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The role of sex in the relationship between fasting adipokines levels, maximal fat oxidation during exercise, and insulin resistance in young adults with excess adiposity

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ABSTRACT

Aim: Previous evidence suggest that a sexual dimorphism in exercise fat oxidation and adipokines levels may explain a lower risk of cardio-metabolic disorders in women. Therefore, we investigated the role of sex in the relationship between adipokines levels, maximal fat oxidation (MFO) during exercise and insulin resistance.
Methods: Fifty young adults with excess adiposity (31 women; body fat: 38.7 ± 5.3%) were included in this study. The fasting levels of leptin, adiponectin, glucose and insulin were determined from blood samples and the homeostatic model assessment of insulin resistance index (HOMA-IR) subsequently calculated. Body fat percentage and visceral adipose tissue (VAT) were assessed through dual-energy X-ray absorptiometry whereas MFO was estimated during an incremental-load exercise test after an overnight fasting through indirect calorimetry.
Results: Men had lower levels of body fat ($d = 1.80$), adiponectin ($d = 1.35$), leptin ($d = 0.43$) and MFO ($d = 1.25$) than women. Conversely, men showed higher VAT ($d = 0.85$) and fasting glucose levels ($d = 0.89$). No sex differences were observed in HOMA-IR ($d = 0.34$). Adipokines levels were not associated with MFO in both sexes ($r < 0.30$), whereas adiponectin levels were inversely related with HOMA-IR in both men ($r = -0.58$) and women ($r = -0.50$). Leptin concentration was associated to HOMA-IR only in men ($r = 0.41$), while no statistically significant relationships were observed between MFO and HOMA-IR in both sexes ($r < 0.44$).
Conclusion: Insulin resistance was similar between sexes regardless of superior levels of adipokines and MFO during exercise in women. Therefore, adiponectin and leptin may regulate glucose homeostasis without altering whole body fat oxidation rate during exercise.

Abbreviations: Fatmax, exercise intensity that elicits maximal fat oxidation; HOMA-IR, homeostatic model assessment of insulin resistance index; MFO, maximal fat oxidation rate during exercise; T2D, type 2 diabetes; VAT, visceral adipose tissue; VO_{2max}, maximum oxygen uptake.

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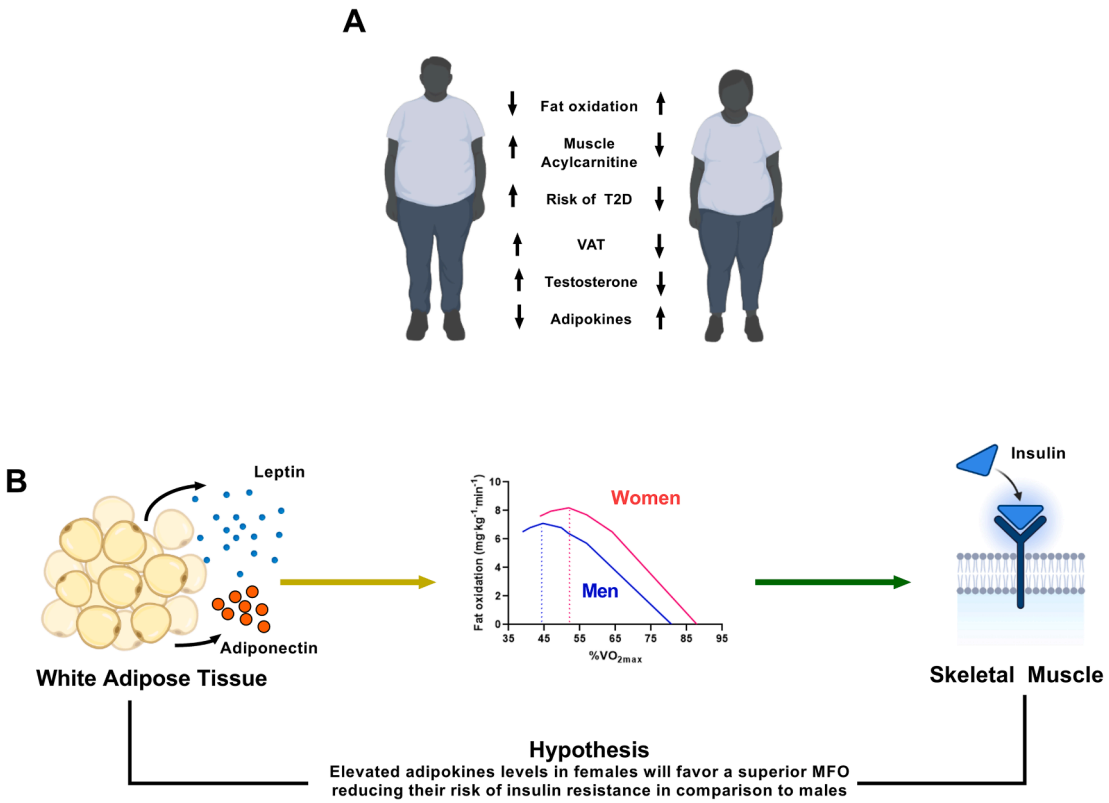

Fig. 1. Study hypothesis (A), based in previous findings reporting a sexual dimorphism in adipokines levels, body composition, maximal fat oxidation (MFO) and the risk of type 2 diabetes mellitus (T2D) (B).

