SL NUTRITION AND METABOLISM Open Access

SCIENTIFIC LITERATURE

RESEARCH ARTICLE

Relation between Changes in Fatty Acid Composition and Release in Perivascular Adipose Tissue and Vascular Smooth Muscle Contractility

María E García[1*], Juan P Fariña[1*], Héctor del Zotto[1], Marina C González[2], Juan D Toledo[2], Alicia Jawerbaum[3], Carolina L Román[1], Mauricio Kraemer[1] and Juan J Gagliardino[1]

[1]Center for Experimental and Applied Endocrinology, School of Medical Sciences UNLP, Argentina
[2]Institute of Biochemical Investigations of La Plata, Faculty of Medical Sciences, Argentina
[3]Center for Pharmacological and Botanical Studies, School of Medicine, Argentina
*These authors have contributed equally to the study's implementation.

ARTICLE INFO

Article history:
Received: 15 January 2022
Accepted: 23 February 2022
Published: 26 February 2022

Keywords:
Aortic rings;
Vascular contractility;
Modulatory role of PVAT on smooth muscle contractility;
PVAT fatty acid composition

Citation this article: García ME, Fariña JP, Zotto HD, González MC, Toledo JD et al., Relation between Changes in Fatty Acid Composition and Release in Perivascular Adipose Tissue and Vascular Smooth Muscle Contractility. SL Nutr Metab. 2022; 2(1):117.

Correspondence:
María Elisa García,
Center for Experimental and Applied Endocrinology, School of Medical Sciences UNLP, 60 y 120 (1900) La Plata, Argentina,
Tel: 54 221 423 6712;
Fax: 54 221 422 2081;
Email: megmuabro@gmail.com

ABSTRACT

Aims: To identify gene enzyme profile of rat thoracic aorta Perivascular Adipose Tissue (PVAT) and correlate K+-induced contractile response of aortic rings (TAR) with changes in its fatty acid composition.

Methods: TAR from normal male rats were suspended in a thermostatted force transducer in KRB buffer and stimulated with K+80mM in the absence/presence of Meclofenamic Acid (MA), including: intact aortic rings (IAR), without PVAT (w/oPVAT), without endothelium (w/oE), and naked Vascular Smooth Muscle (VSM). PVAT pieces were incubated in KRB buffer with low and high K +; thereafter, lipids from PVAT and incubation medium were extracted to determine fatty acid composition(c-GLC) and release. PVAT UCP1/2, CIDEA, Wdnm1-like, TMEM26, and COX1/2 gene expression (qPCR) and K+ -induced thromboxane B2 release were measured.

Results: PVATgene expression profile was compatible with a combination of WAT and BAT characteristicstogether with beige cells. Contraction force and relaxation time (T1) of IAR and w/oE rings were significantly greater than those of w/oPVAT and VSM. Addition of PVAT pieces partially restored both values to those measured in IAR.High K+ significantly decreased and increased arachidonic acid content in PVAT and incubation medium, respectively. MA significantly modified only the T1 in IAR, increasing COX-1 ($p < 0.01$) but not COX-2 gene expression.

Conclusion: PVAT gene enzyme profile comprises WAT, BAT, and beige adipocyte types. PVAT enhanced high K+-induced contractility and T1 of VSM, effect associated with significant changes in its fatty acid composition/release, particularly arachidonic acid. Rest upon to demonstrate the true link between these two phenomena.

Introduction

Much evidence demonstrates that adipose tissue is far more than energy storage and thermoregulator: it is an active secretory organ of multiple mediators known as adipokines [1]. These adipokines include hormones, proinflammatory cytokines and other active metabolic and vascular mediators [2,3].

