Diabetes	−0.055	0.679	–	–
Hypertension	0.153	0.242	–	–
Age (years)	0.177	0.175	–	–
Height (cm)	−0.263	0.042*	–	–
Body weight (kg)	0.091	0.490	–	–
Body mass index (kg/m^2)	0.295	0.022*	0.302	0.008*
SBP (mmHg)	0.061	0.644	–	–
DBP (mmHg)	0.261	0.044*	–	–
Total cholesterol (mg/dl)	0.256	0.048*	–	–
Log-Triglyceride (mg/dl)	0.006	0.964	–	–
HDL-C (mg/dl)	0.101	0.441	–	–
LDL-C (mg/dl)	0.237	0.068	–	–
Log-glucose (mg/dl)	0.019	0.883	–	–
Blood urea nitrogen (mg/dl)	0.187	0.153	–	–
Creatinine (mg/dl)	0.033	0.803	–	–
Total calcium (mg/dl)	−0.105	0.424	–	–
Phosphorus (mg/dl)	0.007	0.956		
iPTH (pg/ml)	0.233	0.073	–	–
Log-CRP (mg/dl)	0.313	0.015*	0.241	0.035*

Data of triglyceride, glucose, CRP, and leptin levels showed a skewed distribution, and therefore, were log-transformed before analysis.
Data was analyzed using univariate linear regression analyses or multivariate stepwise linear regression analysis (adapted factors: gender, height, body mass index, DBP, total cholesterol, and log-CRP).
SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-C, high density lipoprotein-cholesterol; LDL-C, low-density lipoprotein cholesterol; iPTH, intact parathyroid hormone; CRP, C-reactive protein.
*$p < 0.05$ was considered statistically significant.

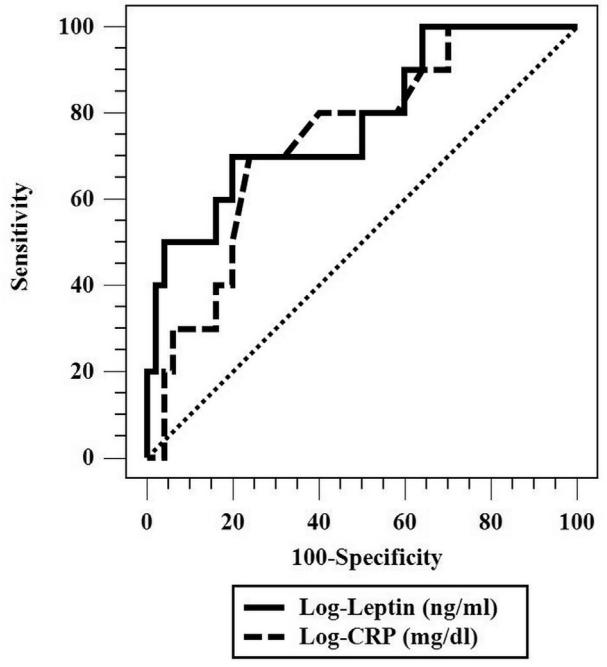

Fig. 1. Receiver operating characteristic (ROC) curve analysis to predict peripheral artery disease of the elderly participants. Area under ROC curve (AUC) indicates the diagnostic potential of log-leptin and log-CRP in predicting peripheral artery disease in geriatric individuals. AUC for log-leptin was 0.782 (95% confidence interval: 0.657−0.878, p = 0.001), and AUC for log-CRP was 0.739 (95% confidence interval: 0.609−0.844, p = 0.004), respectively.

Table 3
Multivariate logistic regression analysis of the factors correlated to peripheral artery disease among the 60 elderly participants.

Variables	Odds ratio	95% confidence interval	p value
Leptin (ng/ml) (each increase of 1 ng/ml)	1.078	1.021−1.138	0.006*
Smoking (yes)	22.460	1.428−353.336	0.027*

*$p < 0.05$ was considered statistically significant in multivariate logistic regression analysis (adapted factors: diabetes mellitus, hypertension, smoking, gender, age, total cholesterol, triglyceride, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, C-reactive protein, and leptin).

cells.[29] In this study, we found that leptin levels had a positive significant association with the female gender and with the BMI and CRP levels.

Several limitations are emphasized in this study. First of all, this was a single-center investigation with a small number of participants. Another limitation is that pharmacological interventions have been shown to influence PAD in humans. Statin used in patients with PAD results in a decreased progression and even a regression in the growth of the atherosclerotic plaque.[30] There was no statistically significant difference based on statins used between elderly population with or without PAD in this study. Moreover, leptin is implicated in the association between sleep and lower leptin level is associated with sleep short sleep duration.[31] Circulating leptin concentration was proportional to body mass with body fat percentage and body fat was decreased in the elderly.[32] Although, blood sampling of this study is in the morning with overnight fasting about 8 h, but did not check body fat mass in elderly adults. Further studies are required before a cause-effect relationship between serum leptin and PAD can be established in elderly adults.

In conclusion, our study showed a significant difference in current smoking, and CRP and leptin levels by comparing a normal ABI group to a low ABI group in the elderly population. A higher serum leptin level and current smoking is associated with PAD in elderly adults. The female gender and the BMI and CRP levels were positively associated with the serum leptin levels in our study.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

This study was supported by a grant from the Buddhist Tzu Chi General Hospital, Hualien, Taiwan (TCRD101-03).

