INSTITUTO POTOSINO DE INVESTIGACIÓN CIENTÍFICA Y TECNOLÓGICA, A.C.

POSGRADO EN CIENCIAS EN BIOLOGÍA MOLECULAR

ALPHA-1-ANTICHYMOTRYPSIN IS EXPRESSED IN HUMAN ADIPOSE TISSUES AND MODULATED BY GENDER AND BODY WEIGHT

Tesis que presenta

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Para obtener el grado de

Maestra en Ciencias en Biología Molecular

Codirectores de la Tesis:

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Constancia de aprobación de la tesis

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Créditos Institucionales

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MAESTRA EN CIENCIAS EN BIOLOGÍA MOLECULAR

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Nataly Guzmán Herrera

sobre la Tesis intitulada:

Alpha-1-antichymotrypsin is expressed in human adipose tissues and modulated by gender and body weight

que se desarrolló bajo la dirección de

Dr. Luis Antonio Salazar Olivo
Dra. Viridiana Candelaria Pérez Nájera

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Dedicatorias

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Contenido

Lista de figuras

Abreviaturas (opcional)

ACT	Alpha-1-Antichymotrypsin
GAPDH	Glyceraldehyde 3-Phosphate Dehydrogenase
BMI	Body Mass Index
T2D	Type 2 Diabetes Mellitus
WHO	World Health Organization

ALFA-1-ANTIQUIMIOTRIPSINA SE EXPRESA EN LOS TEJIDOS ADIPOSOS HUMANOS Y ES MODULA POR EL GÉNERO Y EL PESO CORPORAL

RESUMEN

ANTECEDENTES. La obesidad es una enfermedad crónica caracterizada por una acumulación excesiva de grasa corporal y es uno de los principales factores de riesgo para la hipertensión, diabetes mellitus tipo 2, enfermedades cardiovasculares y algunos tipos de cáncer. Recientemente, la serpina Alfa-1-antiquimiotripsina (ACT) se ha relacionado positivamente con la obesidad, pero su papel en esta enfermedad sigue siendo desconocido.

MÉTODOS. La expresión de *ACT* en muestras de tejido adiposo subcutáneo (n=6) y visceral (n=6) humano se analizó por RT-PCR y se cuantificó por RT-qPCR en muestras de tejido adiposo subcutáneo de donantes con peso normal (n=14) y con obesidad (n=12).

RESULTADOS. *ACT* se expresó en depósitos adiposos humanos subcutáneos y viscerales masculinos y femeninos. En condiciones de normo peso, la expresión de *ACT* fue estadísticamente mayor en el tejido adiposo subcutáneo femenino que en el tejido masculino. La obesidad parece invertir esta relación.

CONCLUSIONES. Nuestros resultados sugieren que *ACT* es un nuevo elemento a considerar en el estudio del metabolismo del tejido adiposo. Otros estudios permitirán evaluar su posible participación en patologías asociadas al tejido adiposo.

PALABRAS CLAVE: Alfa-1-antiquimiotripsina (ACT), obesidad, tejido adiposo subcutáneo humano.

ALPHA-1-ANTICHYMOTRYPSIN IS EXPRESSED IN HUMAN ADIPOSE TISSUES AND MODULATED BY GENDER AND BODY WEIGHT

ABSTRACT

BACKGROUND. Obesity is a chronic disease characterized by an excessive body fat accumulation and a main risk factor for hypertension, type 2 diabetes mellitus, cardiovascular diseases and some types of cancer. Recently, the serpin alpha-1-antichymotrypsin (*ACT*) has been positively related to obesity, but its role in this disease remains unknown.

METHODS. The expression of *ACT* in human subcutaneous (n=6) and visceral (n=6) adipose tissue samples was analyzed by RT-PCR and quantified by RT-qPCR in subcutaneous adipose tissue samples from normal weight (n=14) and obese (n=12) donors.

RESULTS. *ACT* was expressed in both female and male subcutaneous and visceral human adipose depots. Under normal-weight conditions, the expression of *ACT* was statistically higher in female subcutaneous adipose tissue than in male tissues. Obesity seems to invert this relationship.

CONCLUSIONS. Our results suggest *ACT* is a new element to consider in the study of adipose tissue metabolism. Further studies will allow evaluating its possible participation in pathologies associated to adipose tissue.

KEYWORDS: Alpha-1-antichymotrypsin, obesity, human subcutaneous adipose tissue.

