

## Research Article

# Adipokine Concentration in Adipose Tissue of Obese Mice: Location Dependency

Kyaw Thu Moe<sup>1\*</sup>, Katwadi Khairunnisa<sup>2</sup> and John Allen<sup>2</sup>

<sup>1</sup>Newcastle University Medicine Malaysia, Johor, Malaysia

<sup>2</sup>Duke-NUS Graduate Medical School, Singapore

\*Corresponding author: Kyaw Thu Moe, Newcastle University Medicine Malaysia, 79200 Gelang Patah, Johor, Malaysia, Tel: +60 75553885; E-mail: [moe.kyawthu@newcastle.edu.my](mailto:moe.kyawthu@newcastle.edu.my)

Received Date: 11 August, 2017; Accepted Date: 18 September, 2017; Published Date: 3 October, 2017

## Abstract

Hypertrophy and hyperplasia of adipocytes leading to enlargement of adipose tissue are the characteristics of obesity. Adipocytes secrete adipokines, resulting in elevated levels of adipokines in the circulation. However, whether differential secretion of adipokines in obesity is a result of secretion from enlarged adipocytes or due to location-specific production in adipose tissue is not known. Our prior study showed that Perivascular Adipose Tissue (PVAT) plays a crucial role in increased intima-media thickness and vascular smooth muscle cell proliferation. Whether PVAT adipokines play a crucial role in vascular cells changes is unclear. Moreover, PVAT adipokine production compared to other adipose tissue is not known. In the present study, we examined differential adipokine concentrations in PVAT compared to subcutaneous and gonadal adipose tissue and investigated the correlation between adipocyte cell surface area and adipokine concentrations among these tissues. We measured the concentrations of leptin, macrophage chemoattractant protein-1, IL-6, plasminogen activator inhibitor-1, and resistin in plasma and adipose tissues in three locations (subcutaneous, gonadal, perivascular) as well as plasma insulin concentrations in diet-induced obese mice using Milliplex Immunoassays. We also examined the surface area of adipocyte cells in these adipose tissues in obese and lean mice. Finally, we investigated the correlation of the surface area of adipocyte cells in these adipose tissues with adipokine concentrations. Our study revealed that cell surface area of PVAT adipocytes is the smallest among three adipocytes and elevated level of IL-6, but not other four adipokines was observed in PVAT of obese mice. More importantly, differential adipokine concentrations in different adipose tissues were dependent primarily on the location of adipose tissue. Our prior and current studies suggest that increased IL-6 level in PVAT contributes to vascular remodeling.

## Keywords

Adipokines; Obesity; Perivascular Adipose Tissue; IL-6

## Introduction

Obesity is a risk factor for cardiovascular, cerebrovascular and metabolic disorders. Obesity occurs as a result of the enlargement of adipose tissue, due to hypertrophy and hyperplasia of adipocytes. Adipocytes are critical repositories of free fatty acids and also act as an endocrine organ. Adipose tissue secretes several pro-inflammatory cytokines and chemokines (collectively known as “adipokines”). Circulatory levels of inflammatory adipokines are elevated in obese subjects [1].

The notion that elevated levels of circulatory adipokines are a result of secretion of adipokines from hypertrophic adipocytes, or due to different stimuli at a specific location of adipose tissue, is controversial. Some studies have suggested that elevated levels of adipokines are dependent upon the size of adipocytes, whereas other studies have shown that they are dependent upon specific location of adipose tissue, such as Subcutaneous Adipose Tissue (SAT) and Visceral Adipose Tissue (VAT) [2,3]. Levels of leptin, IL-6, IL-8, Tumor Necrosis Factor (TNF)- $\alpha$ , Macrophage Chemoattractant Protein (MCP)-1, Interferon Gamma-Induced Protein (IP)-10, Macrophage Inflammatory Protein (MIP)-1 $\beta$ , and Granulocyte-Colony Stimulating Factor (G-CSF) from freshly isolated adipocytes were positively associated with adipocyte volume and the greatest secretion was observed in the largest adipocyte fraction obtained from the SAT [2]. Other reports have noted location-specific secretion of adipokines from SAT and VAT. Linder et al., have shown that calcyclin, adipsin and PAC clone 12p13.3 are expressed only in SAT, and that Ras homolog gene family member G and phospholipid transfer protein are expressed in VAT [4]. Other reports have shown upregulation of expression of MIP-1 in SAT and IL-6 in VAT [3]. Differential secretion between SAT and VAT, of leptin, TNF- $\alpha$ , angiotensin, and Plasminogen Activator Inhibitor (PAI)-1 has been reported [5]. Leptin mRNA secretion is higher in SAT than VAT in humans, in contrast to rodents, where leptin mRNA levels in Gonadal Adipose Tissue (GAT) and retroperitoneal adipose tissue are higher than those in SAT [6,7]. Another

