

Investigation of the relationship between oxidative stress and SCUBE1 levels in high fat diet-induced obese rats

Akın BODUR^{1*}, Cemil KAHRAMAN², Diler US ALTAY³, Taghi Ahmadi RENDI¹, Ahmet MENTEŞE⁴, Ahmet ALVER⁵

¹Department of Medical Biochemistry, Institute of Health Sciences, Karadeniz Technical University, Trabzon, Turkey

²Department of Nursing, School of Health, Düzce University, Düzce, Turkey

³Laboratory Technology Program, Department of Chemistry and Chemical Processing Technology, Ulubey Vocational School, Ordu University, Ordu, Turkey

⁴Program of Medical Laboratory Techniques, Vocational School of Health Sciences, Karadeniz Technical University, Trabzon, Turkey

⁵Department of Medical Biochemistry, Faculty of Medicine, Recep Tayyip Erdoğan University, Rize, Turkey

Received: 29.09.2017 • Accepted/Published Online: 07.01.2018 • Final Version: 23.02.2018

Background/aim: This study aimed to investigate signal peptide-Cub-EGF domain-containing protein 1 (SCUBE1) levels and oxidative stress (OS) in a high fat diet (HFD)-induced obese rat model.

Materials and methods: Fourteen rats were randomly divided into two groups. Group 1, the control group, was given a standard diet (10% calories of fat) and Group 2, the obese group, was given a HFD (60% calories of fat), both for 70 days. Rats were then sacrificed and serum samples were collected. Serum glucose and triglyceride concentrations were determined using an autoanalyzer. Serum SCUBE1, adiponectin, and plasminogen activator inhibitor-1 levels were determined using an enzyme-linked immunosorbent assay. Serum malondialdehyde levels and superoxide dismutase and glutathione peroxidase enzyme activities were determined using colorimetric methods.

Results: Final body weight was higher in the obese group ($P = 0.007$). Serum malondialdehyde concentrations were also higher in the obese group ($P = 0.021$). Serum glutathione peroxidase activities were higher in the control group ($P = 0.028$). Serum SCUBE1 levels were also higher in the control group ($P = 0.038$).

Conclusion: There may be no connection between the measured OS parameters and SCUBE1. Differences in SCUBE1 levels may therefore be evaluated independently from OS in obesity.

Key words: High fat diet, lipid peroxidation, oxidative stress, obesity, SCUBE1

1. Introduction

Obesity is a serious health problem affecting the global population. It is regarded as a low-grade inflammatory disease based on uncontrolled expansion of adipose tissue (AT) (1). AT is a metabolically active organ that secretes numerous adipokines (such as leptin, adiponectin, and resistin) and proinflammatory cytokines and chemokines (such as tumor necrosis factor alpha, interleukin-6, and monocyte chemoattractant protein-1) (2). AT involves different cell types, but from the metabolic perspective, adipocytes are the most active cell type. Hyperplasia and hypertrophy of adipocytes is the main cause of AT expansion (3). AT expansion limits the use of O₂ and adipocytes in particular move away from capillaries, giving rise to hypoxia. Obesity-related hypoxia disrupts normal levels of adipokines in AT (4). From the biochemical

perspective, obesity is characterized by an imbalance between energy intake and expenditure, and this situation strongly affects the hypertrophic and hyperplastic adipocyte metabolism (2,3).

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced by normal physiological conditions. However, ROS production increases under some pathophysiological conditions, such as obesity, hyperglycemia, and type II diabetes. Increased ROS production and inadequate antioxidant enzyme systems, such as catalase, glutathione peroxidase (GPx), and superoxide dismutase (SOD), generally lead to an imbalance between oxidants and antioxidants, resulting in oxidative stress (OS). Protein carbonyls, malondialdehyde (MDA), and F-2 isoprostanes are well-known biomarkers of OS (5). Excessive fat accumulation, increased fatty acid

* Correspondence: akinbodur28@gmail.com

oxidation, and overconsumption of oxygen enhance ROS generation in obesity. Obesity causes several important pathophysiological conditions, including hypertension, cardiovascular diseases, dyslipidemia, hyperglycemia, and type II diabetes, all of which raise OS levels (6).