1. INTRODUCTION

Obesity is a chronic disease characterized by the excessive body fat accumulation and has become a serious public health problem since it has been estimated that more than 650 million people over the age of 18 suffer from it (1). The main concern about obesity is its association with hypertension, type 2 diabetes mellitus (T2D), cardiovascular diseases and some types of cancer (2–5). The causes that contribute to the development of obesity are multiple and include genetic, physiological, environmental, and social factors. For example, multiple studies have indicated that the adipose tissue itself could be related to development of obesity since it not only acts as an energy reservoir but also as an endocrine organ capable of releasing different hormones, proteins and cytokines, which could affect the cell and tissue functionality (6–10). Likewise, previous studies indicate that the two main depots of white adipose tissue in adults, the visceral and subcutaneous adipose depots, show differences in the expression of genes and proteins associated with obesity and even gender-specific differences have been reported between both types of depots (11–20). Despite the fact that the contribution of visceral depots to pathologies associated with obesity has already been established, some studies indicate that subcutaneous depots may have a neutral or even a protective role against the same pathologies. Therefore, the role of subcutaneous depots in the development and maintenance of various metabolic diseases remains controversial (21–24).

Obesity is considered a disease with an important inflammatory component and many studies have focused on the adipose expression of pro-inflammatory proteins such as tumor necrosis factor alpha (TNF-α) or other cytokines (25–28).

However, to generate a more complete picture of adipose tissue metabolism and its possible participation on the associated pathologies it is necessary to address the role of additional elements which could also play an important role in the regulation of obesity.

Previous work in our laboratory proved that TNF-α induces the overexpression of the gene encoding the serine protease inhibitor SERPINA3g, both in the preadipose and adipose states of 3T3-F442A murine adipose cell line. This work also showed that silencing of *SERPINA3g* prevented the antiadipogenic effect of TNF-α 3T3 preadipocytes and the insulin resistance induced by TNF-α in mature 3T3 adipocytes. These results suggest SerpinA3g plays a role in the modulation of adipose tissue development and metabolism by TNF-α (29). In humans there is the *SERPINA3* gene, which encodes for alpha-1-antichymotrypsin (ACT), a serine protease inhibitor that has been studied primarily in relation to Alzheimer's disease, pathology considered a form of diabetes (30). However, its relationship with human adipose tissue depots has not been addressed.

ACT is a protein that has received major attention in recent years because it has been positively related to T2D and obesity. For example, high levels of ACT have been detected in the serum of animal models with T2D and has been considered as a potential blood biomarker of diabetic retinopathy (31) and a urinary protein biomarker reported in diabetic patients (32). In the case of obesity, ACT protein has been shown to be significantly over-expressed in the serum of obese subjects when compared to their respective healthy controls (33). The mechanisms by which *ACT* appears to be related to obesity involve high levels of pro-inflammatory cytokines such as TNF-α, IL-2 and IL-6 which stimulate the synthesis

of *ACT*, the interaction of ACT with cathepsin G, one of its main targets, promotes the maintenance of an inflammatory environment. Likewise, ACT promotes an increment of ROS, which also seems to stimulate inflammation (34-37). In fact, it has been shown that insulin resistance in obese individuals is related to an increased release of pro-inflammatory cytokines by adipocytes (38). However, despite the aforementioned studies, so far there is no report of the expression of *ACT* in human adipose tissues as well as on its role on the adipose metabolism.

2. METHODS

2.1. Human adipose tissue samples

Samples of subcutaneous adipose tissue were obtained from 14 normal-weight patients corresponding to seven females and seven males who underwent abdominal surgery and from twelve obese patients corresponding to eight women and four males undergoing bariatric surgery at the General Hospital of Mexico in Mexico City. The following inclusion criteria were applied: voluntary patients ages 20-45 years without cancer or HIV diagnosis. The exclusion criteria included pregnancy or treatment with drugs that may alter metabolism and compromise body weight. The Ethics Committee of the General Hospital of Mexico approved the study protocol and adult individuals were recruited during the period from August 2019 to December 2019. Both sexes were classified as obese or lean according to their body mass index (BMI) following the WHO criteria (BMI of ≥30 or <24.9 kg/m^2, respectively) (39).