adipose tissue-specific protein, resistin, is secreted abundantly in GAT. Resistin secretion is reduced as rodents become obese, but it is noteworthy that resistin levels in the circulation may remain elevated [8].

PVAT exerts an anti-contractile effect on vessels through both endothelium-dependent and -independent mechanisms in healthy subjects [9]. However, in obesity, high Free Fatty Acids (FFA) directly cause attenuation of the anti-contractile response of PVAT through an endothelium-dependent pathway [10]. We also reported that PVAT around the abdominal aorta plays a crucial part in vascular remodeling in chronic inflammation and suggested that PVAT produces inflammatory mediators that initiated vascular remodeling [11]. However, adipokine secretion in PVAT, in comparison with adipose tissue in other locations, is not known. Understanding PVAT adipokine secretion and its contribution to vascular remodeling may yield novel, important strategies for management of cardiovascular diseases. We examined the correlation of adipokine concentrations with the cell-surface area of adipocytes from adipose tissue from three locations. We investigated the differential expression of adipokines from the SAT, GAT and PVAT of diet-induced obese mice compared with control lean mice.

## Materials and Methods

### Diet-induced obese mice

All protocols and experimental procedures were undertaken in accordance with the Guidelines for the Care and Use of Research Animals (National Institutes of Health, Bethesda, MD, USA) as approved by the Animal Care and Use Committee of SingHealth (Singapore).

Eight-week-old male C57BL/6 mice were purchased from the National University of Singapore. One group of mice were fed a high-fat diet (60% kcal as fat; Research Diets, New Brunswick, USA) and control mice were fed a low-fat diet (10% kcal as fat; research diet, New Brunswick, USA) for 6 weeks (n=26 in each group) following procedures described previously [12]. After 6 weeks, mice were sacrificed. Samples of SAT, GAT, PVAT and plasma were collected. Plasma was stored at -80°C immediately after isolation.

### Measurement of adipocyte sizes

Sample tissue from SAT, GAT and PVAT were fixed in 4% paraformaldehyde, embedded in paraffin, sectioned and stained with Hematoxylin and Eosin (H&E). Measurement of the surface area of adipocyte cells was undertaken on 10 randomly chosen fields on  $\geq 3$  sections for each mouse. Adipocyte area was traced and obtained manually with Image Pro Plus ver6.0 (Media Cybernetics, Silver Spring, MD, USA) following methods described previously [11].

### Milliplex™ immunoassays

Tissue homogenates from SAT, GAT and PVAT were prepared following methods described previously [13]. Protein concentrations were quantified with a NanoDrop ND-1000 Spectrophotometer (Thermo Scientific, Wilmington, NC, USA). Quantification of plasma levels of insulin and other adipokines (leptin, MCP-1, IL-6, PAI-1, resistin) in plasma and homogenates of adipose tissue was undertaken using Milliplex MAP Mouse Adipokine immunoassays (Millipore, Billerica, MA, USA) following methods described previously [14,15].