1. Introduction

Type 2 Diabetes (T2D) is a major public health challenge with approximately 6.2% of the world's population suffering from this metabolic disease [1]. Interestingly, the prevalence of T2D is lower in young women vs. men in many countries around the world; this difference being potentially explained by: (i) differences in androgens/estrogens levels, (ii) a discrepancy in adipose tissue distribution and functionality, and (iii) dissimilarities in fatty acid oxidation and intramyocellular lipid distribution [2–5]. However, the interrelationship among the aforementioned biological factors has not been deeply examined.

Recently, Broussard et al. [5] reported that acylcarnitine levels in skeletal muscle was lower in women vs. men despite of a similar subcellular lipid distribution, and linked these observations with a superior fat oxidation capacity in women, a fact that may prevent lipotoxicity in skeletal muscle and insulin resistance [6,7]. In this concern, a superior fat oxidation capacity in young women has been also associated with a sexual dimorphism in adipose tissue distribution and functionality [5,8,9]. Specifically, body fat, adipose tissue lipolysis, intramyocellular hydrolysis and serum levels of adiponectin and leptin are superior in young women, whereas visceral adipose tissue (VAT) is commonly greater in men [5,10,11]. This phenomenon is mainly related to elevated androgen levels in men that promotes adipose tissue accumulation into the visceral depots, reduces adiponectin and leptin secretion from adipocytes, and affect leptin sensitivity in skeletal muscle by reducing the abundance of leptin receptors [10–12].

The influence of free fatty acids availability over the fat oxidation capacity in men and women is well documented [5,8,9]. However, although previous studies reported that binding of adiponectin and leptin to their cellular receptors in skeletal muscle could enhance the activity of different enzymes that regulate fatty acid metabolism [13,14], the impact of elevated adipokines levels over fat oxidation rate has not been deeply investigated. Recently, Montes-de-Oca-García et al. [15] reported that cardiorespiratory fitness mediated a modest association between serum leptin concentration and maximal fat oxidation rate (MFO) observed during a graded exercise test in young men and women, although the connection between serum leptin levels and MFO was in opposite direction between sexes (positive association in men vs. negative association in women). The MFO is a physiological marker associated to the abundance of key enzymes that regulate adipose tissue lipolysis, fatty acid trafficking and skeletal muscle oxidative capacity [16]. Nonetheless, Montes-de-Oca-García et al. [15] did not evaluate adiponectin levels nor explored if the sexual dimorphism in leptin and MFO explained a discrepancy on insulin resistance/sensitivity between men and women. Therefore, the role of sex in the relationship between levels of adipokines, MFO during exercise, and insulin resistance merits further analysis (Fig. 1).

In this study, we tested the aforementioned phenomenon on individuals with excess adiposity who exhibit an elevated risk for cardiometabolic disorders associated to (i) an impaired MFO relative to lean mass, (ii) low adiponectin levels, and (iii) elevated leptin levels related to leptin resistance [16–19]. Concretely, we hypothesized that elevated levels of adiponectin and leptin would favor a superior MFO in young women with excess adiposity, reducing their insulin resistance in comparison to men.

2. Materials and methods

2.1. Research design and participants

According to large effect size differences reported for adipokines levels and MFO between men and women (effect size: 0.6–1.4) [11,15,16], a priori analysis in Gpower v.3.1 showed that ~ 20 men and women would be necessary for a statistical power of 0.95 ($\dot{\alpha} < 0.05$; allocation ratio 1/1). Thus, a total of 50 (31 women) young adults (22 ±

2 years) with excess adiposity (i.e., body fat % ≥25 for men and ≥35 for women) were selected from the ACTIBATE study [20] (ClinicalTrials.gov, ID: NCT02365129). The inclusion criteria were as follow: to do <20 min of moderate-vigorous physical activity on <3 days/week, not being involved in an exercise intervention during the previous 12 weeks, being non-smokers, having a stable body weight over the last 12 weeks (i.e., changes < 3 kg), not suffering any cardio-metabolic disease (e.g., hypertension, diabetes, etc.), not being pregnant, and to have no first-order family member with a history of cancer. Data obtained from the ACTIBATE cohort were collected in October-December 2015 and 2016 at our facilities in Granada, Spain. The Human Research Ethics Committees of the University of Granada (n° 924) and the Junta de Andalucía (n° 0838-N-2017) approved the studý protocols, respectively, and oral and written informed consents was obtained by all patients, in accordance with the Declaration of Helsinki (last revision 2013).

2.2. Procedures

2.2.1. Anthropometry and body composition

A SECA model 799 scale and stadiometer (SECA, Hamburg, Germany) was used to determine patients' weight and height, while a Discovery Wi dual energy x-ray absorptiometer (HOLOGIC, Bedford, MA, USA) was selected to obtain fat mass, VAT, fat-free mass, lean mass and the trunk-leg body fat ratio. Body mass index (BMI) was calculated as kg of weight divided by height squared (m^2).