Signal peptide-Cub-EGF domain-containing protein 1 (SCUBE1) is a peptide that involves 10 EGF-like repeats at the amino terminal and 1 CUB domain at the carboxyl terminal side. The SCUBE gene family includes SCUBE1–3. SCUBE1 gene expression is higher in endothelial cells and platelets. SCUBE1 may regulate hematopoiesis in platelets (7,8). Yang et al. showed that SCUBE1 is expressed in the vascular endothelium and may be significant in thrombosis (8). SCUBE1 has been studied in inflammatory and hypoxia-related diseases, such as acute coronary syndrome, acute ischemic stroke, acute mesenteric ischemia, and gastric cancer (9–12). Tu et al. suggested that SCUBE1 may be an adhesive molecule and that this peptide may therefore play a critical role in vascular pathology (13). Obesity-related thrombosis is characterized by chronic inflammation and disrupted fibrinolysis. Activated platelets are the most important cells in obesity-related chronic inflammation and activate the prothrombotic signaling pathway in vascular cells. Plasminogen activator inhibitor-1 (PAI-1) is synthesized in the liver, endothelial cells, and AT. Increased PAI-1 leads to hypofibrinolysis and prothrombosis (14). These then lead to endothelial dysfunction, ruptured atherosclerotic plaque, platelet hyperactivation, and delayed clot lysis (15). This study investigated SCUBE1 levels and OS as a part of measuring SOD, GPx, and MDA levels in a high fat diet (HFD)-induced obese rat model.

2. Materials and methods

2.1. Animals and experimental design

The experimental study commenced following approval from the Karadeniz Technical University Animal Care and Ethics Committee. All experimental and surgical procedures were performed in the Karadeniz Technical University Surgical Application and Research Center. Fourteen Sprague-Dawley rats aged 3–5 weeks and weighing 100–150 g were randomized and divided into two equal groups. The control group received a standard diet and the obese group received a HFD, both for 70 days. Standard and HFD chows were purchased from Research Diets Inc., USA (Catalog # D12450J: 10% calories of fat, 70% carbohydrate, and 20% protein; Catalog # D12492: 60% calories of fat, 20% carbohydrate, and 20% protein). Standard and high fat rat chow diets and water were provided ad libitum for all animals. Rat serum samples were collected and stored at -80°C for measurement of biochemical parameters.

2.2. Serum MDA level measurement

Serum MDA levels were determined following the method described by Yagi (16). This method depends on lipid peroxidation products and thiobarbituric acid. Serum lipids and proteins were precipitated with a phosphotungstic acid and sulfuric acid mixture. Tetramethoxypropane was used as a standard. MDA levels were expressed as nmol/mL.

2.3. Serum SOD enzyme activity measurement

SOD enzyme activities were determined using a colorimetric assay kit (Cayman Chemical Company, USA, Cat. No. 706002, Lot No. 0463319) in line with the manufacturer's instructions. The kit method relies on the reduction of nitroblue tetrazolium by xanthine and the xanthine oxidase enzyme system. Optical densities were measured at 560 nm using a VersaMax tunable microplate reader (Molecular Devices, USA). The results were expressed as U/g protein.

2.4. Serum GPx enzyme activity measurement

GPx was determined using a colorimetric assay kit (Cayman Chemical Company, USA, Cat. No. 703102, Lot No. 0450739), following the kit protocol. GPx activities were measured using the coupling reaction of glutathione reductase, GPx, and oxidation of NADPH to NADP⁺. Decreasing optical densities were measured kinetically at 340 nm using a VersaMax tunable microplate reader. The results were expressed as U/g protein.

2.5. Serum SCUBE1, adiponectin, and PAI-1 measurements

Rat serum SCUBE1, adiponectin, and PAI-1 levels were determined using ELISA kits (Elabscience, China, Cat. No. E-EL-R1103; SunRed, China, Cat. No. 201-11-0759; and SunRed, China, Cat. No. 201-11-0637, respectively) in line with the relevant protocol. Samples were measured at 450 nm using a VersaMax tunable microplate reader. The results were expressed as ng/mL, mg/L, and ng/mL, respectively.

2.6. Serum glucose and triglyceride measurements

Fasting glucose and triglyceride levels were measured using a Roche Hitachi Cobas 8000 autoanalyzer (Switzerland). Both were expressed as mg/dL.

2.7. Statistical analysis

Data were expressed as mean \pm standard error ($X \pm SE$). The Kolmogorov–Smirnov test was used to test normality, and the groups were compared using the Mann–Whitney U test. Statistical significance was set at $P < 0.05$.