2.2. RNA extraction and RT-PCR

Total RNA from subcutaneous and visceral adipose tissue samples was extracted using TRIzol®. RNA samples were quantified by spectrophotometry at a 260 nm UV wavelength; 1 µg of total RNA was used for reverse transcription, 0.5 µg of random primers, 200 U of reverse transcriptase, 25 U of RNAsin, dNTP 0.5 mM, 1x RT buffer in a final volume of 25 µl at 42 ^{o}C, 90 min. Controls without reverse transcriptase were used and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control. The cDNA was amplified using the following *ACT* and gapdh oligonucleotides: 5’ CCAACGTGGACTTCGCTTTC 3’ (sense) 5’ CTCTTGGCATCCTCCGTGAA 3’ (antisense) and 5’

GAAGGTGGTGAAGCAGGCGT 3' (sense) 5' ATGTGGGCCATGAGGTCCACCA 3' (antisense) respectively. Amplification reactions were performed adding 500 µg of cDNA, 1.5 mM $MgCl_2$, *ACT* 0.2 uM primers, dNTP 0.2 uM, 1.25 U Taq polymerase and 1x buffer in a final 15 µL volume. The following conditions were used: an initial denaturation cycle at 95 °C for 5 min, followed by 33 cycles at 95 °C for 30 s, 58.4 °C for 1 min and 72 °C for 1 min, with a final extension cycle at 72 °C for 5 min. The PCR products of 322 bp for *ACT* were visualized on 1.5% agarose gels stained with ethidium bromide.

2.3 RNA extraction and RT-qPCR

Total RNA from subcutaneous adipose tissue samples was extracted using TRIzol®. RNA samples were quantified by spectrophotometry at a 260 nm UV wavelength; for reverse transcription 1 µg of total RNA was used, 0.5 µg of oligo dT primer, 200 U of reverse transcriptase, 25 U of RNAsin, dNTP 0.5 mM, 1x RT buffer in a final volume of 20 µl at 50 ºC 60 min. Controls without reverse transcriptase were used. For the RT-qPCR 200 ng of cDNA, the same *ACT*-specific oligonucleotides used in the RT-PCR and SYBR Green in a final volume of 15 µl was used. The mRNA levels of *ACT* were calculated, with the Ct values normalized to GAPDH as a constitutively expressed gene and compared according to the gender and metabolic condition of subjects.

2.4 Statistical analysis

The correlation between the metabolic condition and mRNA levels was examined using the nonparametric Mann-Whitney U test at statistical significance of $p<0.05$ using GraphPad Prism 5.0 (GraphPad Software, Inc.; San Diego, CA, USA).

3. RESULTS

3.1. ACT is expressed in human adipose tissues

Since the expression of *ACT* in human adipose tissue has not been reported, we first evaluated the expression of *ACT* gene in subcutaneous and visceral human adipose depots, the two main types of mammalian white adipose tissue. Six subcutaneous and six visceral adipose tissue samples obtained from four females and two males normal-weighted donors were analyzed. All the samples, except for a subcutaneous female sample, showed the presence of a 322 bp band corresponding to *ACT* mRNA (Fig. 1). Although this assay was no quantitative, the visceral samples showed a stronger *ACT* expression than subcutaneous ones.