2.2.2. Graded exercise tests

The MFO and Fatmax were calculated through a graded exercise test on a H/P/Cosmos Pulsar treadmill (H/P/Cosmos Sport & Medical GMBH, Traumstein, Germany) which has been previously used and validated [21]. The test was carried out in the afternoon (15:00–20:00 h) and the patients attended our laboratory under standardized conditions: (i) fasting for 6 h, (ii) having slept as usual, (iii) avoiding moderate and vigorous physical activity within the last 24–48 h, respectively, (iv) refraining from alcoholic or stimulant beverages (at least 24 h before the test) or any drugs that could have affected energy metabolism (at least 24 h before performing the trial). The test began calculating the maximum walking speed and, after taking a rest of ~3 min, the graded exercise test started at 3.5 km/h (gradient 0%). Subsequently, increases of 1 km/h every 3 min were programmed until reaching the maximum walking speed. Finally, the speed remained constant incrementing 2% gradient every 3 min. The test finished when a respiratory exchange ratio of 1.0 was attained. VO_2 consumption and VCO_2 production were registered through indirect calorimetric analysis of mixed expired gases using a CPX Ultima CardiO2 ergospirometry system (Medical Graphics Corp., St Paul, MN, USA) and an oronasal mask (model 7400, Hans Rudolph Inc., Kansas City, MO, USA) equipped with a prevent™ metabolic flow sensor (Medical Graphics Corp., St. Paul, MN, USA). VO_2 and VCO_2 were averaged over the last 60 s of each 3 min period to estimate fat oxidation rates through the Frayn stoichiometric equations [22]. Fat oxidation registers obtained in each phase of the graded exercise test were coupled with the exercise intensity, and the method of measured values was used to calculate MFO and Fatmax for each patient [23].

On a different day (separated by 3–10 days), maximal oxygen uptake (VO_{2max}) was obtained by conducting a maximum graded exercise test using the Balke protocol [24]. Criteria for reaching VO_{2max} were (i) to show a respiratory exchange ratio higher than 1.1, (ii) to see a plateau in VO_2 at the final stage of the test, and to reach a heart rate during the last stage within 10 beats/min of the age-predicted maximum [25]. If no plateau in VO_2 was observed, VO_{2peak} was considered.

2.2.3. Biochemical analysis

All patients were instructed to abstain from caffeine the previous 24 h and to refrain from any physical activity of moderate and/or vigorous intensity (i.e., 24–48 h before, respectively). Venous blood samples were obtained in fasting conditions [12 h] from the antecubital vein and collected in ethylenediamine tetra-acetic acid-containing tubes using the Vacutainer SST system (Becton Dickinson, Plymouth, United Kingdom). We centrifuged the samples at 4000 rpm (7 min at 4 °C), and aliquots of plasma were stored at −80 °C until further analysis. Glucose, insulin and testosterone levels were obtained by a chemiluminescent immunometric assay using a Beckman Coulter apparatus (33880) (Beckman Coulter Inc, Brea CA, USA) with a DXI analyzer. Adiponectin and leptin levels were determined using a commercially available ELISA kits (DRG Instruments GmbH, Germany and AdipoGen, Seoul, Korea, respectively). The homeostatic model assessment of insulin resistance index (HOMA-IR) was determined as plasma insulin (UI/mL) × plasma glucose (nmol/L)/22.5. The intra-assay precision was 4.6% and 5.8% for adiponectin and leptin, while inter-assay accuracy was 4.8% and 6.1%, respectively. The reported analytical sensitivity was 100 pg/ml in both cases.

Table 1
Participants characteristics.

	Men (n = 19)	Women (n = 31)
Body composition		
BMI ($kg \bullet m^2$)	28.59 ± 5.49	25.73 ± 3.31
Fat mass (kg)	29.90 ± 8.68	28.51 ± 5.71
Body fat (%)	**34.13 ± 4.86**	**41.62 ± 3.29***
Visceral adipose tissue (g)	**493.09 ± 165.65**	**368.24 ± 125.22#**
Fat-free mass (kg)	**56.21 ± 7.41**	**39.73 ± 6.04***
Lean mass (kg)	**53.54 ± 7.15**	**37.61 ± 5.84***
Trunk-legs body fat ratio	**0.93 ± 0.14**	**0.84 ± 0.11***
Endocrine function		
Adiponectin (mg•l)	**6.77 ± 4.62**	**12.03 ± 6.01***
Leptin (μg•l)	**6.01 ± 4.44**	**12.03 ± 6.01#**
Insulin (μUI•ml)	11.12 ± 7.99	9.40 ± 4.58
Testosterone (ng•dl)	**439.74 ± 152.24**	**52.06 ± 16.15***
Glucose (mg•dl)	**92.89 ± 7.24**	**86.71 ± 6.64***
HOMA-IR	2.64 ± 2.08	2.06 ± 2.17
Whole-body oxidative capacity		
VO_{2max} (mg•kg body mass^{-1}•min^{-1})	**30.7 ± 6.6**	**38.7 ± 6.7**
VO_{2max} (mg•kg lean mass^{-1}•min^{-1})	**48.84 ± 13.51**	**72.03 ± 13.64***

Data is reported as mean ± SD. BMI, body mass index, HOMA-IR, homeostatic model assessment of insulin resistance index; MFO, maximal fat oxidation; VO_{2max}, maximal oxygen uptake. Bold indicates statistically significant differences.
#$p < 0.05$.
*$p < 0.01$.

