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Adipose tissue fatty acids as biomarkers for metabolic dysfunction in obese females: Implication of menopause and ageing

Sara Sousa[a,b], Diogo Pestana[b,c], Gil Faria[b,d], Cristina Delerue-Matos[a], Conceição Calhau[b,c], Valentina Fernandes Domingues[a,*]

[a] REQUIMTE/LAQV, ISEP, Polytechnic of Porto, Rua Dr. António Bernardino de Almeida 431, 4249-015 Porto, Portugal
[b] Center for Research in Health Technologies and Information Systems, Rua Dr. Plácido da Costa, 4200-450 Porto, Portugal
[c] Nutrição e Metabolismo NOVA Medical School Faculdade de Ciências Médicas Universidade Nova de Lisboa, Campo dos Mártires da Pátria 130, 1169-056 Lisboa, Portugal
[d] Faculty of Medicine, University of Porto, Alameda Prof. Hernâni Monteiro, 4200-319 Porto, Portugal

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ABSTRACT

Fatty acids (FA) are biomarkers of metabolic dysfunction. Adipose tissue is the largest reservoir of FA and acts differently in obese individuals. Menopause by itself significantly alters metabolism, lipid metabolism dysregulation, and adipose tissue distribution. How adipose tissue FA alters an obese individual's metabolism depending on a female's menopausal status is yet poorly understood.

Hence, the subcutaneous (scAT) and visceral adipose tissue (vAT) FA profile for 173 obese premenopausal and postmenopausal women was measured and associated with biochemical parameters. scAT and vAT FA profiles were distinct by themselves and in menopause. In total 816 associations were found with biochemical parameters, where only 58 were independent of the menopausal status.

The associations found to emphasize the importance of assessing the adipose tissue FA profile and how their behavior changes with menopause. The FA are crucial in metabolic processes and can be helpful biomarkers in the prevention/treatment and follow-up of female obesity.

Abbreviations

BMI	body index mass
DHEA-S	dehydroepiandrosterone sulphate
FA	fatty acid
FSH	follicle-stimulating hormone
HbA1c,	glycated haemoglobin
HDL	high density lipoprotein
HOMA2-B	homeostasis model assessment of beta cell function
HOMA2-S	homeostasis model assessment of insulin sensitivity
HOMA-IR	homeostasis model assessment of insulin resistance
LDL	low density lipoprotein
LH	luteinizing hormone
MUFA	monounsaturated fatty acids
Post-MW	postmenopausal women
Pre-MW	premenopausal women
PUFA	polyunsaturated fatty acids
RTE	ratio testosterone/estradiol
RWH	ratio waist/hip circumference
scAT	subcutaneous adipose tissue
SFA	saturated fatty acids
T3	triiodothyronine
T4	thyroxine
TSH	thyroid stimulating hormone
vAT	visceral adipose tissue
ω	omega
ω3	omega 3 fatty acids
ω6	omega 6 fatty acids

1. Introduction

Fatty acids (FA) have structural functions and exert effects, in addition to their energy functions. The genomic effects are due to their affinity for nuclear receptors such as peroxisome proliferator-activated receptors, modifying the expression of genes and the non-genomic effects as these are precursors of autacoids. Their study has increased since

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* Corresponding author at: REQUIMTE/LAQV, ISEP, Polytechnic of Porto, Rua Dr. António Bernardino de Almeida 431, 4249-015 Porto, Portugal.
E-mail address: vfd@isep.ipp.pt (V. Fernandes Domingues).

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FA were proven to prevent and treat several metabolic conditions, such as the famous omega (ω) 3 polyunsaturated FA (PUFA) with its anti-inflammatory properties in cardiovascular disease prevention. Nonetheless, high levels of FA specially saturated FA (SFA) were linked to disorders such as diabetes, obesity, autoimmune disease, and atherosclerosis, amongst others [1,2].

Normally the FA profile is assessed in plasma. However adipose tissue is the largest reservoir of FA in humans and during fasting, these are transferred to plasma [3]. Furthermore, FA can regulate preadipocytes' gene expression, influencing adipocyte proliferation and differentiation [4]. Adipose tissue plays a role in obesity and associated complications. Adipocytes can be classified into three varieties: white, brown, and beige. White adipocyte is the more prevalent form in adults. Apart from being the main reservoir of lipids, adipose tissue has a crucial role in metabolism and signaling by producing inflammatory molecules, inflammatory-related adipokines, and releasing hormones. This endocrine organ can yet be sub-categorized according to its location in the body: as subcutaneous adipose tissue (scAT) when close to the skin or as visceral adipose tissue (vAT) when around an internal organ [5]. Their behavior is distinct, vAT is described to be more innervated and vascularized, with more inflammatory and immune cells and consequently more active regarding lipolysis and insulin resistance [6–8].

A high amount of lipids in the circulation triggers hypertrophy and the consequent increase in fat mass causes hyperplasia [4,8]. Obesity is a disease increasing worldwide, characterized by excessive storage of triglycerides in adipose tissue [2] and induced by, amongst others, behavioural, environmental, and genetic factors [9]. The pathogenesis of obesity is associated with an increased risk of chronic inflammation, non-alcoholic fatty liver disease, cardiovascular disease, cancer, and type 2 diabetes mellitus [10]. This is a common disease in women with a higher prevalence after menopause [2]. In addition, menopause is another factor altering the metabolism. The decline of estrogens during menopause can lead to endothelial dysfunction and oxidative stress. Subsequently, levels of ω3 PUFA are reduced contributing to a higher risk of atherosclerosis and cardiovascular disease [2]. Moreover, ageing alters adipose tissue distribution with the development of abdominal adiposity, commonly by vAT and scAT decreased ability to store fat, and a decrease in hip adiposity. The shift in adiposity means a higher circulation of FA that promotes ectopic fat storage, consequently increasing the risk of metabolic disorders, such as insulin resistance, and atherosclerosis [11].

The present study aims to assess the FA profile in scAT and vAT from obese premenopausal (Pre-MW) and postmenopausal women (Post-MW) and evaluate their possible association as individual FA with several biochemical parameters.

2. Materials and methods

2.1. Sampling

Between 2009 and 2010, samples of scAT and vAT from 173 female patients undergoing bariatric surgery were collected at Hospital de São João (Porto, Portugal). The study was performed according to the Declaration of Helsinki and approved by the hospital's ethics committee (CE 146–09). Written informed consent was provided by all the participants. Samples were stored at -80ºC until analysis. Clinical and biochemical data were obtained as described in previous studies [9,12].

2.2. Chemicals

Sodium hydroxide and anhydrous sodium sulphate were obtained from Pronalab (Lisbon, Portugal); boron trifluoride-methanol at 14% in methanol, Supelco 37 Component FAME Mix (containing the fatty acids C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C21:0, C22:0, C23:0, C24:0, C14:1ω5, C15:1ω5, C16:1ω7, C17:1ω7, C18:1ω9*trans*, C18:1ω9*cis*, C20:1ω9, C22:1ω9, C24:1ω9, C18:2ω6*trans*, C18:2ω6*cis*, C18:3ω3, C18:3ω6, C20:2ω6, C20:3ω3, C20:3ω6, C20:4 ω6, C20:5ω3, C22:2ω6 and C22:6ω3) and butylated hydroxytoluene (≥99%) were from Sigma-Aldrich (St. Louis, USA); *n*-hexane (99%) from Merck (Darmstadt, Germany); methanol from VWR Chemicals Prolabo (Fontenay-sous-Bois, France); Tridecanoic acid (C13:0) was obtained from Fluka (Switzerland) and used as internal standard and sodium chloride (99.5%) was from Panreac (Barcelona, Spain).

2.3. Lipid extraction and FA determination

Lipid extraction was performed by ultrasonic assisted extraction as described in Sousa et al. [13]. The lipid content of the extract was obtained gravimetrically and express as g lipid/g of adipose tissue.

FA were analysed as previously described [2] by gas chromatography flame ionization detection GC-FID), using 200 μL of the lipid extract and 60 μL of internal standard C13:0 at 5000 μg/mL. The FA content was expressed as a percentage and obtained by the ratio between each FA peak area and the sum of the peak areas of all FA, after internal normalization of the chromatographic peak areas.

2.4. Gas chromatography flame ionization detector (GC-FID)

A Shimadzu GC-2010 gas chromatograph (Kyoto, Japan) equipped with an FID and a Shimadzu AOC-20i autoinjector was used. The separation was carried out on an Agilent J&W CP-Sil 88 capillary column (100 *m* × 0.25 mm I.D., film thickness 0.20 μm). Operating conditions were as follows: Linear velocity, Split mode, with a split ratio of 1:10. The injection volume of 1.0 μL. The injector and detector temperatures were kept at 260 °C. A flow rate of 30 mL/min of helium as a carrier gas (Linde Sógas purity ≥ 99.999%), 40 mL/min of hydrogen, and 400 mL/min of air. Column thermal gradient was as follows: 100 °C for 5 min, then raise until 180 °C at 8 °C/min and held this temperature for 9 min and raise until 230 °C at 1 °C/min and hold for 1 min. Fatty acid methyl esters were identified by comparison with a known standard mixture (Sigma 47,885-U Supelco 37 Component FAME Mix, USA) and data acquisition and processing was done using the software GCsolution for GC systems (Shimadzu). Examples of GC-FID chromatograms are shown in Supplementary Material Figures SM1 and SM2.