3. Results

The final mean body weight in the obese group (454 ± 11.51 g) was significantly higher than that of the control group (392.25 ± 13.33 g) ($P = 0.007$; Table). Serum glucose concentrations were higher in the control group (112.4 ± 7.70 mg/dL) than in the obese group (104 ± 7.88 mg/

Table. Control and obese group glucose and triglyceride concentrations and body weights.

| Groups | Control (n = 7) | Obese (n = 7) |
|-----------------------|-----------------|---------------|
| Glucose (mg/dL) | 112.43 ± 7.70 | 104.00 ± 7.88 |
| Triglyceride (mg/dL) | 84.57 ± 18.45 | 93.38 ± 10.12 |
| Final body weight (g) | 392.25 ± 13.33 | 454 ± 11.51* |

*: P < 0.05.

dL) (Table). Serum triglyceride concentrations were also higher in the obese group (93.38 ± 10.12 mg/dL) than in the control group (84.57 ± 18.45 mg/dL) (Table). No significant difference was determined between the groups in terms of glucose or triglyceride concentrations (P = 0.536 for both). Serum MDA concentrations were significantly higher in the obese group (1.98 ± 0.35 nmol/mL) than in the control group (1.08 ± 0.13 nmol/mL) (P = 0.021; Figure 1). Serum GPx activities were significantly lower in the obese group (4.43 ± 0.49 U/g protein) than in the control group (7.93 ± 1.32 U/g protein) (P = 0.028; Figure

2). Serum SOD activities were higher in the obese group (2.14 ± 0.49 U/g protein) than in the control group (2.06 ± 0.72 U/g protein), but the difference was not statistically significant (P = 0.491; Figure 2). Serum SCUBE1 levels were significantly increased in the control group (2.51 ± 0.36 ng/mL) compared with the obese group (1.78 ± 0.73 ng/mL) (P = 0.038; Figure 3). Serum adiponectin levels were higher in the obese group (8.28 ± 0.39 mg/L) than in the control group (8.51 ± 0.32 mg/L), although the difference was not statistically significant (P = 0.955; Figure 4). Serum PAI-1 levels were higher in the control

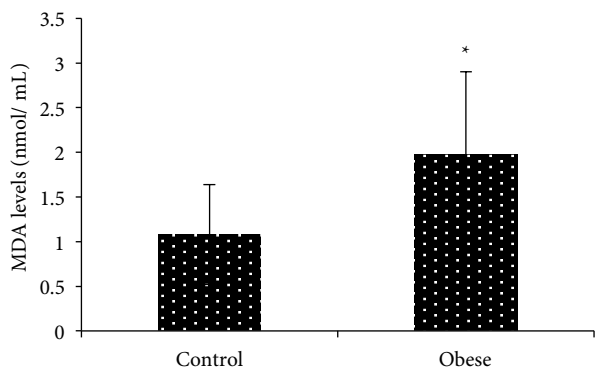


Figure 1. The means of MDA levels of the control and obese groups (*: P = 0.021 compared with control group).

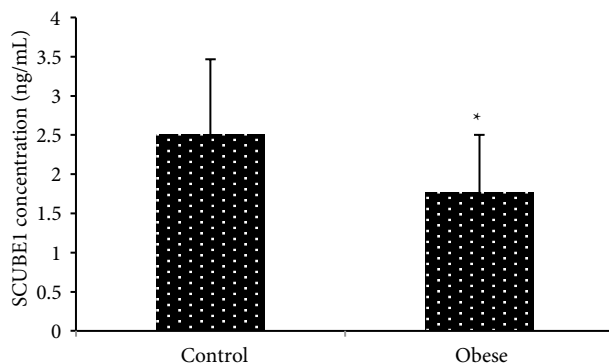


Figure 3. The means of SCUBE1 concentrations of the control and obese groups (*: P = 0.038 compared with control group).