### Statistical analysis

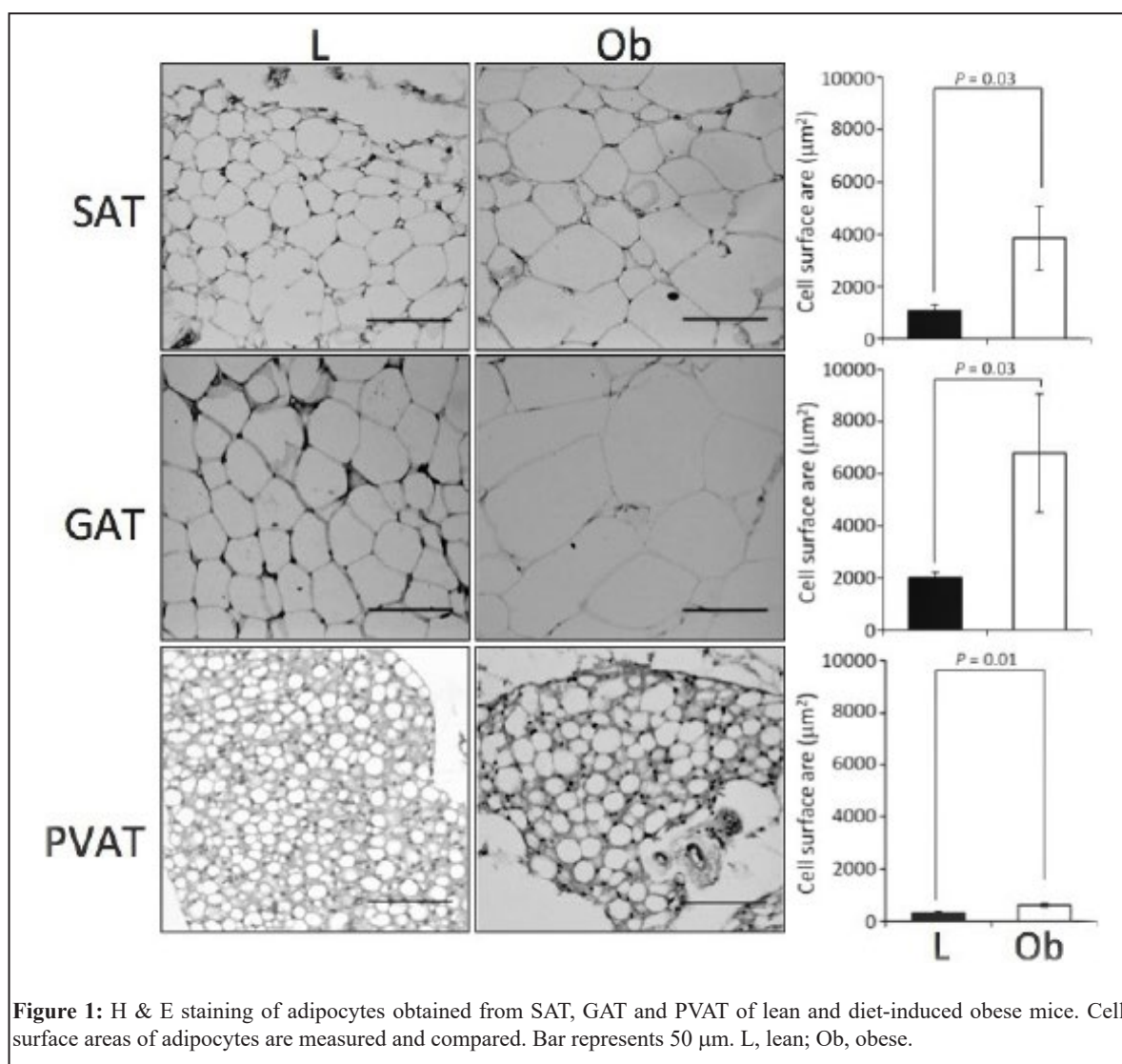
Data are summarized as the mean  $\pm$  and Standard Deviation (SD). Differences in means were tested for statistical significance using Student's *t*-test, and  $P < 0.05$  was considered statistically significant. No adjustment was made for multiple comparisons; however, all *P*-values are reported permitting adjustment at the discretion of the reader. Data were analyzed using GraphPad Prism ver4.02 (GraphPad, San Diego, CA, USA). Least-squares regression was used to fit trend lines to log-transformed data.