2.5. Statistical analysis

Statistical analysis was conducted using Statistical Package for Social Sciences (SPSS, 21.0 version statistical software, IBMCorp.s, NewYork, USA). Data were described as median and respective interquartile range (IQR), since the absence of normal distribution (assessed with Kolmogorov-Smirnov or Shapiro-Wilk test, when n was <50). Samples were divided according to menopausal status. The Mann–Whitney and Kruskal–Wallis tests were used to compare medians between Pre-MW and Post-MW groups and Wilcoxon test was used to compare medians between tissues (scAT vs vAT). Spearman correlation test and multiple linear regression were used to evaluate the associations between different fatty acids and several biochemical parameters. At multiple linear regression analysis, the neperian logarithm was applied when the residuals did not present a linear distribution. All tests were two-tailed and *p* values <0.05 were regarded as significant.

3. Results

3.1. Biochemical parameters

The population includes women from 19 to 65 years old, 121 Pre-MW and 52 Post-MW (Table 1). Pre-MW were obese for fewer years than Post-MW, somewhat expected as Pre-MW are also younger. Menopause had no impact on BMI. The highest vAT adipocyte area in Post-MW has been verified however vAT number of adipocytes was

Table 1
Clinical and biological characteristics of the patients.

	Premenopausal women			Postmenopausal women			*p*
	n	Median	IQR	n	Median	IQR	
Age information							
Age (years)	121	36	11	52	54	8	**<0.001**
Obesity evolution (years)	119	18	12	49	26	17	**<0.001**
Anthropometric parameters							
BMI (kg m^{-2})	121	45	7	52	44	7	0.5
Body fat (%)	40	48	8	9	49	8	0.6
Torso body fat (%)	40	47	6	9	47	5	0.7
Waist circumference (cm)	107	118	14	43	123	16	0.1
Hip circumference (cm)	102	133	13	39	132	15	0.6
vAT adipocyte area (mm^{2})	120	4079	165	51	4430	1814	**<0.001**
scAT adipocyte area (mm^{2})	120	6586	2345	50	6330	1957	0.9
vAT number of adipocyte (x10^{9})	120	82	36	51	70	34	**<0.001**
scAT number of adipocyte (x10^{9})	120	53	22	51	53	18	0.1
Plasma lipid profile							
Total cholesterol (mg dL^{-1})[a]	94	194	39	30	208	38	**0.03**
Total triglycerides (mg dL^{-1})[a]	94	113	61	30	131	56	0.3
HDL cholesterol (mg dL^{-1})[a]	94	51	15	30	54	14	0.2
LDL cholesterol (mg dL^{-1})[a]	94	122	39	30	136	29	**0.001**
Apolipoprotein A1 (mg dL^{-1})	26	153	40	11	158	28	0.8
Apolipoprotein B (mg dL^{-1})	26	93	24	11	91	32	0.5
Lipoprotein A (mg dL^{-1})	25	7	10	10	4	37	0.8
Plasma glucose homeostasis and insulin sensitivity [b]							
Glycaemia (mg dL^{-1})	101	82	14	31	95	14	**<0.001**
HbA1c (%)	82	5.5	0.5	21	5.8	0.4	**<0.001**
HOMA-IR	102	169	90	26	115	71	**<0.001**
HOMA2-B (%)	102	2	1	26	2	2	0.06
HOMA2-S (%)	102	49	32	26	58	82	**0.03**
C-peptide (ng mL^{-1})	63	3	1	13	3	2	0.4
Insulin (μUI L^{-1})	77	16	12	18	13	12	**0.04**
Other parameters							
C-reactive protein (mg L^{-1})	42	8	13	10	3	7	**0.007**
Sedimentation velocity (mm 1^{-1}ah^{-1})	39	25	24	11	17	25	0.7
Cortisol (μg dL^{-1})	40	19	14	13	14	11	**0.004**
FSH (mUI mL^{-1})	80	5	4	32	37	31	**<0.001**
LH (mUI mL^{-1})	83	5	7	32	20	17	**<0.001**
Estradiol (pg mL^{-1})	78	57	65	28	18	31	**<0.001**
Progesterone (ng mL^{-1})	29	0.3	0.5	7	0.6	1.7	0.1
Testosterone (ng mL^{-1})	87	0.4	0.4	33	0.3	0.3	**<0.001**
DHEA-S (μg dL^{-1})	85	210	109	33	91	99	**<0.001**
Free T3 (ng dL^{-1})[c]	72	3.3	0.7	28	2.9	0.5	**<0.001**
Free T4 (ng dL^{-1})[c]	90	1.1	0.1	34	1.1	0.2	0.2
TSH (μUI mL^{-1})[c]	90	2	1	36	2	1	0.05
Albumin (g L^{-1})	111	41	3	49	42	3	**<0.001**

BMI - body index mass; DHEA-S - dehydroepiandrosterone sulphate; FSH - follicle-stimulating hormone; HbA1c - glycated haemoglobin; HDL - high density lipoprotein; HOMA2-B - homeostasis model assessment of beta cell function; HOMA2-S – homeostasis model assessment of insulin sensitivity; HOMA-IR - homeostasis model assessment of insulin resistance; IQR – interquartile range; LDL - low density lipoprotein; LH - luteinizing hormone; scAT - subcutaneous adipose tissue; T3 – Triiodothyronine; T4 – Thyroxine; TSH - thyroid stimulating hormone; vAT - visceral adipose tissue

[a] considered only women who did not take statins

[b] considered only women who did not take anti diabetic medication.

[c] considered only women who did not been diagnosed hypothyroidism;

Statistical analysis performed with Mann-Whitney and Kruskal Wallis tests, $p < 0.05$; Significant *p* values are shown in bold.

higher in Pre-MW.

Post-MW had the lowest levels of estradiol, dehydroepiandrosterone sulphate (DHEA-S), and testosterone, yet the ratio of testosterone/estradiol (RTE) was higher in Post-MW. Cortisol was higher in Pre-MW and the ratio cortisol/DHEA-S increased from 0.09 to 0.15 with menopause. Additionally, Post-MW had higher levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH).

Pre-MW presented lower total and low-density lipoprotein (LDL) cholesterol than the Post-MW. Glycaemia, glycated haemoglobin (HbA1c) and homeostasis model assessment of insulin sensitivity (HOMA2-S) were higher in Post-MW, although homeostasis model assessment of insulin resistance (HOMA-IR) and insulin were higher in Pre-MW. Free triiodothyronine (T3) was higher in Pre-MW. Albumin was higher in Post-MW.

3.2. FA and total lipid composition of vAT and scAT

The FA profile and total lipid concentration are shown in Table 2. The main FA were as follows: C18:1ω9*cis*, C16:0, and C18:2ω6*cis*. The obtained ratio C16:1ω7/C18:0 was approximately 2. C10:0, C12:0, C14:0, C18:0, C20:0, C22:0, C14:1ω5, C16:1ω7, C20:1ω9, C22:1ω9, C24:1ω9 and monounsaturated FA (MUFA) were higher in vAT, while C16:0, C17:0, C20:2ω6, C20:3ω6, C20:4ω6, C20:5ω3, C22:6ω3, SFA, ω3, ω6, and PUFA were higher in scAT. vAT total lipids, SFA, MUFA, C22:1ω9, C22:2ω6 and C24:1ω9 were higher in Pre-MW and differences were found for C12:0, C14:0, C15:0, C16:0, C18:0, and C20:0. Moreover, C20:5ω3, C22:6ω3, C18:2ω6*cis*, C20:2ω6, C20:3ω6, and C20:4ω6, ω3, ω6 and PUFA were higher in Post-MW. The ratio ω6/ω3 was lowest in Post-MW.

Table 2
Total lipid (g g^{-1} AT) and fatty acids profile (%) of scAT and vAT in premenopausal and postmenopausal women.