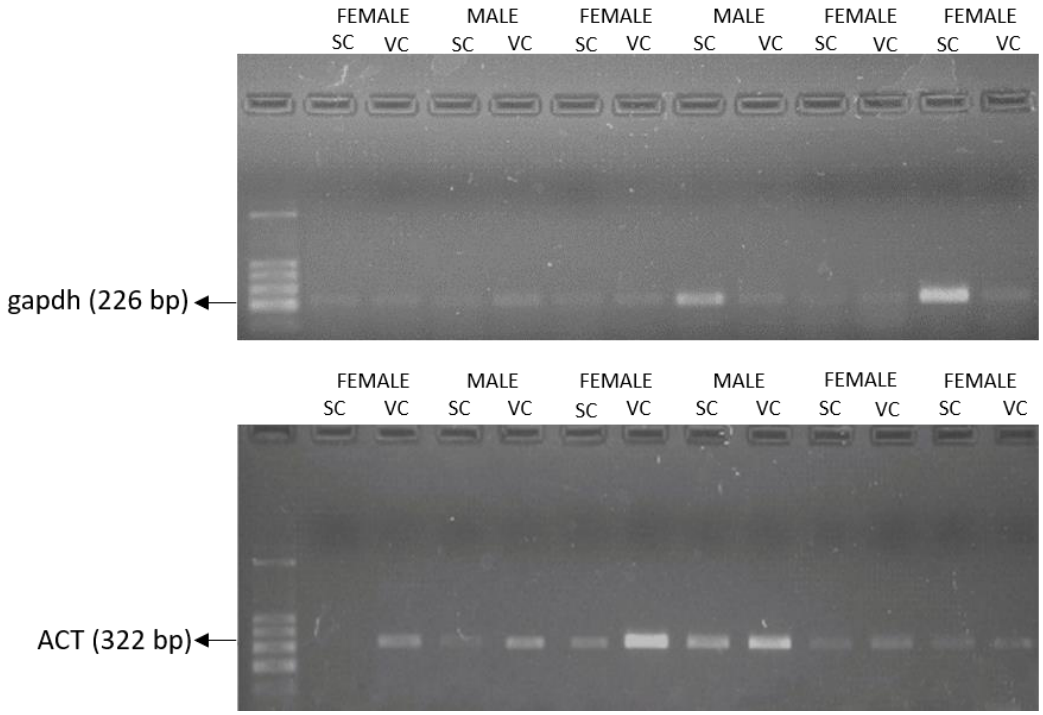

Fig. 1. *ACT* expression in subcutaneous and visceral human adipose tissues. Total RNA (1 μg) from subcutaneous (SC) or visceral (VC) human adipose samples was analyzed by RT-PCR using oligonucleotides specific for *ACT* mRNA. *Gapdh* was used as an internal control and negative controls without reverse transcriptase were included. The PCR products were visualized on 1.5% agarose gels stained with ethidium bromide.

3.2. Human obesity appears to induce act overexpression

Once we demonstrated *ACT* expression in human adipose tissues, to better characterize the *ACT* expression according to body weight, we decided to quantitatively analyze *ACT* expression levels in human subcutaneous adipose tissue samples obtained from fourteen subjects with normal weight and twelve with obesity.

RT-qPCR analyses revealed that despite not presenting statistically significant differences, there is a slight tendency for subjects with obesity to have a higher expression of *ACT* when compared to normal-weight subjects (Fig. 2).

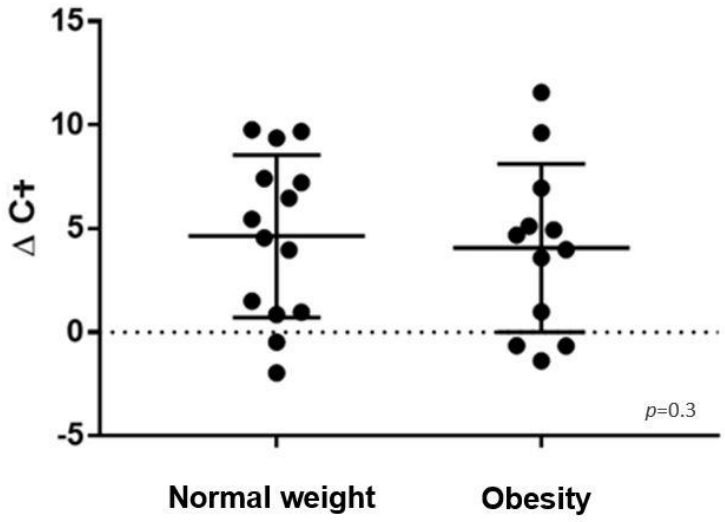

Fig. 2. Quantification of expression levels of *ACT* in subcutaneous adipose tissue of subjects with normal weight or obesity.

Total RNA was isolated from subcutaneous adipose tissue samples of fourteen normal weight and twelve obese patients after undergoing a surgical procedure. The expression of *ACT* was analyzed by RT- qPCR with the Ct values normalized using gapdh. High delta Ct values mean low expression while low delta Ct values mean higher expression.

3.3. Metabolic condition seems to alter act expression patterns and is higher in females with normal weight

Since specific gender differences in the gene expression of human adipose tissue have been reported (40) and to explore the effects of metabolic condition on *ACT* expression, we compared the expression levels of *ACT* between females and males in the obesity or normal weight metabolic conditions and directly between the two genders.

Fig. 3 shows that it is females with normal weight who have a tendency to a higher expression of *ACT*, however when the evaluated metabolic condition changes to a state with obesity, it is males who now show a tendency to a higher expression of *ACT*. Finally, according to gender, significant differences in the level of *ACT* mRNA expression were found, being normal weight females who showed statistically higher levels of *ACT* expression ($p < 0.05$). On the other hand, when the group analyzed is that of obese subjects, it is again males who have a tendency to a higher expression of *ACT* in subcutaneous adipose tissue (Fig. 4).