## Results and Discussion

Several roles for adipose tissue in the regulation of metabolism have been discovered. Among these are hormonal regulation, energy intake, and fat storage as well as endocrine, paracrine and autocrine functions [16]. In obesity, adipose tissue undergoes hypertrophy and hyperplasia, and secretes several adipokines, resulting in elevated levels of circulatory adipokines. However, adipocyte hypertrophy and location of adipose tissue in relation to adipokine secretion are poorly understood. Some studies have shown a positive correlation between adipocyte size and secretion of adipokines from adipocytes, whereas others suggest that the location of adipose tissue is a predominant factor in regulating adipokine secretion [2,3]. In the present study, we investigated the levels of different adipokines in SAT, GAT and PVAT in diet-induced obese mice compared with control lean mice. We further examined correlations of adipocyte cell surface area with adipokine concentrations among these adipose tissues.

Comparison of cell surface area of adipocytes from different adipose tissue in obese mice has not been previously reported. Our study demonstrated that in lean control mice the surface area of adipocyte cells obtained from GAT ( $2007 \pm 198 \mu\text{m}^2$ ) was significantly larger than that from SAT ( $1078 \pm 210 \mu\text{m}^2$ ,  $P=0.001$ ) and PVAT ( $333 \pm 46 \mu\text{m}^2$ ,  $P=0.001$ ). Similarly, the surface area of adipocyte cells in obese mice obtained from GAT ( $6793 \pm 2273 \mu\text{m}^2$ ) was significantly larger than those from SAT ( $3846 \pm 1233 \mu\text{m}^2$ ,  $P=0.001$ ) and PVAT ( $622 \pm 57 \mu\text{m}^2$ ,  $P=0.001$ ). Moreover, the surface areas of adipocyte cells from GAT, SAT, and PVAT of obese mice were significantly larger than those of lean mice ( $P=0.03$ ,  $P=0.03$ , and  $P=0.01$  respectively, figure 1). The surface

areas of gonadal adipocyte cells from lean and obese mice in our study were similar to those reported in other studies [13].



Elevated levels of insulin and adipokines in plasma have been reported in obese subjects as well as several diet-induced and genetically modified animal models. Our study found significantly elevated levels of insulin ( $P=0.009$ ), leptin ( $P=0.03$ ), IL-6 ( $P=0.01$ ) and resistin ( $P=0.002$ ) in plasma of obese mice compared with control lean mice (Figure 2A).

Adipose-derived hormone leptin mRNA levels in obese humans are known to be substantially higher in SAT than in omental adipose tissue. However, in obese rats these levels are significantly higher in GAT and retroperitoneal tissue than in inguinal adipose tissue [6,7]. Our study revealed significantly higher leptin concentrations in GAT than in SAT and PVAT in both lean mice ( $P=0.004$  and  $P=0.005$ , respectively, figure 2B) and obese mice ( $P=0.02$  and  $P=0.01$ ). Moreover, leptin concentrations in GAT, SAT and PVAT of obese mice were significantly higher than those of control lean mice ( $P=0.03$ ,  $P=0.02$ , and  $P=0.001$ ). Our data suggest that leptin concentration in adipose tissue is dependent upon the location of adipose tissue.

MCP-1 is produced predominantly by macrophages and endothelial cells, and is a potent chemotactic factor for monocytes. Adipose tissue secretes MCP-1 in animal models of obesity, suggesting that MCP-1 in adipose tissue contributes to macrophage infiltration into tissue as well as insulin resistance [17]. Longer induction of high fat diet induced obesity (12 weeks) in these studies compared to shorter induction of high fat diet induced obesity (6 weeks) in our study could explain different severity of obesity and inflammatory changes in these mice. Our study did not detect a significant increase in MCP-1 levels in three adipose tissues.