	Premenopausal women							Postmenopausal women							scAT	vAT
	scAT			vAT			p^1	scAT			vAT			p^1	Pre vs Postmenopausal women	
	n	Median	IQR	n	Median	IQR		n	Median	IQR	n	Median	IQR		p^2	p^2
Total lipids (g g^{-1})																
	121	0.9	0.2	121	1.00	0.09	**<0.001**	52	1.0	0.2	52	1.00	0.06	0.8	0.2	0.2
Fatty acid (%)																
C10:0	22	0.008	0.008	83	0.01	0.01	**<0.001**	22	0.008	0.009	30	0.01	0.01	**<0.001**	0.7	0.1
C12:0	121	0.2	0.2	121	0.3	0.2	**<0.001**	52	0.15	0.11	52	0.18	0.18	**<0.001**	**<0.001**	**<0.001**
C14:0	121	2.1	0.7	121	2.2	0.8	**<0.001**	52	1.7	0.6	52	1.8	0.6	**<0.001**	**<0.001**	**<0.001**
C15:0	121	0.20	0.07	121	0.19	0.06	**<0.001**	52	0.183	0.063	52	0.176	0.059	**<0.001**	**0.001**	**0.004**
C16:0	121	23.5	3.4	121	22.6	3.3	**<0.001**	52	23	4	52	21	4	**<0.001**	**0.002**	**<0.001**
C17:0	121	0.20	0.05	121	0.19	0.05	**<0.001**	52	0.19	0.06	52	0.18	0.05	**<0.001**	0.3	0.09
C18:0	121	2.9	1.0	121	3.2	1.2	**<0.001**	52	3	1	52	3	1	1	0.2	**0.001**
C20:0	121	0.05	0.02	121	0.10	0.03	**<0.001**	51	0.05	0.02	52	0.10	0.03	**<0.001**	0.2	**0.04**
C22:0	45	0.009	0.004	84	0.014	0.007	**<0.001**	21	0.008	0.004	38	0.014	0.006	**<0.001**	0.6	0.2
C14:1 ω5	121	0.19	0.13	121	0.22	0.16	**<0.001**	52	0.18	0.13	52	0.21	0.14	**<0.001**	0.1	0.08
C15:1 ω5	1	0.0001	na	nd			na	nd							na	na
C16:1 ω7	121	5	2	121	6	3	**<0.001**	52	5.6	2.9	52	5.8	2.4	**<0.001**	0.8	0.7
C18:1 ω9 *cis*	121	41	4	121	42	4	**<0.001**	52	42	5	52	44	5	**<0.001**	0.1	0.06
C20:1ω9	121	0.5	0.1	121	0.6	0.2	**<0.001**	52	0.5	0.2	52	0.6	0.2	**<0.001**	0.5	0.5
C22:1 ω9	84	0.013	0.005	92	0.019	0.006	**<0.001**	38	0.01	0.01	40	0.017	0.006	**<0.001**	**0.04**	**0.02**
C24:1 ω9	4	0.006	0.006	22	0.008	0.004	**0.03**	2	0.003	0.001	14	0.006	0.002	0.3	0.07	**0.001**
C18:2 ω6 *cis*	121	19.9	4.6	121	19.7	4.1	**<0.001**	52	20.9	5.8	52	21.0	5.5	**<0.001**	0.09	**0.002**
C18:3 ω3	121	0.6	0.1	121	0.6	0.1	0.8	52	0.6	0.2	52	0.6	0.2	0.7	0.8	0.9
C18:3 ω6	120	0.08	0.04	119	0.07	0.04	**<0.001**	52	0.08	0.04	52	0.06	0.04	**<0.001**	0.1	0.05
C20:2 ω6	121	0.35	0.11	121	0.31	0.09	**<0.001**	52	0.39	0.14	52	0.35	0.11	**<0.001**	**0.008**	**<0.001**
C20:3 ω3	97	0.03	0.01	96	0.019	0.008	**<0.001**	44	0.03	0.02	42	0.020	0.009	**<0.001**	0.1	0.1
C20:3 ω6	121	0.6	0.3	121	0.3	0.2	**<0.001**	52	0.6	0.3	52	0.4	0.2	**<0.001**	0.7	**<0.001**
C20:4 ω6	121	1.0	0.3	121	0.5	0.2	**<0.001**	52	1.0	0.4	52	0.6	0.2	**<0.001**	0.6	**<0.001**
C20:5 ω3	117	0.11	0.06	111	0.08	0.03	**<0.001**	50	0.13	0.06	50	0.11	0.04	**<0.001**	**<0.001**	**<0.001**
C22:2 ω6	nd			5	0.008	0.005	na	nd			6	0.007	0.002	na	na	**0.02**
C22:6 ω3	121	0.3	0.2	121	0.2	0.1	**<0.001**	52	0.5	0.3	52	0.4	0.2	**<0.001**	**<0.001**	**<0.001**
ΣSFA	121	29.4	4.2	121	28.8	4.0	**<0.001**	52	28	5	52	27	4	**<0.001**	**0.001**	**<0.001**
ΣMUFA	121	47	4	121	49	5	**<0.001**	52	48	6	52	51	6	**<0.001**	0.3	0.1
ΣPUFA	121	23	5	121	22	4	**<0.001**	52	24.3	6.0	52	23.6	5.4	**<0.001**	**0.02**	**<0.001**
Σω3	121	1.0	0.3	121	0.9	0.3	**<0.001**	52	1.2	0.5	52	1.1	0.5	**<0.001**	**<0.001**	**<0.001**
Σω6	121	22	5	121	21	4	**<0.001**	52	23	6	52	22	6	**<0.001**	0.06	**<0.001**

IQR – interquartile range; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; SFA – saturated fatty acids; scAT - subcutaneous adipose tissue; vAT - visceral adipose tissue; na – not applicable; nd – not detected; ω – omega; ω3– omega 3 fatty acids; ω6 – omega 6 fatty acids.

[1] Statistical analysis performed with Wilcoxon test, $p < 0.05$.

[2] Statistical analysis performed with Mann-Whitney and Kruskal Wallis tests, $p < 0.05$; Significant p values are shown in bold.

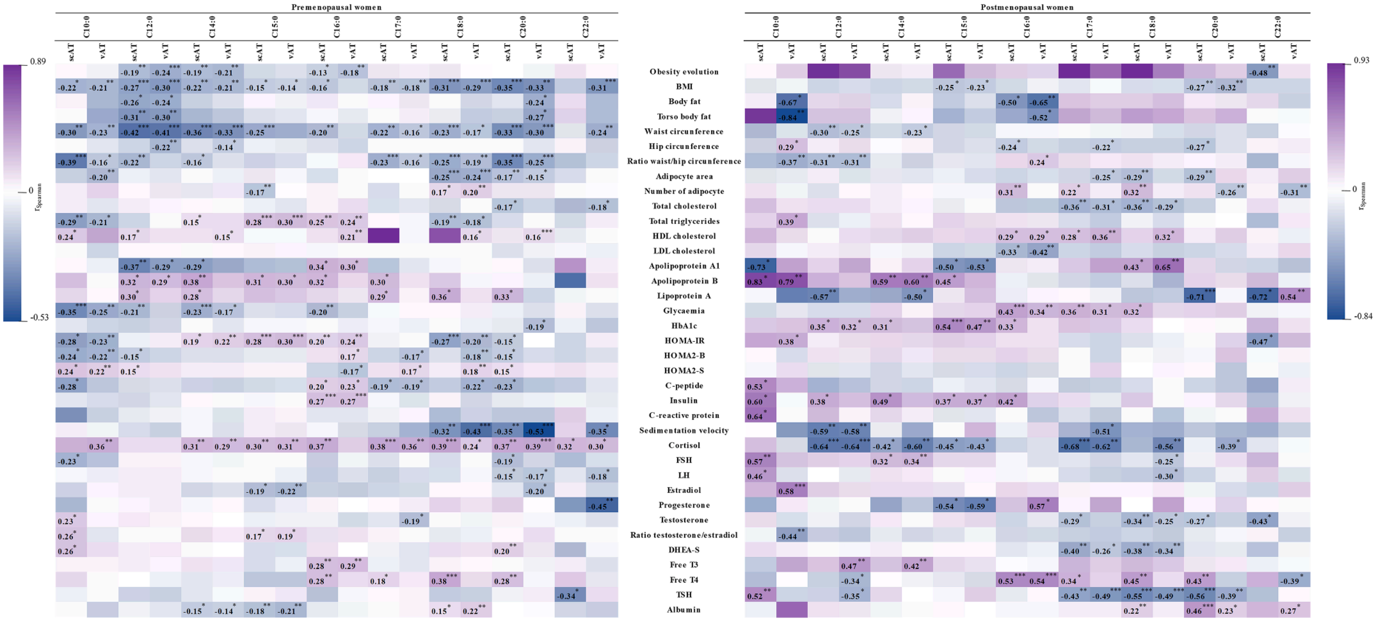

Fig. 1. Heatmap of the associations found between SFA in adipose tissue and the biochemical parameters for Pre-MW and Post-MW. The color code corresponds to Spearman's correlation coefficient ($r_{Spearman}$), from lower values in blue to higher values in purple. Values in tiles are $r_{Spearman}$ for significant associations, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$.

3.3. FA associations with biochemical parameters independent of menopausal status

This section will only be addressed Spearman's correlations obtained between FA (vAT and scAT) and the females' biochemical parameters that are independent of the menopausal status (Figs. 1 to 3). In total 58 correlations were obtained where the effect of the same FA on the same biochemical parameter showed the same tendency (positive or negative correlation) in Pre-MW and Post-MW.