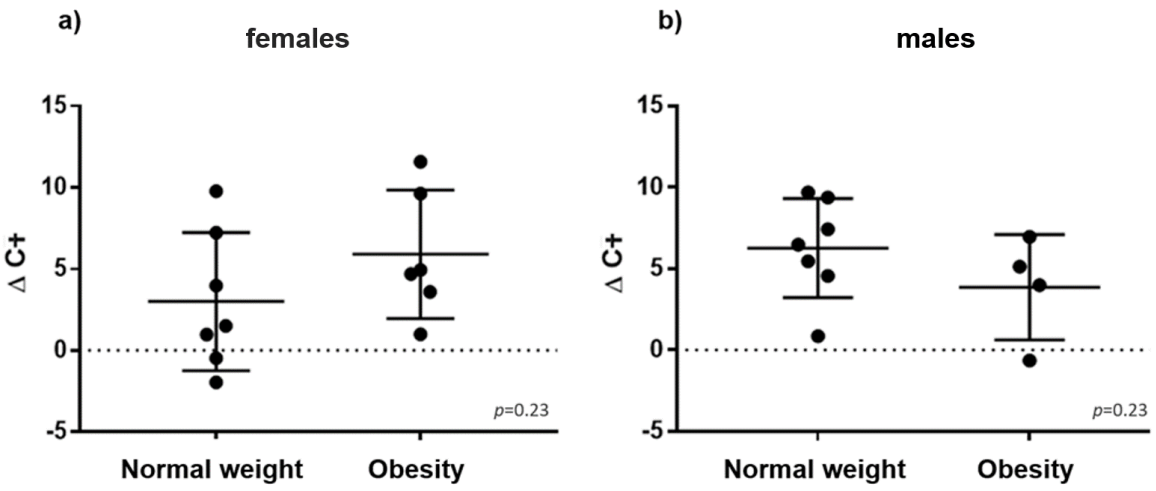

Fig. 3. Effects of gender and metabolic condition on *ACT* expression levels.

The expression of *ACT* mRNA was analyzed in subcutaneous adipose tissue samples by RT-qPCR as described in Methods. The comparison of *ACT* expression levels according to the metabolic condition (normal weight or obesity) presented by males and females is show. High delta CT values mean low expression while low delta CT values mean higher expression.

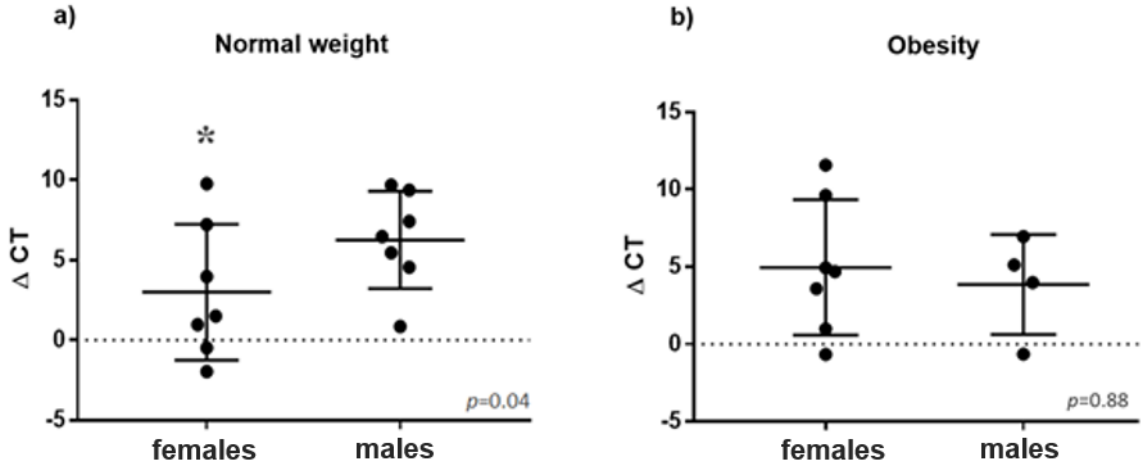

Fig. 4. Differences in *ACT* expression according to gender.