IL-6 is a proinflammatory cytokine and is elevated in chronic low-grade inflammation such as obesity. Adipose tissue in obese humans and in animal models produces several pro-inflammatory cytokines, including IL-6. IL-6 is secreted by T cells and macrophages to stimulate immune response. It is also produced by skeletal muscle in response to exercise and reported to have beneficial effects in regulating metabolism in tissue and organs [18]. Accumulating evidence indicates that IL-6 is produced abundantly by visceral

adipose tissue. In addition, plasma concentrations of IL-6 correlate with insulin resistance. In our study, IL-6 concentrations in lean mice did not differ significantly among SAT, GAT and PVAT. However, IL-6 concentrations in PVAT of obese mice were significantly elevated compared with those of lean mice ( $P=0.03$ ). Moreover, IL-6 concentrations in PVAT were significantly higher than those of SAT and GAT in obese mice ( $P=0.03$  and  $P=0.005$ , respectively, figure 2D). Our present study confirms our previous finding of elevated PVAT IL-6 level in chronic inflammation and is consistent with PVAT inflammatory mediation in the development of increased intima-media thickness of abdominal aorta [11]. Our data suggest that higher IL-6 levels in the PVAT of obese mice contribute to elevated IL-6 concentrations in the circulation, but not to IL-6 concentrations in SAT and GAT. Furthermore, our data indicate that increased IL-6 secretion in obesity is dependent upon adipose tissue location.

PAI-1 plays a crucial role in fibrinolytic activity and contributes to the pathogenesis of atherothrombosis. PAI-1 is produced primarily by SAT in human and rodent models of obesity following insulin

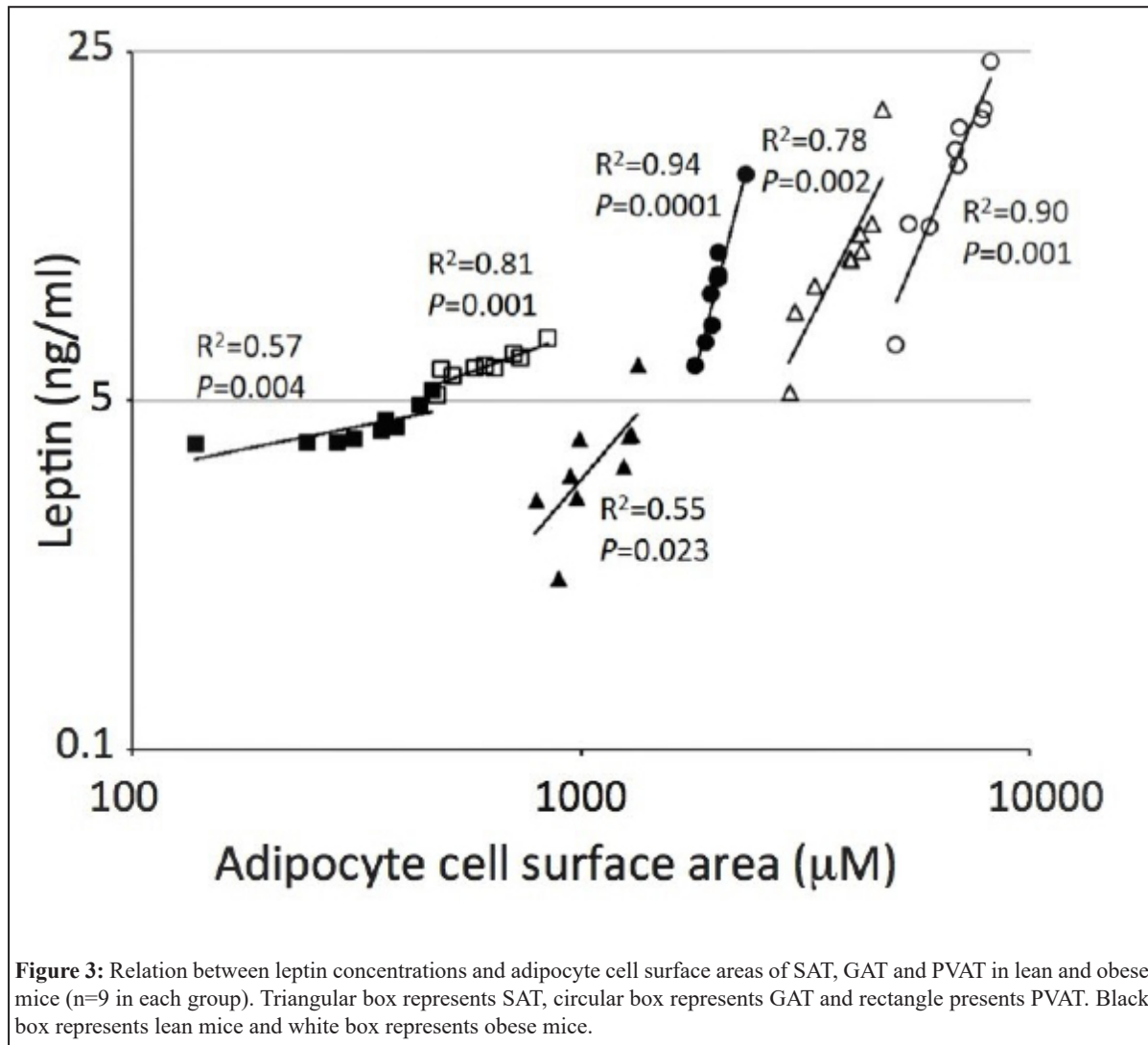
resistance. Our data are consistent with published work and show that PAI-1 is produced by SAT as mice become obese [19]. In obese mice, PAI-1 concentrations were significantly elevated in SAT but not in GAT or PVAT, ( $P=0.01$ , figure 2E), suggesting that PAI-1 production from adipose tissue is dependent upon adipose tissue location.