Anthropometric parameters had mainly negative associations with SFA (Fig. 1): BMI showed correlations with C15:0 and C20:0, waist circumference displayed correlations with C12:0 and vAT C14:0, the ratio waist/hip circumference (RWH) had correlations with vAT C10:0 and scAT C12:0 and scAT adipocyte area displayed correlations with C18:0 and C20:0. scAT adipocyte number presented a positive correlation with C18:0. Waist circumference, RWH, and vAT adipocyte area also showed positive correlations with PUFA, namely with vAT C20:2ω6, C20:3ω6 and C20:4ω6 for waist circumference, C20:3ω6, and vAT C20:4ω6 for RWH and vAT C20:3ω6 and C20:4ω6 for adipocyte area (Fig. 3). Adipocyte number displayed a positive correlation with scAT C20:2ω6 and a negative correlation with vAT C20:3ω6.

High-density lipoprotein (HDL) cholesterol presented positive correlations with vAT C16:0 and C18:0, and apolipoprotein B showed positive correlations with scAT C14:0 and C15:0 (Fig. 1). Whereas total cholesterol had a positive correlation with scAT C16:1ω7 (Fig. 2) and triglycerides displayed three negative correlations and two positive correlations with PUFA (Fig. 3).

HOMA-IR showed a negative correlation with scAT C20:1ω9 and positive correlations with C20:4ω6 (Figs. 2 and 3). HOMA2-B and HOMA2-S exhibited correlations with the same FA but with opposite tendencies, in which HOMA2-B displayed positive correlations with C20:3ω6 and scAT C20:4ω6 (Fig. 3). Insulin and albumin had positive correlations with scAT C16:0 and scAT C18:0, respectively (Fig. 1). Sedimentation velocity displayed positive correlations with C20:3ω6 (Fig. 3).

Concerning hormonal parameters, FSH showed positive correlations with scAT C16:1ω7 and scAT C18:3ω6 and a negative correlation with vAT C24:1ω9 (Figs. 2 and 3). LH had a positive correlation with scAT C16:1ω7 (Fig. 2) and progesterone displayed a negative correlation with vAT C20:3ω6 (Fig. 3). Free T3 showed a positive correlation with vAT C16:1ω7 (Fig. 2) and free T4 presented positive correlations with scAT C16:0, C17:0, C18:0, C20:0 (Fig. 1) and negative correlations with scAT C14:1ω5 and C18:1ω9*cis* (scAT and vAT, Fig. 2).

3.4. Implications of menopause on FA associations with biochemical parameters

This section addressed Spearman's correlations achieved between FA and the biochemical parameters which were dependent on menopausal status (Figs. 1 to 4). In total 700 distinct correlations were obtained, representing the impact of menopause on FA and their subsequent effect on biochemical parameters (397 correlations in Pre-MW versus 303 correlations in Post-MW). Furthermore, will be highlighted within this section correlations between the same FA and the same biochemical parameter displayed opposite tendencies (positive versus negative correlation) depending on the female menopausal status.

Obesity evolution presented negative correlations with four SFA (three in Pre-MW, Fig. 1), nine correlations with MUFA in Pre-MW (three negatives, Fig. 2), eight positive correlations with PUFA (five in Pre-MW, Fig. 3), and a negative correlation with scAT total lipids in

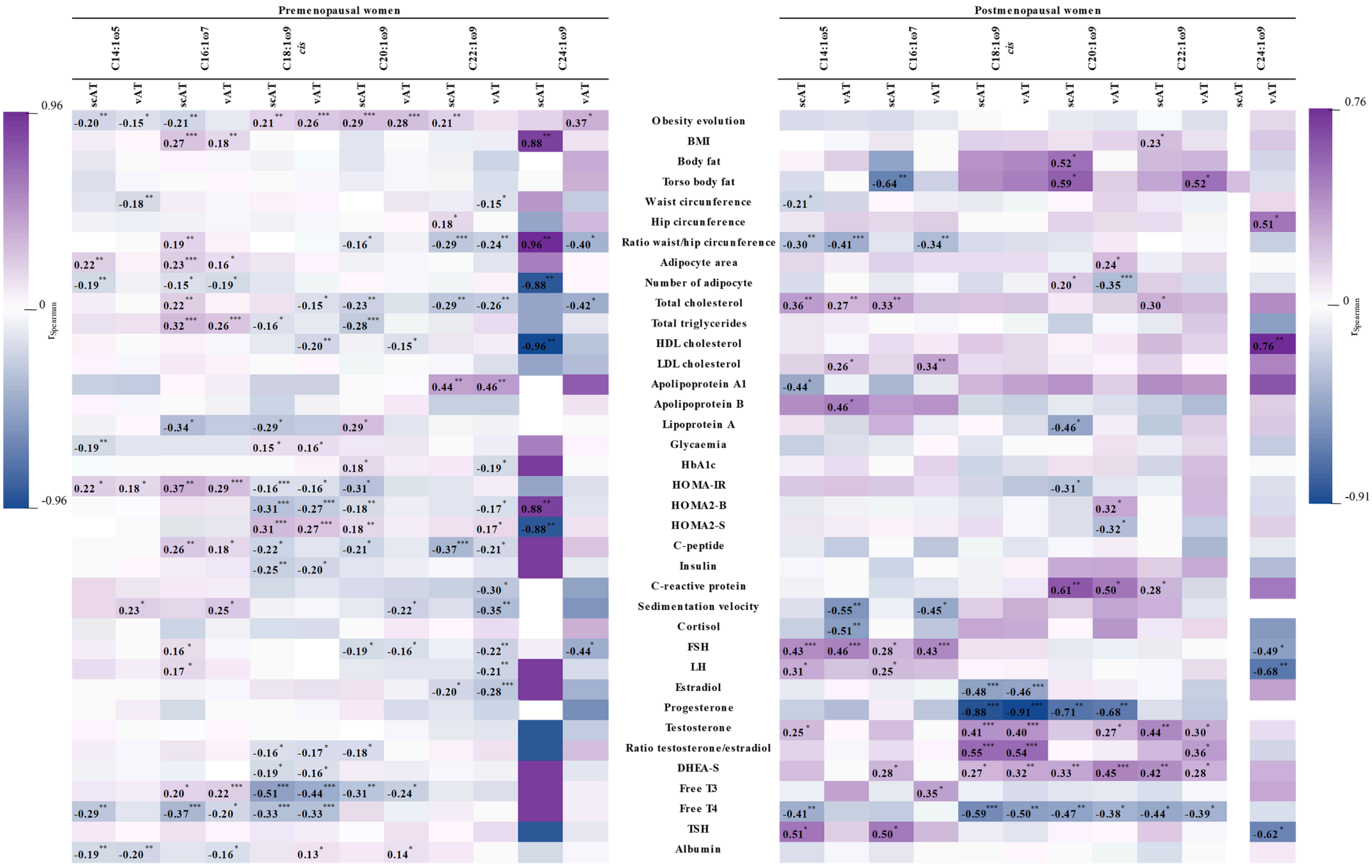

Fig. 2. Heatmap of the associations found between MUFA in adipose tissue and the biochemical parameters for Pre-MW and Post-MW. The color code corresponds to Spearman's correlation coefficient ($r_{Spearman}$), from lower values in blue to higher values in purple. Values in tiles are $r_{Spearman}$ for significant associations, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$.

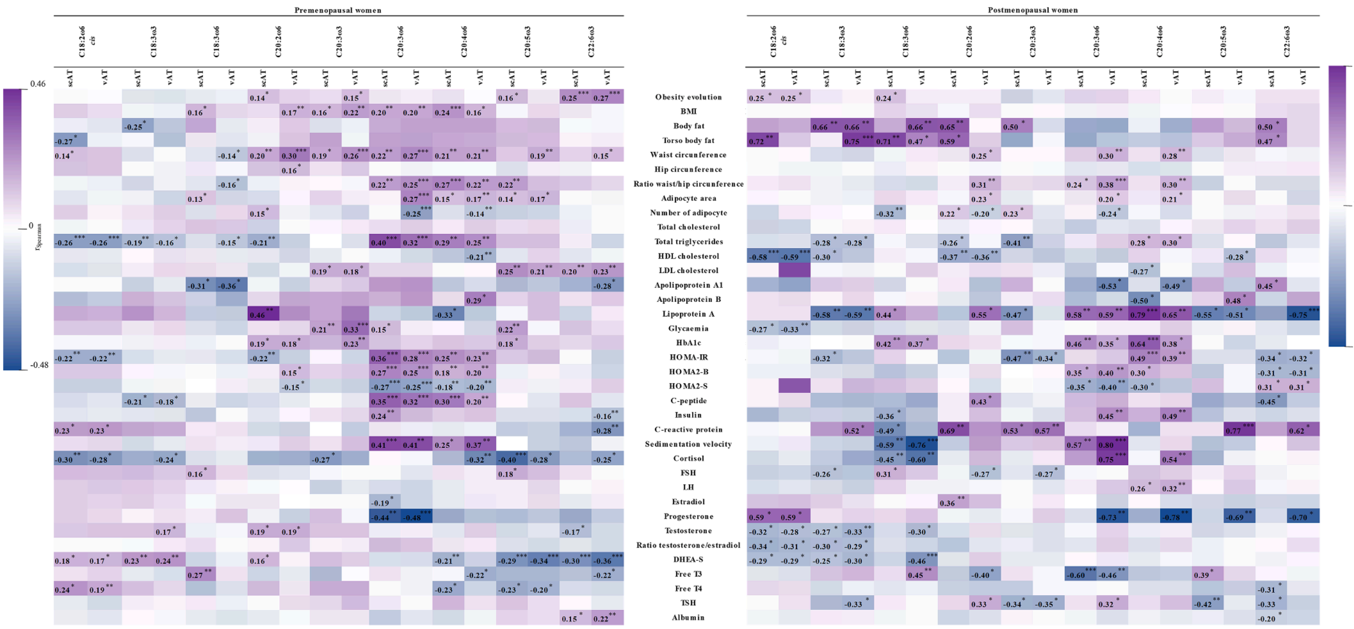

Fig. 3. Heatmap of the associations found between PUFA in adipose tissue and the biochemical parameters for Pre-MW and Post-MW. The color code corresponds to Spearman's correlation coefficient ($r_{Spearman}$), from lower values in blue to higher values in purple. Values in tiles are $r_{Spearman}$ for significant associations, $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$.