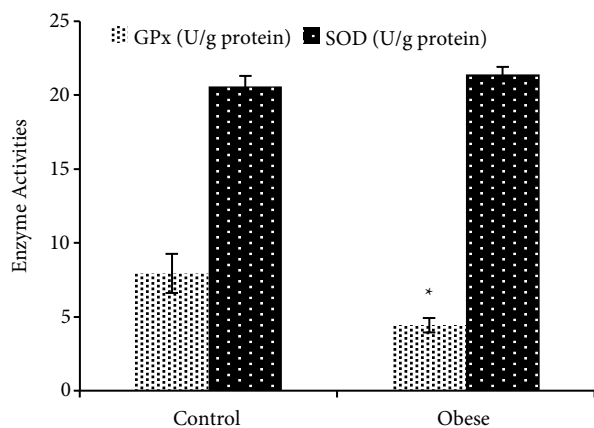


Figure 2. The means of GPx and SOD activities of the control and obese groups (*: P = 0.028 compared with control group).

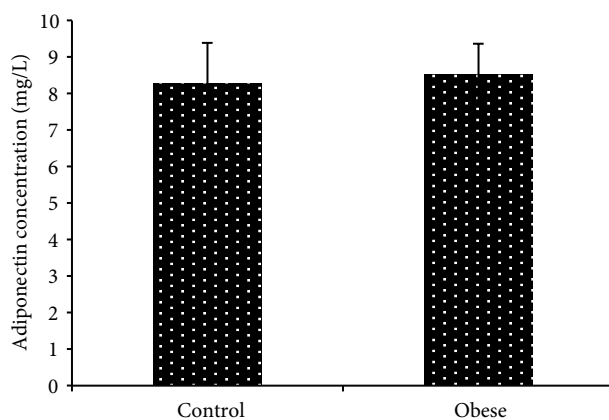


Figure 4. The means of adiponectin concentrations of the control and obese groups.

group (7.60 ± 0.40 ng/mL) than in the obese group (6.83 ± 0.37 ng/mL), but not statistically significantly higher ($P = 0.232$; Figure 5).

4. Discussion

A HFD has been used to induce obesity in experimental models. Studies have reported that when at least 30% of energy is supplied by fat content the diet exhibits HFD effects. These HFD effects vary depending on different animal species, feeding periods, and fat types, and there is no standardized fatty acid content of HFDs (17). Buettner et al. demonstrated that HFDs contain different types of fatty acids causing different metabolic and molecular effects in rat models (18). In the present study, Sprague-Dawley rats received a 60% fat content diet for 70 days. The final body weight of the obese group was significantly higher than that of the control group ($P = 0.007$). In addition, a HFD causes metabolic outcomes such as adipocyte hypertrophy, hyperplasia, and insulin resistance (17). In our study, serum glucose and triglyceride levels were measured for general metabolic assessment of HFD-fed rats. Serum triglyceride levels increased but glucose levels decreased in the obese group. Both triglyceride and glucose levels exhibited no significant difference between the control and obese groups ($P = 0.536$ for both). This suggested that serum glucose and triglyceride levels are not affected by a HFD for 70 days. Some previous studies have reported serum glucose and triglyceride findings similar to our results (19,20). One comprehensive study demonstrated that results for metabolic parameters varied depending on the animal model strain, sex, and feeding period. The authors concluded that animal characterization is important for nutrition studies in animal models (21).

Obesity is a low chronic inflammatory condition that proceeds with hypoxia, OS, endoplasmic reticulum (ER) stress, and mitochondrial dysfunction (22). Hypertrophied adipocytes and preadipocytes increase in expanded AT,

which causes enlarged adipocytes. The oxygenation of these adipocytes decreases and proinflammatory cytokine synthesis thus increases (23). Cytokines stimulate ROS and RNS synthesis, and obesity-induced OS therefore occurs with cytokine concentration elevation. If obesity is prolonged, the antioxidant defense system becomes insufficient against ROS and RNS generation in AT (24,25). Inadequate antioxidant defenses, hyperglycemia, enhanced muscle activity, high tissue lipid levels, and enhanced endothelial ROS generation contribute to OS in obesity (5). Emami et al. demonstrated that HFD-induced obesity alters the redox balance by reducing antioxidant enzyme activities and enhances MDA levels in rats (26). Higher MDA levels have been reported in 15-week-old HFD-induced obese rats. The same study also reported lower catalase, SOD, and GPx activities in HFD-fed rats than in control diet-fed rats (27). In our study, MDA, a lipid peroxidation end product and a biomarker of OS, was significantly elevated in the obese group compared with the control group ($P = 0.021$). GPx levels decreased significantly in the obese group ($P = 0.028$). SOD levels were higher in the obese group, but the difference was not statistically significant ($P = 0.491$). Our results show that HFD induces OS in AT, but that the antioxidant defense system is insufficient under conditions of OS.