2.3. Statistical analyses

The Shapiro–Wilk test, Q–Q, and box plots were used to analyze data distribution. Homoscedasticity was checked by Levene test. Of note, data from adiponectin, leptin and HOMA-IR were non-normally distributed and, therefore, these data were log transformed prior to further analysis. Initially, differences in body composition, cardiorespiratory fitness, hormone levels and HOMA-IR between men and women were analyzed through independent *t*-test. Subsequently, analysis of covariance (ANCOVA) was employed to corroborate sex differences in adiponectin (adjusting for VAT and testosterone) and leptin levels (adjusting for body fat percentage). Likewise, ANCOVA was used to examine if differences in MFO relativized to lean mass between men and women were explained by discrepancies in VO_{2max} and adipokines levels. After discarding that VO_{2max} influenced the association between MFO and adipokines levels, Pearson correlation coefficient was then used to determine the relationship between adipokines levels, MFO and HOMA-IR, independently of sex.

The Cohen's *d* was computed with Gpower v. 3.1 [26] to determine the effect size of the differences retrieved from independent *t* test (small = 0.3, moderate d = 0.5, large = 0.8). Moreover, partial eta square (n_p^2) was used to represent the effect size of the differences retrieved from

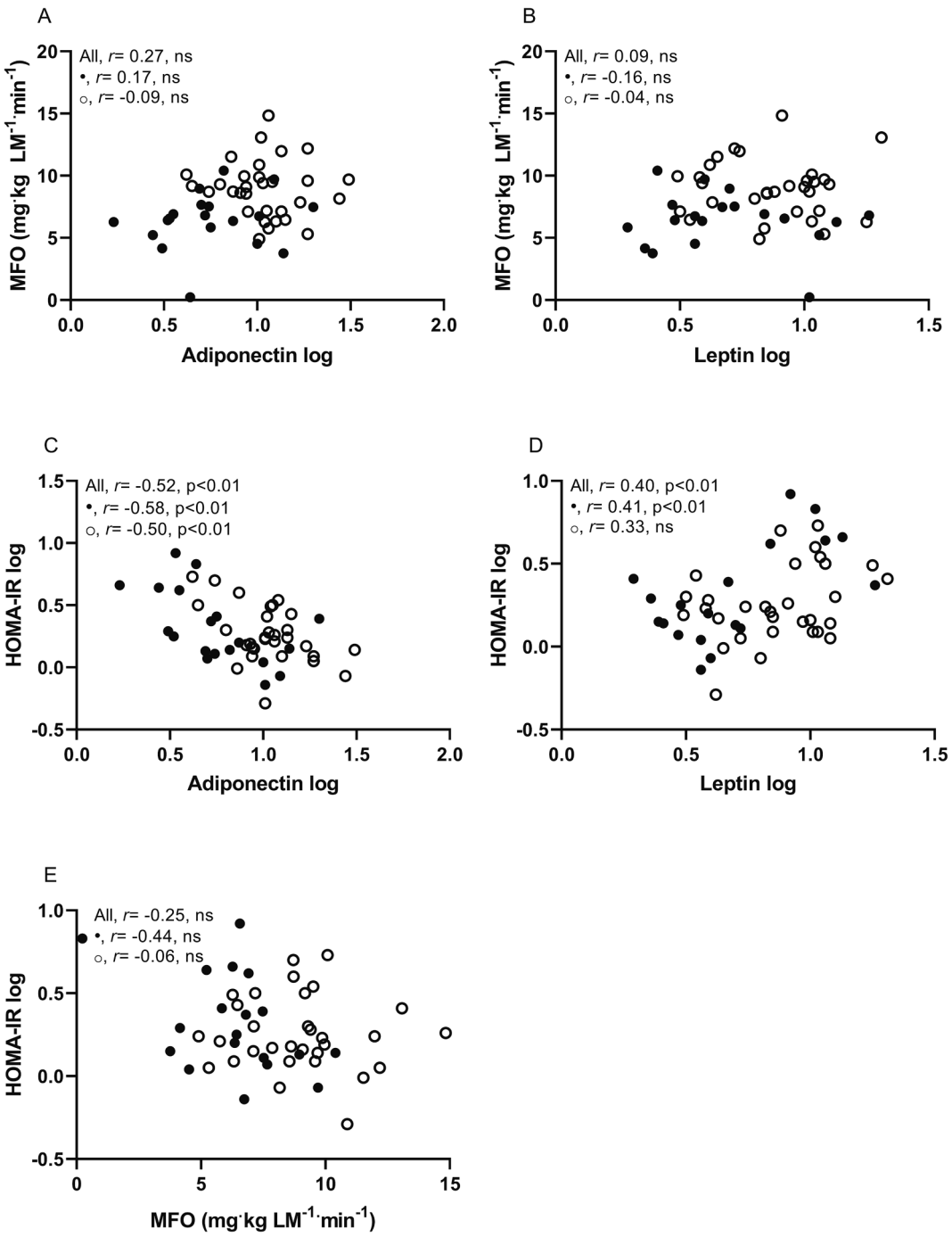

Fig. 2. Association between adipokines levels, maximal fat oxidation rate (MFO) during exercise and the homeostatic model assessment of insulin resistance index (HOMA-IR) in men (●) and women (○) with excess adiposity. r, Pearson correlation coefficient; ns, not significant.

ANCOVA. The analyses were performed in SPSS 22 (IBM corporation, NY, USA) and the statistical significance was set at $p < 0.05$. All the data were reported as mean ± SD. Figures were elaborated in GraphPad Prism v. 8.1.

3. Results

The descriptive characteristics of the study participants are shown in Table 1. Men had lower body fat ($d = 1.80$) and cardiorespiratory fitness ($d = 1.63$) than women; however, men had higher levels of VAT ($d = 0.85$), trunk-legs body fat ratio ($d = 0.71$) and fasting glucose ($d = 0.89$). A large difference in plasma testosterone ($d = 3.58$) and adiponectin ($d = 1.35$) levels were observed between men and women. Furthermore, levels of leptin were lower in men vs. women ($d = 0.43$). After adjusting for VAT and testosterone levels, the difference in adiponectin levels between men and women was attenuated ($F = 3.8$, $n_p^2 = 0.07$, $p = 0.06$). Likewise, differences in leptin levels disappeared after controlling for body fat ($F = 0.14$, $n_p^2 = 0.19$, $p = 0.71$). A small difference in MFO relative to lean mass was observed between men and women even after adjusting for VO_{2max} and adipokines levels ($F = 8.3$, $n_p^2 = 0.15$, $p < 0.05$). However, no difference between sexes was observed regarding HOMA-IR ($d = 0.34$, $p = 0.71$).