Resistin (adipose tissue-specific secretory factor) is produced by adipose tissue in humans and rodents. In obesity, plasma levels of resistin are elevated and several studies have shown a positive correlation with insulin resistance. Our study confirmed significantly elevated resistin plasma levels in obese mice and demonstrated the complex nature of resistin levels in adipose tissue in different locations. In lean mice, resistin concentrations in GAT were significantly higher than those of SAT and PVAT ( $P=0.001$  in both, figure 2F). However, in obese mice, significantly increased concentrations of resistin were observed in SAT ( $P=0.02$ ) but not in GAT and PVAT. Our data suggest that adipose tissues at different locations have different functions as obesity develops. The secretion of adipokines from adipose tissue may be dependent upon the location of adipose tissue.



The correlation between adipokine concentration and surface area of adipocyte cells was examined. Among the five adipokine concentrations in three types of adipose tissues in our study, adipokine concentrations of MCP-1, IL-6, PAI-1 and resistin did not correlate with adipocyte cell surface area (data not shown).

However, leptin concentration was significantly correlated with adipocyte cell surface area (Figure 3) in both lean and obese mice. The high  $r^2$  values obtained from the regression analysis demonstrated a strong association between leptin concentration and adipocyte cell surface area for all three types of adipose tissues.



**Figure 3:** Relation between leptin concentrations and adipocyte cell surface areas of SAT, GAT and PVAT in lean and obese mice (n=9 in each group). Triangular box represents SAT, circular box represents GAT and rectangle presents PVAT. Black box represents lean mice and white box represents obese mice.

Moreover, the highest concentrations of leptin and the strongest correlations with adipocyte cell surface area were found in GAT in both lean and obese mice. Our study demonstrated that leptin concentration was primarily dependent upon location of the adipose tissues rather than the surface area of adipocyte cells.

In conclusion, the present study clearly demonstrated that adipocyte tissue cell surface area was primarily dependent upon location-subcutaneous, gonadal or perivascular. Moreover, we suggest that differential adipokine concentrations in different types of adipose tissues may lead to elevated levels of adipokine concentrations in plasma in a mouse model of obesity. Furthermore, our prior and current studies suggest that increased IL-6 level in PVAT may contribute to vascular remodeling.

## Acknowledgements

This work was supported by SingHealth Research Foundation

grant (SHF/FG436P/2010) to KTM.

## Conflict of Interest

The authors declare that they have no competing interests.