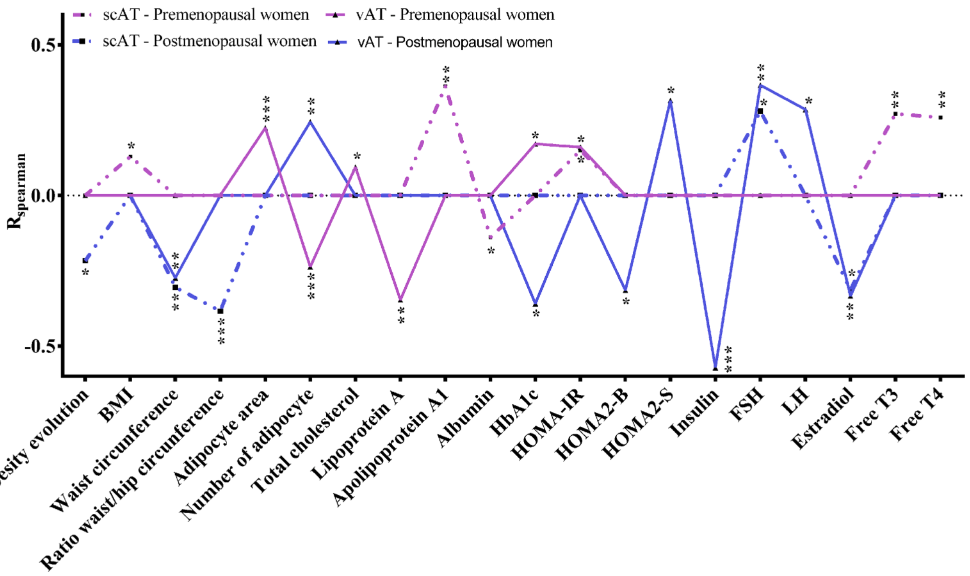

Fig. 4. Line plot of the significant Spearman's correlation coefficient between the total lipids and each metabolic marker, $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$.

Post-MW (Fig. 4). In Pre-MW, BMI was further correlated with all other SFA analysed (twelve negative correlations, Fig. 1), had eight positive correlations with PUFA (Fig. 3), and a positive correlation with scAT total lipid (Fig. 4). BMI also showed four positive correlations with MUFA (three in Pre-MW, Fig. 2). The body fat and torso body fat presented negative correlations with four SFA (two in each cohort, Fig. 1). While only in Post-MW were found correlations with MUFA (one for body fat and three for torso body fat, Fig. 2). PUFA had seven correlations with each parameter (one each in Pre-MW, Fig. 3). Furthermore, the correlations achieved between scAT C18:3ω3 and body fat and between scAT C18:2ω6*cis* and torso body fat had opposite tendencies depending on menopausal status. The vAT adipocyte area had negative correlations with SFA (three in Pre-MW, Fig. 1), positive correlations with MUFA and PUFA (one in each cohort, Figs. 2 and 3), and a positive correlation with vAT total lipid in Pre-MW (Fig. 4). While in Pre-MW scAT adipocyte area showed positive correlations with two scAT MUFA (Fig. 2) and three scAT PUFA (Fig. 3). For adipocyte number were found six correlations with SFA (two in Pre-MW, Fig. 1), six correlations with MUFA (four in Pre-MW, Fig. 2), four correlations with PUFA (one in Pre-MW, Fig. 3), and correlations with vAT total lipids, which had an opposite tendency depending on the menopausal status (Fig. 4). Waist circumference showed further nine negative correlations with SFA in Pre-MW (Fig. 1), three negative correlations with MUFA (one in Post-MW, Fig. 2), more nine correlations with PUFA in Pre-MW (Fig. 3), and negative correlations with total lipid in Post-MW (Fig. 4). The hip circumference had six correlations with SFA (four in Post-MW, Fig. 1), two correlations with MUFA (one in each cohort, Fig. 2), and a positive correlation with vAT C20:2ω6 in Pre-MW (Fig. 3). RWH showed more ten correlations with SFA (eight in Pre-MW all negative, Fig. 1), nine correlations with MUFA (three in Post-MW all negative, Fig. 2), four correlations with PUFA (three in Pre-MW, Fig. 3), and a negative correlation with scAT total lipids in Post-MW (Fig. 4).

Cortisol had correlations with all the SFA analysed, in which, all correlations were positive in Pre-MW, and, all were negative in Post-MW (Fig. 1). The two cohorts achieved correlations with the same SFA, i.e. C14:0, C15:0, C17:0, vAT C18:0, and vAT C20:0, showing opposite tendencies depending on the menopausal status. Furthermore, this parameter achieved a negative correlation with vAT C14:1ω5 in Post-MW (Fig. 2) and twelve correlations with PUFA (eight in Pre-MW all negative, Fig. 3). Where the correlation found with vAT C20:4ω6 displayed an opposite tendency according to menopausal status.

Concerning inflammatory parameters, sedimentation velocity presented eight negative correlations with SFA (five in Pre-MW, Fig. 1), six correlations with MUFA (four in Pre-MW, Fig. 2), and four correlations with PUFA (two in each cohort, Fig. 3). The correlations found with vAT C14:1ω5 and C16:1ω7 had opposite tendencies according to menopausal status (Fig. 2). The C-reactive protein showed a positive correlation with scAT C10:0 in Post-MW, four correlations with MUFA (one in Pre-MW, Fig. 2), and nine correlations with PUFA (three in Pre-MW, Fig. 3). In which, the correlation with vAT C22:6ω3 displayed an opposite tendency according to menopausal status.

Total cholesterol displayed only negative correlations with SFA (different FA to each cohort, Fig. 1), eight more correlations with MUFA (five in Pre-MW all negative, Fig. 2) and a positive correlation with vAT total lipids in Pre-MW (Fig. 4). Being that the correlation with scAT C16:1ω7 showed an opposite tendency according to menopausal status. Regarding LDL, correlations were found with C16:0, vAT C14:1ω5 and vAT C16:1ω7 in Post-MW (Figs. 1 and 2) and with four PUFA (three in Pre-MW all positive, Fig. 3). For lipoprotein A were obtained ten correlations with SFA (five in each cohort, Fig. 1), four correlations with scAT MUFA (three in Pre-MW, Fig. 2), fourteen with PUFA (two in Pre-MW, Fig. 3) and a negative correlation with vAT total lipids in Pre-MW (Fig. 4). In which, an opposite effect (dependent on the menopausal status) was observed for the correlations with scAT C12:0, C20:0, C20:1ω9 and C20:4ω6. Triglycerides had ten correlations with SFA (one in Post-MW, Fig. 1), four correlations with MUFA in Pre-MW (two positive, Fig. 2), and further six correlations with PUFA (five in Pre-MW, Fig. 3). Where the correlation with vAT C10:0 displayed an opposite tendency depending on the menopausal status. Apolipoprotein B and HDL presented further fifteen positive correlations with SFA (nine in Pre-MW, Fig. 1). HDL showed negative correlations with MUFA and PUFA in Pre-MW, whereas a positive correlation with vAT C24:1ω9 and negative correlations with PUFA were found in Post-MW (Figs. 2 and 3). Apolipoprotein B had a positive correlation with vAT C14:1ω5 in Post-MW (Fig. 2) and three correlations with PUFA (one in Pre-MW, Fig. 3). The apolipoprotein A1 showed five correlations with distinct SFA in each cohort (Fig. 1), three correlations with MUFA (two in Pre-MW, Fig. 2), six correlations with PUFA (three in Pre-MW all negative, Fig. 3), and a positive correlation with scAT total lipid in Pre-MW (Fig. 4).

Albumin only had correlations with SFA, MUFA, and scAT total lipid in Pre-MW (Figs. 1, 2, and 4). As for PUFA, three correlations were achieved with C22:6ω3 (two in Pre-MW all positive, Fig. 3), showing an opposite tendency depending on menopausal status.