Analysis of the levels of ACT expression obtained from samples of adipose tissue from seven females and seven men with normal weight and from twelve patients with obesity corresponding to eight females and four males. High delta Ct values mean low expression while low delta Ct values mean higher expression. Asterisk indicates significant differences according to an ANOVA test (P< 0.05).

4. DISCUSSION

We demonstrate for the first time that *ACT* gene is expressed in subcutaneous and visceral human adipose tissues (Fig 1); suggesting that ACT serpin could play a role on the human adipose metabolism. To strengthen this hypothesis, the presence of the ACT protein must be experimentally corroborated, especially considering that even though the expression of ACT has been confirmed in the liver, activated astrocytes and monocytes (35, 41, 42), there are no reports that indicate the same for human adipose tissue; these results might suggest hat the inhibitor may carry out different functions depending on the local microenvironment.

It is important to mention that this study focused only on mRNA levels, so some of the differences in gene expression that were observed may not occur at the protein level. Conversely, significant differences in protein regulation could occur without alteration in mRNA levels, so it is recommended to continue with the analysis of the ACT protein in the adipose tissue**.**

Although initial evaluations comparing *ACT* expression in normal weight versus obesity samples showed no statistically significant differences (Fig. 2), more detailed analyses grouping samples first by gender and then by metabolic condition showed clear although yet no statistically significant tendencies in the expression of *ACT* (Fig. 3). These tendencies in the *ACT* expression appeared contrasting as obesity seems to decrease *ACT* expression in female subcutaneous tissue while the same condition seems to induce the overexpression in male samples. Grouping of samples first by metabolic condition (normal weight vs obesity) and then by gender (female vs male) confirmed that under normal weight

conditions *ACT* showed a higher expression in the female subcutaneous tissue than their male counterparts. On the contrary, under obesity conditions we observed a clear although not statically significant tendency for a higher *ACT* expression in male subcutaneous adipose tissue. These results suggest that there is a specific gender regulation in the ACT adipose tissue expression, it should be taken into account that during obesity the levels of pro-inflammatory molecules that can regulate ACT expression may not change or increase in the case of women, while in the case of men they always increase (43), which could partially explain the results obtained in the present study. However, it is necessary to increase the number of samples in each of the analyzed groups to obtain more reliable results.

On the other hand, we consider that it is important in the future to carry out an analysis focused on the expression of *ACT* in visceral adipose tissue since it has been pointed out that visceral depots are more strongly associated to diverse co-morbidities of obesity such as hypertension, T2D and metabolic syndrome (44). Furthermore, since SERPINA3g seems to play a role in anti-adipogenesis and insulin resistance induced by TNF-α in murine adipocytes (29), it would also be important to analyze *ACT* expression in the course of human adipogenesis *in vitro.* Addressing these issues will generate a broader picture of the functioning of adipose tissue, its relationship with *ACT* and the contribution of both to obesity.

5. CONCLUSION

Our study demonstrates that *ACT* is expressed in subcutaneous and visceral human adipose tissues and suggests *ACT* expression depends on both the gender and metabolic condition of the patient. Further studies on a larger population sample will allow us to confirm the role of gender and body weight on *ATC* expression and its role in the terminal adipose differentiation.

REFERENCES

1. World Health Organization, WHO. Obesity and overweight. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight. Updated, April 1, 2020. Accessed April 27, 2020.
2. López-Jiménez F, Cortés-Bergoderi M. Obesidad y corazón. *Rev Esp Cardiol*. 2011;64(2):140–9.
3. Stone T, McPherson M, Gail Darlington L. Obesity and cancer: existing and new hypotheses for a causal connection. *EBioMedicine*. 2018;30(1):14–28.
4. Bhushan I, Kour M, Kour G, Gupta S, Sharma S, Yadav A. Alzheimer's disease: Causes & treatment–A review. *Ann Biotechnol*. 2018;1(1):1–8.
5. Al-Goblan A, Al-Alfi M, Khan M. Diabetes, metabolic syndrome and obesity: targets and therapy dovepress mechanism linking diabetes mellitus and obesity. *Diabetes, Metab Syndr Obes Targets Ther*. 2014;7–587.
6. Booth A, Magnuson A, Fouts J, Foster MT. Adipose tissue: an endocrine organ playing a role in metabolic regulation. *Horm Mol Biol Clin Investig*. 2016;26(1):25–42.
7. Kershaw E, Flier J. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab. 2004;89(6):2548–56.
8. Wright S, Aronne L. Causes of obesity. Abdom Imaging. 2012;37(5):730–2.
9. Trayhurn P. Adipose tissue in obesity-An inflammatory issue. *Endocrinology*. 2005;146(3):1003–5.
10. Longo M, Zatterale F, Naderi J, Parrillo L, Formisano P, Raciti G, et al. Adipose tissue dysfunction as determinant of obesity-associated metabolic complications. *Int J Mol Sci*. 2019;20(9).