Adiponectin is an AT-derived hormone with antiinflammatory properties that protects insulin sensitivity (2). In our study, adiponectin levels were higher in the obese group than in the control group, but the difference was not statistically significant ($P = 0.955$). Adiponectin levels have been shown to differ depending on the feeding period, animal species, and diet content in HFDs in previous studies (28,29).

The tissue distribution profiles of SCUBE1 and SCUBE1 mRNA expression and function are still uncertain (30). Obesity increases the risk of atherosclerosis, and adipokines may regulate the progression of atherosclerosis development (31). Prothrombotic conditions lead to endothelial dysfunction, ruptured atherosclerotic plaque, platelet hyperactivation, and delayed clot degradation in obesity (15). Platelet activation is closely linked to inflammation and thrombosis. PAI-1 is associated with a prothrombotic state and is related to obesity (32). In our study, PAI-1 levels decreased in the obese group, but the difference between the two groups was not statistically significant ($P = 0.232$). SCUBE1 is a glycoprotein located in the vascular system and endothelium. Previous studies have described the importance of SCUBE1 in thrombosis, inflammation, and hypoxia. SCUBE1 is stored in α -granules of inactivated platelets. This protein produces an increase in the adhesion of platelets to the matrix. When platelet activation occurs, SCUBE1 is released into circulation

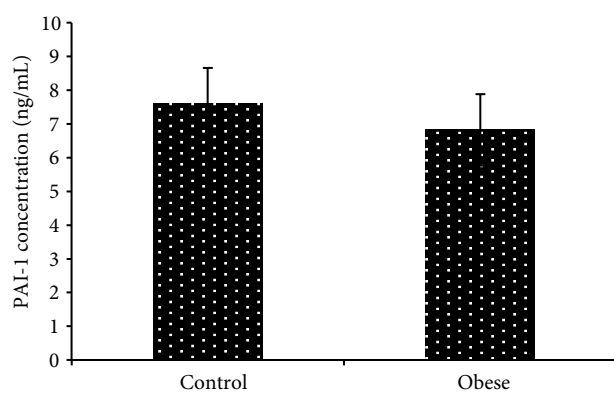


Figure 5. The means of PAI-1 concentrations of the control and obese groups.

under hypoxia and inflammatory conditions and enters thrombi (8,13). Liao et al. recently demonstrated that N-glycosylation is essential for SCUBE1 functions in a zebrafish model (33). However, the functions of SCUBE1 are still not fully understood. Wu et al. demonstrated that SCUBE1 is involved in platelet aggregation and provides a link between activated platelets in thrombosis (34). Elevated plasma SCUBE1 concentrations have been observed in acute coronary syndrome and ischemic stroke in humans (9). Higher serum SCUBE1 levels have been reported in patients with aneurysmal subarachnoid hemorrhage compared to healthy controls (35). Ours is the first study to examine the relationship between SCUBE1 and obesity in a HFD-induced obese rat model. Serum SCUBE1 levels were significantly lower in the obese group compared to the control group ($P = 0.038$) (Figure 3). This may be attributed to HFD consumption for 70 days being inadequate for the development of chronic inflammatory conditions in our diet-induced obesity model. In addition, the prothrombotic signaling pathway was not activated in the inadequate chronic inflammatory environment, and no alteration was therefore observed in serum PAI-1 levels in the obese group compared to the control group (Figure 5). It should also be noted that SCUBE1 could not

be released into the systemic circulation, and that small peptides were able to be degraded in α -granules.

MDA levels increased in serum specimens in our study, but SCUBE1 levels decreased (Figures 1 and 3). However, no correlation was observed between the measured OS parameters and SCUBE1. Since no previous studies have investigated the relation between obesity-induced OS and SCUBE1, we are unable to compare our results with findings from other research. OS is a factor that dominates the procoagulant and prothrombotic state in obesity (36). It may be inferred from our results that prothrombosis was not stimulated by OS. SCUBE1 therefore cannot be used as a target molecule for suppressing OS in obesity. Variations in SCUBE1 levels may therefore be evaluated independently from OS parameters. It may be speculated that severe ER stress leads to the degradation of SCUBE1 protein and that this may lead to a decrease in SCUBE1 levels in HFD-fed obese rats. Additionally, AT-derived factors may stimulate SCUBE1 levels, and our review of the literature revealed no previous study of the association between diet type and SCUBE1. We intend to design further studies to investigate the relation between SCUBE1 and obesity, with particular reference to obesity-derived ER stress and hypoxia.