Fasting levels of adiponectin and leptin were not associated to MFO in both men and women (Fig. 2A, B). On the contrary, adiponectin levels

were inversely associated to HOMA-IR in both sexes (Fig. 2C). Leptin concentration was positively associated to HOMA-IR only in men (Fig. 2D), whereas no statistically significant associations were observed between the MFO and HOMA-IR in men and women (Fig. 2E).

4. Discussion

In this study, we hypothesized that elevated levels of adiponectin and leptin would favor a superior MFO in young women, reducing their risk of insulin resistance in comparison to men. In this regard, we observed that both adipokines and MFO levels were higher in women vs. men, yet, contrary to our hypothesis, adipokines levels were not related to the sexual dimorphism in MFO. In addition, the HOMA-IR was similar between sexes while no association was observed neither between adipokines levels and MFO or HOMA-IR and MFO in men or women. Hence, our findings do not support a connection between adipokines levels, MFO and insulin resistance in young adults with excess adiposity.

To our knowledge this is the first study that investigates the connection of adipose tissue distribution and functionality with the sexual dimorphism in fat oxidation rates during exercise and insulin resistance. Our findings concur with previous studies in young individuals that reported higher adipokines levels in women vs. men [10,11]. Furthermore, we confirm that men exhibit lower adiponectin levels along with elevated VAT and androgen levels, whereas women showed higher leptin levels and increased body fat [4,10,11]. Interestingly, elevated levels of adiponectin and leptin in women were unrelated to a superior MFO as we hypothesized. Moreover, we observed that adiponectin was inversely related to HOMA-IR regardless of MFO levels and sex, whereas leptin levels were positively associated to HOMA-IR only in men. Hence, it seems that adipokines levels could regulate glucose homeostasis without altering whole body fat oxidation rate. In this regard, both adiponectin and leptin upregulate glucose uptake in skeletal muscle through the activation of AMP-activated protein kinase [27,28], and the stimulation of the ventromedial hypothalamic nucleus [29], respectively. Nevertheless, further analyses at the molecular level are necessary to corroborate the metabolic effects of adipokines over skeletal muscle.