11. Rakotoarivelo V, Lacraz G, Mayhue M, Brown C, Rottembourg D, Fradette J, et al. Inflammatory cytokine profiles in visceral and subcutaneous adipose tissues of obese patients undergoing bariatric surgery reveal lack of correlation with obesity or diabetes. *EBioMedicine*. 2018;30:237–47.
12. Passaro A, Miselli M, Sanz J, Dalla Nora E, Morieri M, Colonna R, et al. Gene expression regional differences in human subcutaneous adipose tissue. *BMC Genomics*. 2017;18(1):1–11.
13. Vohl M, Sladek R, Robitaille J, Gurd S, Marceau P, Richard D, et al. A survey of genes differentially expressed in subcutaneous and visceral adipose tissue in men. *Obes Res*. 2004;12(8):1217–22.
14. Vatier C, Kadiri S, Muscat A, Chapron C, Capeau J, Antoine B. Visceral and subcutaneous adipose tissue from lean women respond differently to lipopolysaccharide-induced alteration of inflammation and glyceroneogenesis. *Nutr Diabetes*. 2012;2(12):e51-7.
15. Klimčáková E, Roussel B, Márquez-Quiñones A, Kováčová Z, Kováčiková M, Combes M, et al. Worsening of obesity and metabolic status yields similar molecular adaptations in human subcutaneous and visceral adipose tissue: Decreased metabolism and increased immune response. *J Clin Endocrinol Metab*. 2011;96(1):73–82.
16. Roca-Rivada A, Bravo S, Pérez-Sotelo D, Alonso J, Castro AI, Baamonde I, et al. CILAIR-based secretome snalysis of obese visceral and subcutaneous adipose tissues reveals distinctive ECM remodeling and inflammation mediators. *Sci Rep*. 2015;5(June):1–13.
17. Zha J, Di W, Zhu T, Xie Y, Yu J, Liu J, et al. Comparison of gene transcription

between subcutaneous and visceral adipose tissue in Chinese adults. *Endocr J*. 2009;56(8):935–44.

18. Azizian M, Mahdipour E, Mirhafez S, Shoeibi S, Nematy M, Esmaily H, et al. Cytokine profiles in overweight and obese subjects and normal weight individuals matched for age and gender. *Ann Clin Biochem*. 2016;53(6):663–8.
19. Ronquillo M, Mellnyk A, Cárdenas-Rodríguez N, Martínez E, Comoto D, Carmona-Aparicio L, et al. Different gene expression profiles in subcutaneous & visceral adipose tissues from Mexicans patients with obesity. *Indian J Med Res*. 2019;149(2014):616–26.
20. Linder K, Arner P, Flores-Morales A, Tollet-Egnell P, Norstedt G. Differentially expressed genes in visceral or subcutaneous adipose tissue of obese men and women. *J Lipid Res*. 2004;45(1):148–54.
21. Patel P, Abate N. Role of subcutaneous adipose tissue in the pathogenesis of insulin resistance. J Obes. 2013;2013.
22. Hamdy O, Parramatikul S, Al-Ozairi E. Metabolic obesity: the paradox between visceral and subcutaneous fat. *Curr Diabetes Rev*. 2006;2(4):367–73.
23. Wajchenberg B. Subcutaneous and visceral adipose tissue: their relation to the Metabolic Syndrome*. Endocr Rev*.2000;21(6):697–738.
24. McLaughlin T, Lamendola C, Liu A, Abbasi F. Preferential fat deposition in subcutaneous versus visceral depots is associated with insulin sensitivity. *J Clin Endocrinol Metab*. 2011;96(11):E1756-60.
25. Schmidt F, Weschenfelder J, Sander C, Minkwitz J, Thormann J, Chittka T, et

al. Inflammatory cytokines in general and central obesity and modulating effects of physical activity. *PLoS One*. 2015;10(3):1–17.