## References

1. Nakamura K, Fuster JJ, Walsh K (2014) Adipokines: a link between obesity and cardiovascular diseases. J Cardiol 63: 250-259.
2. Skurk T, Alberti-Huber C, Herder C, Hauner H (2007) Relationship between adipocyte size and adipokine expression and secretion. J Clin Endocrinol Metab 92: 1023-1033.
3. Dolinková M, Dostálová I, Lacinová Z, Michalský D, Haluzíková D, et al. (2008) The endocrine profile of subcutaneous and visceral adipose tissue of obese patients. Mol Cell Endocrinol 291: 63-70.
4. Linder K, Arner P, Flores-Morales A, Tollet-Egnell P, Norstedt G (2004)

- Differentially expressed genes in visceral or subcutaneous adipose tissue of obese men and women. *J Lipid Res* 45: 148-154.
5. Samaras K, Botelho NK, Chisholm DJ, Lord RV (2010) Subcutaneous and visceral adipose tissue gene expression of serum adipokines that predict type 2 diabetes. *Obesity* 18: 884-889.
  6. Li H, Matheny M, Nicolson M, Tümer N, Scarpace PJ (1997) Leptin gene expression increases with age independent of increasing adiposity in rats. *Diabetes* 46: 2035-2039.
  7. Montague CT, Prins JB, Sanders L, Digby JE, O'Rahilly S (1997) Depot- and sex-specific differences in human leptin mRNA expression: implications for the control of regional fat distribution. *Diabetes* 46: 342-347.
  8. Way JM, Görgün CZ, Tong Q, Uysal KT, Brown KK, et al. (2001) Adipose tissue resistin expression is severely suppressed in obesity and stimulated by peroxisome proliferator-activated receptor gamma agonists. *J Biol Chem* 276: 25651-25653.
  9. Gao YJ, Lu C, Su LY, Sharma AM, Lee RMKW (2007) Modulation of vascular function by perivascular adipose tissue: the role of endothelium and hydrogen peroxide. *Br J Pharmacol* 151: 323-331.
  10. Sun X, Hou N, Han F, Guo Y, Hui Z, et al. (2013) Effect of high free fatty acids on the anti-contractile response of perivascular adipose tissue in rat aorta. *J Mol Cell Cardiol* 63: 169-174.
  11. Moe KT, Naylynn TM, Yin NO, Khairunnisa K, Allen JC, et al. (2013) Tumor necrosis factor-alpha induces aortic intima-media thickening via perivascular adipose tissue inflammation. *J Vasc Res* 50: 228-237.
  12. Sitnick M, Bodine SC, Rutledge JC (2009) Chronic high fat feeding attenuates load-induced hypertrophy in mice. *J Physiol* 587: 5753-5765.
  13. Nickelson KJ, Stromsdorfer KL, Pickering RT, Liu T-W, Ortinou LC, et al. (2012) A comparison of inflammatory and oxidative stress markers in adipose tissue from weight-matched obese male and female mice. *Exp Diabetes Res* 2012: 1-8.
  14. Barbosa-da-Silva S, Fraulob-Aquino JC, Lopes JR, Mandarim-de-Lacerda CA, Aguilá MB (2012) Weight cycling enhances adipose tissue inflammatory responses in male mice. *PLoS One* 7: 39837.
  15. Moe KT, Yin NO, Naylynn TM, Khairunnisa K, Wutyi MA, et al. (2011) Nox2 and Nox4 mediate tumor necrosis factor-alpha-induced ventricular remodeling in mice. *J Cell Mol Med* 15: 2601-2613.
  16. Greenberg AS, Obin MS (2006) Obesity and the role of adipose tissue in inflammation and metabolism. *Am J Clin Nutr* 83: 461-465.
  17. Kanda H, Tateya S, Tamori Y, Kotani K, Hiasa K, et al. (2006) MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. *J Clin Invest* 116: 1494-1505.
  18. Pedersen BK, Fischer CP (2007) Beneficial health effects of exercise--the role of IL-6 as a myokine. *Trends Pharmacol Sci* 28: 152-156.
  19. Eriksson P, Van Harmelen V, Hoffstedt J, Lundquist P, Vidal H, et al. (2000) Regional variation in plasminogen activator inhibitor-1 expression in adipose tissue from obese individuals. *Thromb Haemost* 83: 545-548.