Glycaemia and HbA1c obtained opposite correlations with SFA in each cohort (Fig. 1). The effect of scAT C16:0 on glycaemia is depending on the menopausal status. Only in Pre-MW were found correlations with MUFA for these two parameters (three correlations with glycaemia and two with HbA1c, Fig. 2). Glycaemia had six correlations with PUFA (two in Post-MW all negative, Fig. 3). While for HbA1c were found ten positive correlations with PUFA (four in Pre-MW, Fig. 3) and two correlations with vAT total lipids (one in cohorts, Fig. 4) with an opposite tendency depending on menopausal status. HOMA-IR showed fourteen correlations with SFA (twelve in Pre-MW, Fig. 1), more six correlations with MUFA in Pre-MW (four positive, Fig. 2), ten additional correlations with PUFA (five in Pre-MW, Fig. 3), and two positives with total lipids in Pre-MW (Fig. 4). Being that the correlations with vAT C10:0 showed an opposite tendency depending on the menopausal status. HOMA2-B and HOMA2-S exhibited correlations with the same FA however in opposite tendencies. Which, HOMA2-B had seven correlations with SFA in Pre-MW (Fig. 1), six correlations with MUFA (five in Pre-MW, Fig. 2), more four correlations with PUFA (two in each cohort, Fig. 3), and a negative correlation with vAT total lipids in Post-MW (Fig. 4). Insulin had further six positive correlations with SFA (one in Pre-MW, Fig. 1), negative correlations with C18:1ω9*cis* in Pre-MW (Fig. 2), five correlations with PUFA (two in Pre-MW, Fig. 3), and a negative correlation with vAT total lipids in Post-MW (Fig. 4). C-peptide showed eight correlations with SFA (seven in Pre-MW, Fig. 1), six correlations with MUFA in Pre-MW (four negatives, Fig. 2), and eight correlations with PUFA (six in Pre-MW, Fig. 3). In which the correlation with scAT C10:0 presented an opposite tendency depending on the menopausal status.

In Pre-MW, FSH, LH, estradiol, and progesterone only presented negative correlations with SFA (Fig. 1). FSH had two correlations in each cohort, where scAT C10:0 exhibited an opposite effect depending on menopausal status. LH, estradiol, and progesterone had, respectively, five (two in Post-MW), four (one in post-MW), and four correlations (three in Post-MW) with SFA (Fig. 1). FSH had more three correlations with MUFA in each cohort (Fig. 2), a further four correlations with PUFA (three in Post-MW, Fig. 3), and a positive correlation with scAT total lipids in Post-MW (Fig. 4). While LH showed more three correlations with MUFA (two in Post-MW, Fig. 2) and two correlations with C20:4ω6 in Post-MW (Fig. 3). Moreover, FSH and LH had positive correlations with vAT total lipids in Post-MW (Fig. 4). Estradiol also showed four negative correlations with MUFA (two in each cohort, Fig. 2), two correlations with PUFA (one in each cohort, Fig. 3), and a negative correlation with total lipids in Post-MW (Fig. 4). Progesterone also had negative correlations with MUFA in Post-MW (Fig. 2) and further six correlations with PUFA (one in Pre-MW, Fig. 3). Testosterone showed seven correlations with SFA (five in Post-MW, Fig. 1), six positive correlations with MUFA in Post-MW (Fig. 2), and nine correlations with PUFA (four in Pre-MW, Fig. 3). Which, the correlation found with vAT C18:3ω3 displayed an opposite tendency depending on menopausal status. The RTE had four correlations with SFA (three in Pre-MW all positive, Fig. 1), six correlations with MUFA (three in each cohort, Fig. 2), and four negative correlations with PUFA in Post-MW (Fig. 3). Furthermore, the correlations with C18:1ω9*cis* had an opposite tendency, depending on menopausal status. DHEA-S achieved six correlations with SFA (two in Pre-MW all positive, Fig. 1), nine correlations with MUFA (two in Pre-MW all negative, Fig. 2), and fifteen correlations with PUFA (ten in Pre-MW, Fig. 3). Where the correlations with C18:1ω9*cis,* C18:2ω6*cis,* and C18:3ω3 displayed opposite tendencies depending on the menopausal status.

TSH showed, in Pre-MW, a negative correlation with scAT C22:0 and, in Post-MW, eight correlations with SFA, three correlations with MUFA, and seven with PUFA (Figs. 1, 2, and 3). Regarding free T3 were found two positive correlations with SFA in each cohort (Fig. 1), further five correlations with MUFA in Pre-MW (Fig. 2), and eight correlations with PUFA (three in Pre-MW, Fig. 3). Free T4 showed further three correlations with SFA in Post-MW (Fig. 1), six correlations with MUFA (four in Post-MW, Fig. 2), five correlations with PUFA in Pre-MW (two positives), and one negative correlation with scAT C22:6ω3 in Post-MW (Fig. 3). Moreover, scAT total lipid had positive correlations with free T3 and T4 in Pre-MW (Fig. 4).

3.5. *Multiple linear regression*

The FA with significant Spearman's correlations ($p < 0.05$) were added to multiple linear regression models. Figs. 5 and 6 present the 45 models achieved for each biochemical parameter divided into the two cohorts (27 in Pre-MW). vAT total lipids fitted three models as a negative

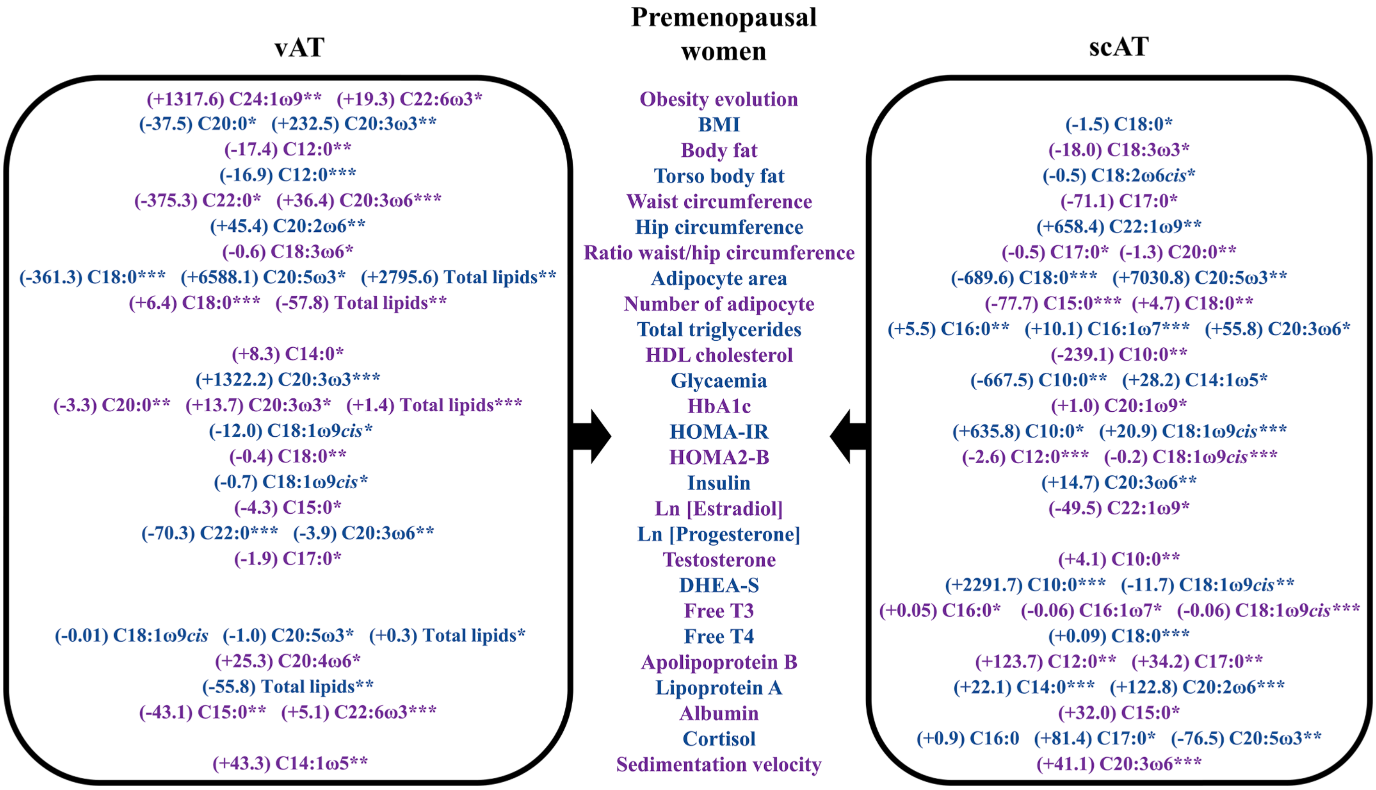

Fig. 5. Multiple linear regressions obtained for Pre-MW, $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$.