References

1. Via MA, Mechanick JI. Obesity as a disease. *Curr Obes Rep* 2014; 3: 291-297.
2. Galic S, Oakhill JS, Steinberg GR. Adipose tissue as an endocrine organ. *Mol Cell Endocrinol* 2010; 316: 129-139.
3. Vazquez-Vela ME, Torres N, Tovar AR. White adipose tissue as endocrine organ and its role in obesity. *Arch Med Res* 2008; 39: 715-728.
4. Ruskovska T, Bernlohr DA. Oxidative stress and protein carbonylation in adipose tissue - implications for insulin resistance and diabetes mellitus. *J Proteomics* 2013; 92: 323-334.
5. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol* 2007; 39: 44-84.
6. Vincent HK, Taylor AG. Biomarkers and potential mechanisms of obesity-induced oxidant stress in humans. *Int J Obes (Lond)* 2006; 30: 400-418.
7. Wu BT, Su YH, Tsai MT, Wasserman SM, Topper JN, Yang RB. A novel secreted, cell-surface glycoprotein containing multiple epidermal growth factor-like repeats and one CUB domain is highly expressed in primary osteoblasts and bones. *J Biol Chem* 2004; 279: 37485-37490.
8. Yang RB, Ng CK, Wasserman SM, Colman SD, Shenoy S, Mehraban F, Komuves LG, Tomlinson JE, Topper JN. Identification of a novel family of cell-surface proteins expressed in human vascular endothelium. *J Biol Chem* 2002; 277: 46364-46373.
9. Dai DF, Thajeb P, Tu CF, Chiang FT, Chen CH, Yang RB, Chen JJ. Plasma concentration of SCUBE1, a novel platelet protein, is elevated in patients with acute coronary syndrome and ischemic stroke. *J Am Coll Cardiol* 2008; 51: 2173-2180.
10. Mentese A, Fidan E, Sumer AU, Karahan SC, Sonmez M, Altay DU, Kavgaci H, Alver A. Is SCUBE 1 a new biomarker for gastric cancer? *Cancer Biomark* 2012; 11: 191-195.
11. Turkmen S, Eryigit U, Karaca Y, Mentese A, Sumer UA, Yulug E, Aksut N, Gazioglu S, Gunduz A. Diagnostic value of plasma signal peptide-Cub-Egf domain-containing protein-1 (SCUBE-1) in an experimental model of acute ischemic stroke. *Am J Emerg Med* 2015; 33: 262-265.
12. Turkmen S, Mentese S, Mentese A, Sumer AU, Saglam K, Yulug E, Turedi S, Gunduz A. The value of signal peptide-CUB-EGF domain-containing protein 1 and oxidative stress parameters in the diagnosis of acute mesenteric ischemia. *Acad Emerg Med* 2013; 20: 257-264.
13. Tu CF, Su YH, Huang YN, Tsai MT, Li LT, Chen YL, Cheng CJ, Dai DF, Yang RB. Localization and characterization of a novel secreted protein SCUBE1 in human platelets. *Cardiovasc Res* 2006; 71: 486-495.