It should be noted that only young adults without metabolic disorders participated in this study. Thus, we cannot discard that adipose tissue distribution and functionality influences fat oxidation capacity and insulin resistance in elders or patients with metabolic syndrome. Moreover, given the cross-sectional design of the present study, we cannot overrule that altering adipose tissue distribution and functionality through an exercise or dietary intervention could be a therapeutic approach to enhance fat oxidation rate during exercise, preventing insulin resistance. Indeed, previous studies conducted in middle-aged and elderly women with excess of adiposity reported that MFO, insulin resistance, VAT and adipokines levels improved after 8 to 12-weeks of aerobic exercise intervention at Fatmax [30–32]. Likewise, the increment of MFO observed after 8 weeks of exercise intervention at Fatmax combined with a hypocaloric diet was significantly correlated with changes in adiponectin ($r = 0.43$), leptin ($r = -0.41$) and HOMA-IR ($r = -0.66$) in adolescent girls with excess of adiposity [33]. Moreover, Bordenave et al. [34] reported that a 10-week aerobic exercise intervention at Fatmax enhanced MFO and mitochondrial respiratory capacity in subjects with T2D, an effect that might reduce acylcarnitine accumulation and insulin resistance in skeletal muscle. At present, sex differences in the effect of Fatmax training over adipose tissue distribution, adipokines levels, MFO and insulin resistance have not been investigated. Thus, further clinical trials are necessary to elucidate the role of sex in the relationship between adipokines levels, MFO and insulin resistance.

The present findings should be interpreted with caution given that previous studies in healthy women reported that intake of oral contraceptives affects the MFO values whiles leptin (not adiponectin) concentration fluctuates across the different phases of the menstrual cycle [35,36]. At the moment, these observations have not been replicated in women with excess adiposity, thus, future studies need to investigate if adipokines variations across the menstrual cycle and the intake of oral contraceptives also influence exercise energy metabolism in this population. In addition, Fletcher et al. [37] and Jurado-Fasoli et al. [38] informed that fat and carbohydrate content of the diet explain around 3% of intra-individual variation in MFO. Nonetheless, we did not provide a standardized diet before the graded exercise test. Future studies are thus necessary to investigate the role of dietary intake on the sexual dimorphism in MFO, adipokines levels and insulin resistance. In conclusion, insulin resistance was similar between sexes regardless of superior levels of adipokines and MFO during exercise in women. Therefore, it seems that adiponectin and leptin could regulate glucose homeostasis without altering whole body fat oxidation rate in both men and women with excess adiposity.

5. Submission declaration and verification

All the authors have approved the final version of this work which has not been published previously nor is under consideration elsewhere.

CRediT authorship contribution statement

Isaac A. Chávez-Guevara: Conceptualization, Formal analysis, Writing – original draft. **Francisco J. Amaro-Gahete:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Writing – original draft. **Francisco J. Osuna-Prieto:** Investigation, Writing – review & editing. **Idoia Labayen:** Writing – review & editing. **Concepcion M. Aguilera:** Writing – review & editing. **Jonatan R. Ruiz:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

[1] M.A.B. Khan, M.J. Hashim, J.K. King, R.D. Govender, H. Mustafa, K.J. Al, Epidemiology of type 2 diabetes - global burden of disease and forecasted trends, J Epidemiol Glob Health. 10 (1) (2020) 107–111, https://doi.org/10.2991/jegh.k.191028.001.

[2] A. Nordström, J. Hadrévi, T. Olsson, P.W. Franks, P. Nordström, Higher prevalence of type 2 diabetes in men than in women is associated with differences in visceral fat mass, J. Clin. Endocrinol. Metab. 101 (10) (2016) 3740–3746, https://doi.org/10.1210/jc.2016-1915.

[3] A. Kautzky-Willer, J. Harreiter, G. Pacini, Sex and gender differences in risk, pathophysiology and complications of type 2 diabetes mellitus, Endocr. Rev. 37 (3) (2016) 278–316, https://doi.org/10.1210/er.2015-1137.

[4] E.B. Geer, W. Shen, Gender differences in insulin resistance, body composition, and energy balance, Gend. Med. 6 (Suppl 1) (2009) 60–75, https://doi.org/10.1016/j.genm.2009.02.002.

[5] G.H. Goossens, J.W.E. Jocken, E.E. Blaak, Sexual dimorphism in cardiometabolic health: the role of adipose tissue, muscle and liver, Nat. Rev. Endocrinol. 17 (1) (2021) 47–66, https://doi.org/10.1038/s41574-020-00431-8.

[6] J.L. Broussard, L. Perreault, E. Macias, S.A. Newsom, K. Harrison, H.H. Bui, P. Milligan, K.D. Roth, T. Nemkov, A. D'Alessandro, J.T. Brozinick, B.C. Bergman, Sex differences in insulin sensitivity are related to muscle tissue acylcarnitine but not subcellular lipid distribution, Obesity (Silver Spring) 29 (3) (2021) 550–561.

[7] S.S. Park, Y.K. Seo, Excess accumulation of lipid impairs insulin sensitivity in skeletal muscle, Int. J. Mol. Sci. 21 (6) (2020) 1949, https://doi.org/10.3390/ijms21061949.

[8] A. Cano, L. Ventura, G. Martinez, L. Cugusi, M. Caria, F. Deriu, A. Manca, Analysis of sex-based differences in energy substrate utilization during moderate-intensity aerobic exercise, Eur. J. Appl. Physiol. 122 (1) (2022) 29–70.

[9] A.M. Lundsgaard, B. Kiens, Gender differences in skeletal muscle substrate metabolism - molecular mechanisms and insulin sensitivity, Front. Endocrinol. (Lausanne) 5 (2014) 195, https://doi.org/10.3389/fendo.2014.00195.