26. Samaras K, Botelho N, Chisholm DJ, Lord R. Subcutaneous and visceral adipose tissue gene expression of serum adipokines that predict type 2 diabetes. *Obesity*. 2010;18(5):884–9.

27. Hamid A, Laway B, Dil A, Wan AI. Evaluation of proinflammatory cytokines in obese vs nonobese patients with metabolic syndrome. *J Clin Endocrinol Metab*. 2016;11(1):3348–55.

28. Spoto B, Di Betta E, Mattace-Raso F, Sijbrands E, Vilardi A, Parlongo R, et al. Pro- and anti-inflammatory cytokine gene expression in subcutaneous and visceral fat in severe obesity. *Nutr Metab Cardiovasc Dis.* 2014;24(10):1137–43.

29. Salazar-Olivo LA, Mejia-Elizondo R, Alonso-Castro AJ, Ponce-Noyola P, Maldonado-Lagunas V, Melendez-Zajgla J, et al. SerpinA3g participates in the antiadipogenesis and insulin-resistance induced by tumor necrosis factor-α in 3T3-F442A cells. *Cytokine*. 2014;69(2):180–188.

30. Abraham CR, Potter H. Alpha 1-antichymotrypsin in brain aging and disease. *Prog Clin Biol Res*. 1989;317:1037-1048.

31. Takahashi E, Okumura A, Unoki-Kubota H, Hirano H, Kasuga M, Kaburagi Y. Differential proteome analysis of serum proteins associated with the development of type 2 diabetes mellitus in the KK-Ay mouse model using the iTRAQ technique. *J Proteomics*. 2013;84:40–51.

32. Riaz S. Study of protein biomarkers of diabetes mellitus type 2 and therapy with vitamin B1. *J Diabetes Res*. 2015;2015(5):1–10.

33. Al-Daghri N, Torretta E, Capitanio D, Fania C, Guerini FR, Sabico SB, et al. Intermediate and low abundant protein analysis of Vitamin D deficient obese and non-obese subjects by MALDI-profiling. *Sci Rep*. 2017;7(1):1–10.
34. Tyagi E, Fiorelli T, Norden M, Padmanabhan J. Alpha 1-antichymotrypsin, an inflammatory protein overexpressed in the brains of patients with Alzheimer's disease, induces Tau hyperphosphorylation through c-Jun N-terminal kinase activation. *Int J Alzheimers Dis*. 2013;2013:1–12.
35. Janciauskiene S. Conformational properties of serine proteinase inhibitors (serpins) confer multiple pathophysiological roles. *Biochim Biophys Acta - Mol Basis Dis*. 2001;1535(3):221–35.
36. Oguntibeju O. Type 2 diabetes mellitus, oxidative stress and inflammation: examining the links. *Int J Physiol Pathophysiol Pharmacol*. 2019;11(3):45–63. 35.
37. Lin Y, Berg A, Iyengar P, Lam T, Giacca A, Combs T, et al. The hyperglycemia-induced inflammatory response in adipocytes: the role of reactive oxygen species. *J Biol Chem*. 2005;280(6):4617–26.
38. Shoelson S, Lee J, Goldfine A. Inflammation and insulin resistance. *Diabetes*. 2006;116(7):1793–801.
39. World Health Organization, WHO. Mean Body Mass Index (BMI). https://www.who.int/gho/ncd/risk_factors/bmi_text/en/. Accessed August 1, 2019.
40. Iglesias J, Eiras S, Piñeiro R, González-Juanatey J. Gender differences in adiponectin and leptin expression in epicardial and subcutaneous adipose tissue. Findings in patients undergoing cardiac surgery. *Rev Espa Cardiol*.

2006;59(12):1252–60.

41. Chelbi S, Wilson M, Veillard A, Ingles S, Zhang J, Mondon F, et al. Genetic and epigenetic mechanisms collaborate to control SERPINA3 expression and its association with placental diseases. *Hum Mol Genet*. 2012;21(9):1968–78.

42. Abraham C. Reactive astrocytes and α1-antichymotrypsin in Alzheimer's disease. *Neurobiol Aging*. 2001;22(6):931–6.

43. White U, Tchoukalova Y. Sex dimorphism and depot differences in adiposetissue function. *Biochim Biophys Acta*. 2013;1842(3):377–92.

44. Fox C, Massaro J, Hoffmann U, et al. Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. *Circulation*. 2007;116(1):39-48.