14. Maury E, Brichard SM. Adipokine dysregulation, adipose tissue inflammation and metabolic syndrome. *Mol Cell Endocrinol* 2010; 314: 1-16.
15. Samad F, Ruf W. Inflammation, obesity, and thrombosis. *Blood* 2013; 122: 3415-3422.
16. Yagi K. Lipid peroxides and related radicals in clinical medicine. *Adv Exp Med Biol* 1994; 366: 1-15.
17. Buettner R, Scholmerich J, Bollheimer LC. High-fat diets: modeling the metabolic disorders of human obesity in rodents. *Obesity (Silver Spring)* 2007; 15: 798-808.
18. Buettner R, Parhofer KG, Woenckhaus M, Wrede CE, Kunz-Schughart LA, Scholmerich J, Bollheimer LC. Defining high-fat-diet rat models: metabolic and molecular effects of different fat types. *J Mol Endocrinol* 2006; 36: 485-501.
19. Bake T, Morgan DG, Mercer JG. Feeding and metabolic consequences of scheduled consumption of large, binge-type meals of high fat diet in the Sprague-Dawley rat. *Physiol Behav* 2014; 128: 70-79.
20. Öner-İyidoğan Y, Koçak H, Seyidhanoğlu M, Gürdöl F, Gülçubuk A, Yildirim F, Çevik A, Uysal M. Curcumin prevents liver fat accumulation and serum fetuin-A increase in rats fed a high-fat diet. *J Physiol Biochem* 2013; 69: 677-686.
21. Gallou-Kabani C, Vigé A, Gross MS, Rabès JB, Boileau C, Larue-Achagiotis C, Tomé D, Jais JP, Junien C. C57BL/6J and A/J mice fed a high-fat diet delineate components of metabolic syndrome. *Obesity (Silver Spring)* 2007; 15: 1996-2005.
22. Kusminski CM, Scherer PE. Mitochondrial dysfunction in white adipose tissue. *Trends Endocrinol Metab* 2012; 23: 435-443.
23. Trayhurn P, Wang B, Wood IS. Hypoxia in adipose tissue: a basis for the dysregulation of tissue function in obesity? *Br J Nutr* 2008; 100: 227-235.
24. Fernández-Sánchez A, Madrigal-Santillán E, Bautista M, Esquivel-Soto J, Morales-González Á, Esquivel-Chirino C, Durante-Montiel I, Sánchez-Rivera G, Valadez-Vega C, Morales-González JA. Inflammation, oxidative stress, and obesity. *Int J Mol Sci* 2011; 12: 3117-3132.
25. Le Lay S, Simard G, Martinez MC, Andriantsitohaina R. Oxidative stress and metabolic pathologies: from an adipocentric point of view. *Oxid Med Cell Longev* 2014; 2014: 908539.
26. Emami SR, Jafari M, Haghshenas R, Ravasi A. Impact of eight weeks endurance training on biochemical parameters and obesity-induced oxidative stress in high fat diet-fed rats. *J Exerc Nutrition Biochem* 2016; 20: 29-35.
27. Carmiel-Haggai M, Cederbaum AI, Nieto N. A high-fat diet leads to the progression of non-alcoholic fatty liver disease in obese rats. *FASEB J* 2005; 19: 136-138.
28. Haluzik M, Gavriloova O, LeRoith D. Peroxisome proliferator-activated receptor-alpha deficiency does not alter insulin sensitivity in mice maintained on regular or high-fat diet: hyperinsulinemic-euglycemic clamp studies. *Endocrinology* 2004; 145: 1662-1667.
29. Ye JM, Iglesias MA, Watson DG, Ellis B, Wood L, Jensen PB, Sørensen RV, Larsen PJ, Cooney GJ, Wassermann K et al. PPARα/γ ragaglitazar eliminates fatty liver and enhances insulin action in fat-fed rats in the absence of hepatomegaly. *Am J Physiol Endocrinol Metab* 2003; 284: E531-540.
30. Tu CF, Yan YT, Wu SY, Djoko B, Tsai MT, Cheng CJ, Yang RB. Domain and functional analysis of a novel platelet-endothelial cell surface protein, SCUBE1. *J Biol Chem* 2008; 283: 12478-12488.
31. Yoo HJ, Choi KM. Adipokines as a novel link between obesity and atherosclerosis. *World J Diabetes* 2014; 5: 357-363.
32. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet* 2005; 365: 1415-1428.
33. Liao WJ, Tsao KC, Yang RB. Electrostatics and N-glycan-mediated membrane tethering of SCUBE1 is critical for promoting bone morphogenetic protein signalling. *Biochem J* 2016; 473: 661-672.
34. Wu MY, Lin YC, Liao WJ, Tu CF, Chen MH, Roffler SR, Yang RB. Inhibition of the plasma SCUBE1, a novel platelet adhesive protein, protects mice against thrombosis. *Arterioscler Thromb Vasc Biol* 2014; 34: 1390-1398.
35. Ding YS, Sun B, Jiang JX, Zhang Q, Lu J, Gao GZ. Increased serum concentrations of signal peptide-Cub-Egf domain-containing protein-1 in patients with aneurysmal subarachnoid hemorrhage. *Clin Chim Acta* 2016; 459: 117-122.
36. Morange PE, Alessi MC. Thrombosis in central obesity and metabolic syndrome: mechanisms and epidemiology. *Thromb Haemost* 2013; 110: 669-680.