[10] K.M. Gavin, D.H. Bessesen, Sex differences in adipose tissue function, Endocrinol. Metab. Clin. North Am. 49 (2) (2020) 215–228, https://doi.org/10.1016/j.ecl.2020.02.008.

[11] T. Christen, S. Trompet, R. Noordam, J.B. van Klinken, K.W. van Dijk, H.J. Lamb, C.M. Cobbaert, M. den Heijer, I.M. Jazet, J.W. Jukema, F.R. Rosendaal, R. de Mutsert, Sex differences in body fat distribution are related to sex differences in serum leptin and adiponectin, Peptides 107 (2018) 25–31.

[12] B. Guerra, T. Fuentes, S. Delgado-Guerra, A. Guadalupe-Grau, H. Olmedillas, A. Santana, J.G. Ponce-Gonzalez, C. Dorado, J.A.L. Calbet, A. Lucia, Gender dimorphism in skeletal muscle leptin receptors, serum leptin and insulin sensitivity, PLoS One 3 (10) (2008), e3466, https://doi.org/10.1371/journal.pone.0003466.

[13] D.J. Dyck, Adipokines as regulators of muscle metabolism and insulin sensitivity, Appl. Physiol. Nutr. Metab. 34 (3) (2009) 396–402, https://doi.org/10.1139/H09-037.

[14] M.P. Krause, K.J. Milne, T.J. Hawke, Adiponectin-consideration for its role in skeletal muscle health, Int. J. Mol. Sci. 20 (7) (2019) 1528, https://doi.org/10.3390/ijms20071528.

[15] A. Montes-de-Oca-García, A. Perez-Bey, J. Corral-Pérez, A. Marín-Galindo, M. Calderon-Dominguez, D. Velázquez-Díaz, C. Casals, J.G. Ponce-Gonzalez, Influence of gender on plasma leptin levels, fat oxidation, and insulin sensitivity in young adults: the mediating role of fitness and fatness, Nutrients 15 (11) (2023) 2628.

[16] I.A. Chávez-Guevara, R.P. Hernández-Torres, E. González-Rodríguez, A. Ramos-Jiménez, F.J. Amaro-Gahete, Biomarkers and genetic polymorphisms associated with maximal fat oxidation during physical exercise: implications for metabolic health and sports performance, Eur. J. Appl. Physiol. 122 (8) (2022) 1773–1795.

[17] F.J. Amaro-Gahete, G. Sanchez-Delgado, I. Ara, R.J. Ruiz, Cardiorespiratory fitness may influence metabolic inflexibility during exercise in obese persons, J. Clin. Endocrinol. Metab. 104 (12) (2019) 5780–5790, https://doi.org/10.1210/jc.2019-01225.

[18] M.F. Landecho, C. Tuero, V. Valentí, I. Bilbao, M. de la Higuera, G. Frühbeck, Relevance of leptin and other adipokines in obesity-associated cardiovascular risk, Nutrients 11 (11) (2019) 2664, https://doi.org/10.3390/nu11112664.

[19] M.C. Fava, R. Agius, S. Fava, Obesity and cardio-metabolic health, Br. J. Hosp. Med. (Lond.) 80 (8) (2019) 466–471, https://doi.org/10.12968/hmed.2019.80.8.466.

[20] G. Sanchez-Delgado, B. Martinez-Tellez, J. Olza, C.M. Aguilera, I. Labayen, F. B. Ortega, P. Chillon, C. Fernandez-Reguera, J.M.A. Alcantara, W.D. Martinez-Avila, V. Muñoz-Hernandez, F.M. Acosta, J. Prados-Ruiz, F.J. Amaro-Gahete, L. Hidalgo-Garcia, L. Rodriguez, Y.-K. Ruiz, A. Ramirez-Navarro, M.A. Muros-de Fuentes, Y. García-Rivero, R. Sanchez-Sanchez, J. de Dios Beas Jimenez, C. de Teresa, S. Navarrete, R. Lozano, E. Brea-Gomez, J. Rubio-Lopez, M.R. Ruiz, A. Cano-Nieto, J.M. Llamas-Elvira, J.A. Jimenez Rios, A. Gil, J.R. Ruiz, Activating brown adipose tissue through exercise (ACTIBATE) in young adults: Rationale, design and methodology, Contemp. Clin. Trials 45 (2015) 416–425.

[21] F.J. Amaro-Gahete, G. Sanchez-Delgado, J.M.A. Alcantara, B. Martinez-Tellez, F. M. Acosta, J.W. Helge, J.R. Ruiz, Impact of data analysis methods for maximal fat oxidation estimation during exercise in sedentary adults, Eur. J. Sport Sci. 19 (9) (2019) 1230–1239.

[22] K.N. Frayn, Calculation of substrate oxidation rates in vivo from gaseous exchange, J. Appl. Physiol. Respir. Environ. Exerc. Physiol. 55 (2) (1983) 628–634, https://doi.org/10.1152/jappl.1983.55.2.628.