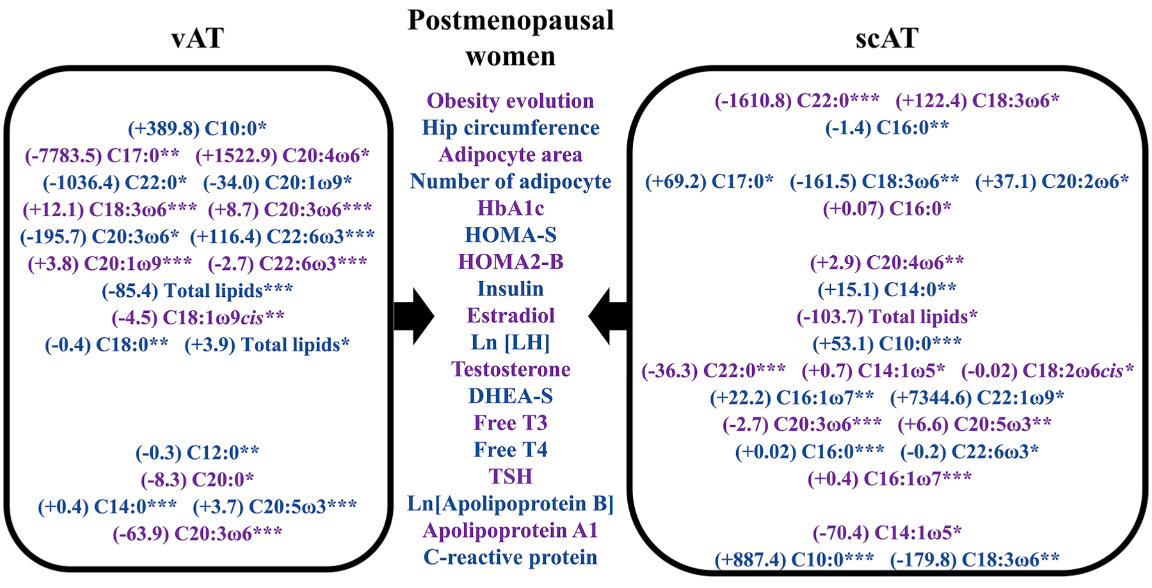

Fig. 6. Multiple linear regressions obtained for Post-MW, $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$.

factor (two in Pre-MW) and four models as a positive factor (three in Pre-MW). scAT total lipids, on the other hand, fitted only one model as a negative factor in Post-MW. SFA were included in 38 models (25 in Pre-MW), specifically with twenty-seven scAT and twenty vAT SFA. Twenty models were achieved containing MUFA (13 in Pre-MW), in which eight scAT and thirteen vAT MUFA were included. Finally, PUFA were included in 32 models (20 in Pre-MW), namely with eighteen vAT and fifteen scAT PUFA.

4. Discussion

This study intended to understand how total lipids and FA profiles in adipose tissue alter biochemical biomarkers in obese women according to menopausal status. The scAT and vAT FA profile are in accordance with the literature [4,14–17], MUFA are the dominant FA in both adipose tissues and the ratio C16:1ω7/C18:0 was similar to previous reports [16]. However, slight differences between the scAT and vAT FA profile were described [6]. Moreover, some studies described C22:0 [6,16], C16:1ω7 and MUFA [4] highest in scAT, together with highest SFA [4], and PUFA [14] in vAT. SFA (in plasma) [18] had been previously reported as highest in Pre-MW and the ratio ω6/ω3 obtained in our study agrees with other studies [11]. Opposite to our study, MUFA (in plasma) were highest in Post-MW [19,20]. vAT seems to have a role in Pre-MW obesity evolution while in Post-MW scAT has a more marked contribution. Our results showed no impact on BMI due to menopause as already reported [21]. Contrary to this study, obese females had positive correlations between BMI and C16:0 and C18:0 (in serum of normal weight Pre-MW [22] and both gender [23]) and C14:0 [22]. Which highlights the need for more studies in this particular population. The positive correlations between BMI and C16:1ω7 in both adipose tissues in Pre-MW were also reported in other studies, namely: in serum of normal weight Pre-MW [22]; normal weight and obese Post-MW [24]; and both genders [23]. Moreover, high scAT MUFA has been associated with BMI in morbidly obese women (without any correlation between serum and vAT MUFA) [17]. A negative association between BMI and MUFA was also described [7]. Regarding PUFA and BMI, positive associations with serum C20:3ω6 and C20:4ω6 were reported [22,23] (agreeing with our findings). Likewise, a positive association between BMI and C18:3ω6 was described (in both genders) [23], while others report a negative association between these factors in normal weighted Pre-MW [22].

The shift in fat distribution is mainly the consequence of a drastic decline of estrogen during menopause together with increased hormonal levels of testosterone, androsterone, and androstenedione, as the receptors of estrogen and androgen are expressed in vAT and scAT cells. While estrogen favours hip and thigh subcutaneous fat, androgens boost visceral abdominal fat accumulation [11]. Body fat had also a negative association with serum C22:6ω3 (normal-weighted women) [25] and with C18:2ω6*cis* (normal-weighted Pre-MW) [22]. Furthermore, a negative association between MUFA and body fat was described [7]. Waist circumference had a negative association with C18:0, C20:0, and total SFA (in serum, normal-weighted Pre-MW) [22], consistent with our findings. Contradictory, positive correlations were reported for C16:0 and C18:0 (in serum of both genders) [23]. Even though we did not achieve a correlation between waist circumference and C16:1ω7, some reported it in blood, namely: a negative correlation (normal weight and obese Post-MW) [24] and a positive correlation (both genders) [23]. In this study, the RWH showed a correlation with C16:1ω7 (positive with scAT in Pre-MW and negative with vAT in Post-MW), indicative of the influence of this MUFA. Towards PUFA were reported a negative association in vAT with RWH [7] and positive associations between waist circumference and C20:3ω6 and C20:4ω6 (in serum) [22,23]. Furthermore, a negative correlation (in serum of both genders) between waist circumference and C18:2ω6 (opposite to this study), a positive correlation with C18:3ω6 (opposite to this study), and a positive correlation with C20:5ω3 (as in our study) were described [23]. BMI, waist circumference, RWH, body fat, and torso body fat only achieved multiple linear regressions in Pre-MW, which can be explained by the fact that Pre-MW are subject to high hormonal changes. Nonetheless, menopause is associated with changes in fat distribution and the highest abdominal fat [26], which were verified here by the increase of vAT adipocyte area in Post-MW. A correlation between the number of adipocytes and body fat has been described previously [27] however in our work, Pre-MW had the lowest vAT adipocyte area and highest vAT number of adipocytes. vAT adipocyte area was negatively associated with C18:0 in adipose tissue of overweight women [28], as in our study. Moreover, the sum of C14:0, C16:0, and C18:0 had positive associations with increased fat cell size and number [4]. Opposite to our findings, a negative correlation between adipocyte size and scAT ω3 and ω6 was reported in Pre-MW [4]. As described, C16:0, C17:0, and C18:0 were associated with hyperplasia nonetheless some SFA were also linked to lower adipocyte area, BMI, waist circumference, and RWH. These regressions agree with the induction of expression of miR-126b-5p associated with adipogenesis, and FA preadipocytes composition changes namely: C10:0, C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C16:1, C17:1, C18:1ω9*cis* and C18:3ω6 [10]. MUFA was linked to decreased visceral adiposity [7] and ω9 was associated with a lower number of adipocytes [4], which suggests a reduction in hyperplasia although MUFA only fitted in multiple linear regressions in Post-MW.

Cortisol and obesity seemed linked to fat accumulation by increasing the secretion of glucocorticoids [29] and chronic exposure to glucocorticoids induces lipogenesis, resulting in increased fat storage particularly, abdominal adiposity [30] and is associated with inflammatory activity [31]. Alterations in body fat distribution might be a consequence of SFA interaction in cortisol metabolism [7], as cortisol increases abdominal obesity [8]. This could explain, in our study, the positive correlation between SFA and cortisol in Pre-MW. Also, C12:0 and C14:0 increase in the breast milk of mothers with high cortisol [32]. Regarding long-chain PUFA, negative correlations were reported with cortisol (in breast milk) [33], concordant with our study, namely for: C18:2ω6*cis,* C18:3ω3, C20:3ω3, C20:4ω6, C20:5ω3, and C22:6ω3.

A negative correlation between C-reactive protein and PUFA specially ω3 has been described [34,35], which agrees with our findings however the multiple linear regression model was obtained only in Post-MW and did not include any ω3.

A study with ulcerative colitis patients showed the intake of olive oil decreases inflammatory markers including sedimentation velocity [36]. In our study, a negative correlation between C18:0 and sedimentary velocity was found.