References

1. Libby P. Inflammation in atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2012;32:2045−2051.
2. Criqui MH, Fronek A, Barrett-Connor E, et al. The prevalence of peripheral arterial disease in a defined population. *Circulation*. 1985;71:510−515.
3. Diehm C, Schuster A, Allenberg JR, et al. High Prevalence of peripheral arterial disease and co-morbidity in 6880 primary care patients: cross-sectional study. *Atherosclerosis*. 2004;172:95−105.
4. Hooi JD, Stoffers HE, Kester AD, et al. Risk factors and cardiovascular diseases associated with asymptomatic peripheral arterial occlusive disease. The Limburg PAOD study. Peripheral arterial occlusive disease. *Scand J Prim Health Care*. 1998;16:177−182.
5. Lijmer JG, Hunink MG, van den Dungen JJ, et al. Roc analysis of noninvasive tests for peripheral arterial disease. *Ultrasound Med Biol*. 1996;22:391−398.
6. Rooke TW, Hirsch AT, Misra S, et al. 2011 ACCF/AHA focused update of the guideline for the management of patients with peripheral artery disease. *J Am Coll Cardiol*. 2011;58:2020−2045.
7. Smith CC, Mocanu MM, Davidson SM, et al. Leptin, the obesity-associated hormone, exhibits direct cardioprotective effects. *Br J Pharmacol*. 2006;149:5−13.

8. Abel ED, Litwin SE, Sweeney G. Cardiac remodeling in obesity. *Physiol Rev*. 2008;88:389–419.
9. Pieterse C, Schutte R, Schutte AE. Leptin relates to prolonged cardiovascular recovery after acute stress in Africans: the SABPA study. *Nutr Metab Cardiovasc Dis*. 2016;26:45–52.
10. Beltowski J. Role of leptin in blood pressure regulation and arterial hypertension. *J Hypertens*. 2006;24:789–801.
11. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. I. Diagnosis and classification of diabetes mellitus: provisional report of a WHO consultation. *Diabet Med*. 1998;15:539–553.
12. Tsai JP, Wang JH, Chen ML, et al. Association of serum leptin levels with central arterial stiffness in coronary artery disease patients. *BMC Cardiovasc Disord*. 2016;18:80.
13. Chen MC, Hsu BG, Lee CJ, et al. Hyperleptinemia positively correlates with cardiometabolic syndrome in hypertensive patients. *Int J Clin Exp Pathol*. 2016;9:12959–12967.
14. Hsu BG, Lee CJ, Yang CF, et al. High serum resistin levels are associated with peripheral artery disease in the hypertensive patients. *BMC Cardiovasc Disord*. 2017;17:80.
15. Ferreira AC, Macedo FY. A review of simple, non-invasive means of assessing peripheral arterial disease and implications for medical management. *Ann Med*. 2010;42:139–150.
16. Hirsch AT, Criqui MH, Treat-Jacobson D, et al. Peripheral arterial disease detection, awareness, and treatment in primary care. *JAMA*. 2001;286:1317–1324.
17. Fowkes FG, Rudan D, Rudan I, et al. Comparison of global estimates of prevalence and risk factors for peripheral artery disease in 2000 and 2010: a systematic review and analysis. *Lancet*. 2013;382:1329–1340.
18. Ness J, Aronow WS, Ahn C. Risk factors for symptomatic peripheral arterial disease in older persons in an academic hospital-based geriatrics practice. *J Am Geriatr Soc*. 2000;48:312–314.
19. Silbernagel G, Rein P, Saely CH, et al. Prevalence of type 2 diabetes is higher in peripheral artery disease than in coronary artery disease patients. *Diab Vasc Dis Res*. 2015;12:146–149.
20. Cauley JA, Kassem AM, Lane NE, et al. Prevalent peripheral arterial disease and inflammatory burden. *BMC Geriatr*. 2016;16:213.
21. Shankar A, Li J, Nieto FJ, et al. Association between C-reactive protein level and peripheral arterial disease among US adults without cardiovascular disease, diabetes, or hypertension. *Am Heart J*. 2007;154:495–501.
22. Criqui MH, Ho LA, Denenberg JO, et al. Biomarkers in peripheral arterial disease patients and near- and longer-term mortality. *J Vasc Surg*. 2010;52:85–90.
23. Singh M, Bedi US, Singh PP, et al. Leptin and the clinical cardiovascular risk. *Int J Cardiol*. 2010;140:266–271.
24. Gonzalez M, Lind L, Söderberg S. Leptin and endothelial function in the elderly: the prospective investigation of the vasculature in Uppsala seniors (PIVUS) study. *Atherosclerosis*. 2013;228:485–490.
25. Quehenberger P, Exner M, Sunder-Plassmann R, et al. Leptin induces endothelin-1 in endothelial cells in vitro. *Circ Res*. 2002;90:711–718.
26. Apter D. The role of leptin in female adolescence. *Ann N Y Acad Sci*. 2003;997:64–76.
27. Owecki M, Nikisch E, Miczke A, et al. Leptin, soluble leptin receptors, free leptin index, and their relationship with insulin resistance and BMI: high normal BMI is the threshold for serum leptin increase in humans. *Horm Metab Res*. 2010;42:585–589.
28. Hribal ML, Fiorentino TV, Sesti G. Role of C reactive protein (CRP) in leptin resistance. *Curr Pharm Des*. 2014;20:609–615.
29. Calabró P, Willerson JT, Yeh ET. Inflammatory cytokines stimulated C-reactive protein production by human coronary artery smooth muscle cells. *Circulation*. 2003;108:1930–1932.
30. Markel A. Statins and peripheral arterial disease. *Int Angiol*. 2015;34:416–427.
31. Grandner MA, Seixas A, Shetty S, Shenoy S. Sleep duration and diabetes risk: population trends and potential mechanisms. *Curr Diab Rep*. 2016;16:106.
32. Friedman J. 20 years of leptin: leptin at 20: an overview. *J Endocrinol*. 2014;223:T1–T8.