[23] F.J. Amaro-Gahete, G. Sanchez-Delgado, L. Jurado-Fasoli, A. De-la-O, M.J. Castillo, J.W. Helge, J.R. Ruiz, Assessment of maximal fat oxidation during exercise: A systematic review, Scand. J. Med. Sci. Sports (2019), https://doi.org/10.1111/sms.13424.

[24] B. Balke, R.W. Ware, An experimental study of physical fitness of Air Force personnel, U S Armed Forces Med J. 10 (6) (1959) 675–688.

[25] J. Pallarés, R. Morán-Navarro, Methodological approach to the cardiorespiratory endurance training, J. Sport Health Res. 4 (2012) 119–136.

[26] F. Faul, E. Erdfelder, A.G. Lang, A. Buchner, G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences, Behav. Res. Methods 39 (2) (2007) 175–191, https://doi.org/10.3758/bf03193146.

[27] L.D. Høeg, K.A. Sjøberg, A.-M. Lundsgaard, A.B. Jordy, N. Hiscock, J.F. P. Wojtaszewski, E.A. Richter, B. Kiens, Adiponectin concentration is associated with muscle insulin sensitivity, AMPK phosphorylation, and ceramide content in skeletal muscles of men but not women, J. Appl. Physiol. 114 (5) (2013) 592–601.

[28] Y. Minokoshi, C. Toda, S. Okamoto, Regulatory role of leptin in glucose and lipid metabolism in skeletal muscle, Indian J. Endocrinol. Metab. 16 (Suppl 3) (2012) S562–S568, https://doi.org/10.4103/2230-8210.105573.

[29] Achari AE, Jain SK. Adiponectin, a Therapeutic Target for Obesity, Diabetes, and Endothelial Dysfunction. Int J Mol Sci. 2017;18(6):1321. Published 2017 Jun 21. doi:10.3390/ijms18061321.

[30] S. Tan, J. Wang, L. Cao, Z. Guo, Y. Wang, Positive effect of exercise training at maximal fat oxidation intensity on body composition and lipid metabolism in overweight middle-aged women, Clin. Physiol. Funct. Imaging 36 (3) (2016) 225–230.

[31] S. Tan, P. Du, W. Zhao, J. Pang, J. Wang, Exercise training at maximal fat oxidation intensity for older women with type 2 diabetes, Int. J. Sports Med. 39 (5) (2018) 374–381, https://doi.org/10.1055/a-0573-1509.

[32] Chávez-Guevara IA, Urquidez-Romero R, Pérez-León JA, et al. Chronic Effect of Fatmax Training on Body Weight, Fat Mass, and Cardiorespiratory Fitness in Obese Subjects: A Meta-Analysis of Randomized Clinical Trials. Int J Environ Res Public Health. 2020;17(21):7888. Published 2020 Oct 28. doi:10.3390/ijerph17217888.

[33] O. Ben Ounis, M. Elloumi, G. Lac, E. Makni, E. Van Praagh, H. Zouhal, Z. Tabka, M. Amri, Two-month effects of individualized exercise training with or without caloric restriction on plasma adipocytokine levels in obese female adolescents, Ann. Endocrinol. 70 (4) (2009) 235–241.

[34] S. Bordenave, L. Metz, S. Flavier, K. Lambert, E. Ghanassia, A.-M. Dupuy, F. Michel, A.-M. Puech-Cathala, E. Raynaud, J.-F. Brun, J. Mercier, Training-induced improvement in lipid oxidation in type 2 diabetes mellitus is related to alterations in muscle mitochondrial activity. Effect of endurance training in type 2 diabetes, Diabetes Metab. 34 (2) (2008) 162–168.

[35] L. Isacco, D. Thivel, B. Pereira, M. Duclos, N. Boisseau, Maximal fat oxidation, but not aerobic capacity, is affected by oral contraceptive use in young healthy women, Eur. J. Appl. Physiol. 115 (5) (2015) 937–945, https://doi.org/10.1007/s00421-014-3075-7.

[36] K. Wyskida, G. Franik, T. Wikarek, A. Owczarek, A. Delroba, J. Chudek, J. Sikora, M. Olszanecka-Glinianowicz, The levels of adipokines in relation to hormonal changes during the menstrual cycle in young, normal-weight women, Endocr. Connect. 6 (8) (2017) 892–900.

[37] G. Fletcher, F.F. Eves, E.I. Glover, S.L. Robinson, C.A. Vernooij, J.L. Thompson, G. A. Wallis, Dietary intake is independently associated with the maximal capacity for fat oxidation during exercise, Am. J. Clin. Nutr. 105 (4) (2017) 864–872.

[38] L. Jurado-Fasoli, F.J. Amaro-Gahete, E. Merchan-Ramirez, I. Labayen, J.R. Ruiz, Relationships between diet and basal fat oxidation and maximal fat oxidation during exercise in sedentary adults, Nutr. Metab. Cardiovasc. Dis. 31 (4) (2021) 1087–1101, https://doi.org/10.1016/j.numecd.2020.11.021.