Negative correlations between total cholesterol and SFA (in the blood) have been reported [37] specially with C16:0 (normal-weight population). However, in our study correlations were only obtained with C17:0 and C18:0 in Post-MW. There is controversy about the effects of MUFA on total cholesterol, HDL, and LDL [38]. In our study were obtained mostly negative correlations with MUFA in Pre-MW (concordant with the literature [37]) and positive correlations in Post-MW. Hormonal changes during menopause can alter the lipids metabolism [11] and lower total cholesterol and LDL are effects associated with menopause [19,26]. In our study, an increment of apolipoprotein B was not observed in Post-MW as described by others [19,26]. This apolipoprotein is the primary protein of LDL and the effects of replacing dietary SFA with MUFA on lipoprotein metabolism appear to be almost exclusively limited to LDL [39]. PUFA and MUFA consumption is described to promote triglycerides decrease, whereas SFA increases it [40]. In Pre-MW, the correlations found here with C14:0, C15:0, C16:0, C18:1ω9*cis,* C20:1ω9, C18:2ω6*cis*, C18:3ω3, C18:3ω6 and C20:2ω6 agree with that premise. However, the correlations with C10:0, C18:0, C16:1ω7, C20:3ω6, and C20:4ω6 do not. The multiple linear regression model revealed the role of scAT C16:1ω7 and C20:3ω6 in triglycerides in Pre-MW. In Post-MW, the correlations with C10:0, C18:3ω3, C20:3ω3, and C20:2ω6 agree with the literature whereas the correlation with C20:4ω6 does not. The highest albumin in Post-MW was found and also previously reported in the literature [18,19].

Free FA transferred from adipose tissue into the circulation in a high flow can result in diminished glucose clearance from blood, triggering hyperinsulinemia and β-cell dysfunction [41]. HbA1c, HOMA-IR, and HOMA2-B are usually correlated with BMI [42] and, in our work, total lipids were correlated to HbA1c and HOMA-IR in Pre-MW and to HOMA2-B and HOMA2-S in Post-MW. These results are corroborated by a study on Iraqis obese, where visceral adiposity was correlated with the risk of impaired glucose tolerance [43]. High free SFA release from adipose tissue may induce insulin resistance, namely C16:0 and C14:0 (non-obese population) [44]. Considering that the SFA release is proportional to SFA adipose tissue content, the correlations obtained here with SFA and glycaemia, HbA1c, HOMA-IR, and HOMA2-B for Post-MW are corroborated. In Pre-MW, however, opposite correlations were obtained with C10:0, C12:0, C18:0, and C20:0. Positive associations between HOMA-IR, C-peptide, and C16:0 (serum, pregnant women) were described [1] and a similar correlation was found in our study in Pre-MW. Other authors reported opposite correlations with C14:0 and C18:0 (serum) in healthy and gestational diabetic pregnant women [1] or in normal-weight individuals [45]. In an overweight pregnant women cohort, serum C18:1ω9*cis* had negative associations with HOMA-IR and C-peptide [1], corroborating our findings in Pre-MW. Positive associations between C16:1ω7 and C-peptide were described (in serum, normal weight individuals) [45], similar to ours in Pre-MW. However, negative associations between C16:1ω7 and HOMA-IR and C-peptide are found in the literature [1], opposite to our findings. Inverse associations between insulin resistance and ω3 have been described [46]. A negative association between the C18:3ω3 in scAT and HOMA-IR was described (both genders) [41], concordant with our findings in Post-MW. Moreover, a negative correlation between C-peptide and this PUFA was obtained in Pre-MW. Regarding C22:6ω3, a negative correlation with HOMA-IR was described (serum, women) [25] (similar to our study in Post-MW). However, a positive association between HOMA-IR and C22:6ω3 (serum, pregnant women) was reported [1]. A negative association between HOMA-IR and C18:2ω6*cis* was reported in erythrocytes (overweight cohort) [47], similar to our findings in Pre-MW and with glycaemia in Post-MW. Positive associations between C20:3ω6 and HOMA-IR and C-peptide were reported (normal weight population [45] and serum, pregnant women [1]), which are concordant with our findings. Additionally, we found positive associations with this PUFA and HOMA2-B and insulin. Furthermore, a negative correlation with C-peptide and C18:3ω3 was described (serum) [1], similar to ours in Pre-MW. The multiple linear regression models for HOMA2-B included distinct FA in each cohort. Insulin resistance was shown to increase with visceral adipocyte, as more free FA are released into the liver [11]. Additionally, the FA increase, lipoproteins, and lipids triggered by menopause can alter liver enzymes, which are also associated with increased glucose (as in our study), insulin resistance, and insulin (opposite to our study) [19–21,48]. Although Dehghan et al. [26] did not find differences in glycaemia or insulin levels between Pre-MW and Post-MW.

The lowering of estradiol and DHEA-S, coupled with elevated FSH and LH in Post-MW are well-documented [18,20,48,49], as is the observed low testosterone and high RTE in Post-MW [50]. This seems to imply that fat redistribution is not altered by testosterone itself but by estradiol. Studies suggest that high RTE may indicate dysregulated lipid metabolism and obesity in menopause [11]. The ratio cortisol/DHEA-S is used to determine how the hypothalamic-pituitary-adrenal axis is affected by chronic stress and linked to metabolic syndrome. During menopause this ratio increases due to DHEA-S decline and age-related increase of cortisol [49], contrary to our study. In Post-MW, a correlation between total lipids and FSH was observed. During the menopausal transition period, ovarian follicular functions reduce, and estrogens (estradiol and estrone) and progesterone become fluctuating and then diminish, whereas FSH increases [51]. Dietary SFA affects hormonal levels and expression of genes related to steroid metabolism [51], corroborating our findings of negative correlations in Pre-MW and positive correlations in Post-MW. The negative correlations in Pre-MW between MUFA and FSH, LH, and estradiol are concordant with a study showing loss of ovarian steroidogenesis, oocyte ability for fertilization, lowered ovulation rates, and diminished production of estradiol [52]. The menopause transition is associated with changes in fat metabolism and the association between MUFA and hormone levels is also altered in Post-MW [53]. To note, the ω3 effect on FSH is dependent on BMI [54]. In our study, positive correlations with C18:3ω6 and C20:5ω3 in Pre-MW and negative correlations with C18:3ω3, C20:2ω6, and C20:3ω3 in Post-MW were obtained with FSH. Estradiol could modulate the metabolism of PUFA in SH-SY5Y cells [55], which corroborates our findings in Pre-MW. The negative correlation found between FSH and PUFA in Post-MW might be a result of the anti-inflammatory properties of PUFA in opposition to FSH pro-inflammatory properties. Some PUFA can regulate the pituitary gonadotropin β-subunit expression (FSHβ and LHβ), in which high PUFA lowers Fshβ expression [56]. Multiple linear regression models were obtained only with negative β coefficients for Ln [estradiol] and Ln [Progesterone] in Pre-MW and for estradiol in Post-MW. The multiple linear regressions model for testosterone shows that while in Post-MW the scAT FA plays a greater role, in Pre-MW both tissues fitted the model. In Pre-MW, C10:0 influences both testosterone and DHEA-S levels. While in Post-MW, only scAT MUFA fitted DHEA-S, a multiple linear regression model.

Visceral obesity can cause an increase in the circulatory levels of T3, an adjustment to the increase in the energy outflow [57]. Decrease-free T4 and T3 synthesis is observed with ageing, while TSH levels stay unaltered [48], which corroborates our findings for T3 and TSH. Moreover, the prevalence of high levels of TSH increases with age, especially after menopause [58].

5. Conclusions

FA are essential in several metabolic processes, and their behavior changes with menopause. Understanding their pathways can help to protect women from menopause-related outsets, which was already amplified by obesity in this cohort. The fatty acid profile are distinct in scAT and vAT, and it changes after menopause. Overall, SFA, MUFA and the ratio ω6/ω3 were higher in Pre-MW, while PUFA were higher in Post-MW. A total of 816 individual correlations and 45 multiple linear regression models were achieved. Confirming the influence of adipose tissue FA profile on several metabolic processes. Furthermore, only 58 individual correlations were independent of the female's menopausal status, and 36 menopause-dependents correlations displayed opposite tendencies between the two cohorts, showing how menopause strongly alters the FA metabolism and their subsequent relationship with biochemical parameters.

The authors are aware of the limitations of this study as the non-existent nonobese controls, making the results less generalizable and the cross-sectional design employed did not permit the assessment of the temporal and potentially causal relation of variables. Nonetheless, most of the studies available focused on the general effects of FA groups, while in this study the effect of each specific FA was addressed. Our findings revealed further insights into the effect of FA on several biochemical parameters from a wide range of metabolic processes in obese women according to menopausal status. This study shows that specific FA in different types of adipose tissue behaves differently, therefore extrapolation must be made with caution. The assessment of FA in adipose tissue can be an important tool for health care professionals. As biomarkers, these can aid the assessment of metabolic dysfunction in obese females, improving the follow-up of obesity and post-menopause. Future research is needed to further explore each FA role in metabolic processes and how specific FA behaves in nonobese women.

Institutional review board statement

The study was conducted in accordance with the Declaration of Helsinki and approved by Ethics Committee of Hospital de São João (CE 146-09, approved in 2009).

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

CRediT authorship contribution statement

Sara Sousa: Conceptualization, Methodology, Validation, Investigation, Visualization, Formal analysis, Writing – original draft. **Diogo Pestana:** Writing – review & editing. **Gil Faria:** Resources, Writing – review & editing. **Cristina Delerue-Matos:** Resources, Writing – review & editing. **Conceição Calhau:** Writing – review & editing. **Valentina Fernandes Domingues:** Conceptualization, Validation, Supervision, 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.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.plefa.2023.102581.

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