The thin-fat layeroutside vascular adventitia surrounding most systemic blood vessels -commonly referred to as Perivascular Adipose Tissue (PVAT)-possesses these properties [4]. Several reports have demonstrated that it may modulate vascular function through paracrine mechanisms not fully identified. In fact, PVAT is a source of relaxing factors such as hydrogen sulfide, adiponectin, and Adipose-Derived Relaxing Factor (ADRF) [5-9]; this anti-contractile activity is lost in obesity despite an associated increase in perivascular adipose tissue mass [10-14]. This fact suggests the existence of a counteracting vasoconstriction mechanism that might be activated when obesity is established.

Coincidentally, PVAT also promotes vasoconstriction in response to stimuli by producing compounds (the "perivascular adipocyte-derived constricting factor") whose activity is mediated through activation of tyrosine kinase and MAPK/ERK pathways [15]. Therefore, PVAT exerts a dual modulatory role in vessel function: it attenuates vasoconstriction to agonists by producing relaxing factors but also promotes constriction by producing the opposite compounds [16]. This issue was reviewed recently by Meyer MR et al. [17].

Striving to clarify some of these last issues, the present study was designed to test the possible correlation between the effect of PVAT on Vascular Smooth Muscle (VSM) contractile properties and simultaneous changes in its fatty acid composition under normal conditions.

Materials and Methods

1. Materials

organic and inorganic components for buffer preparations were purchased from Sigma Chemical Co. (St Louis, MO, USA). All solvents were HPLC-grade and provided by Carlo Erba (Milan, Italy). Lipid standards for c-gas liquid chromatography (c-GLC) were from Serdary Research Laboratories (London, Ontario, Canada). All of these were stored in appropriate solvent solutions in a nitrogen atmosphere at -20° C. Concentrations and purities were routinely checked by HPLC and/or c-GLC. Other chemicals used were reagent grade, obtained from local commercial sources.

2. Animals

we used normal male Wistar rats weighing 340-370 g, kept in a temperature-controlled environment (23°C) with a fixed 12hour light/dark cycle and fed a standard laboratory rat diet (Ganave, Argentina). The composition analysis of this diet, described previously [18], follows American Institute of Nutrition prescriptions [19].The animals were killed by anesthesia inhalation (Isofluorane, Piramal Critical Care, Inc., Bethlehem, Pennsylvania, USA.). All experimental procedures followed the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health [20] and were approved by the Animal Welfare Committee (CICUAL. Comité Institucional para el Cuidado y Uso de Animales de Laboratorio) of the La Plata University Medical School.

3. Enzyme characterization

3.1. RNA isolation: Total RNA was obtained from fat tissue from different locations such as perivascular (PVAT), abdominal (WAT), and interscapular region (BAT) by modification of the single-step acid guanidinium isothiocyanate-phenol-chloroform extraction method (Trizol; Invitrogen, Life Tech., USA) [21]. Concentration and purity of isolated RNA was determined by measuring the extinction coefficient at 260 and 280 nm and electrophoresis performed in denaturing conditions on 1% agarose gel.

3.2. Quantitative Real-Time PCR: 1µg of total RNA was used for reverse transcription with Super Script III Reverse Transcriptase (Invitrogen) and oligo-dT. Real-time PCRs were run in triplicate using Fast Start SYBR Green Master (Roche) in the iCycler 5 (BioRad). The cycling profile used was: 1 cycle of 1 minute at 95° C, 40 cycles of 30 seconds at 95° C, 30 seconds at 60° C and 30 seconds at 72° C followed by a melting curve from 55° to 90° C. Quantified values were normalized against the housekeeping gene β actin, using individual efficiency calculated with a standard curve for each gene.

4. Quantitative real-time PCR was performed using the primers listed in (Table 1)

4.1. Vascular response studies: after the animals'euthanasia, the thoracic aortas were quickly

removed, trimmed, and immersed in cold Krebs-Ringer-Bicarbonate (KRB) solution. The aortas were thereafter cut into 2-3 mm wide rings under a light magnifier and gently suspended between two stainless steel wires in a water-jacketed organ bath containing KRB solution continuously bubbled with a mixture of 5% CO2 and 95% O2, pH 7.4, and held at 37°C. The lower side was fixed to a vertical plastic rod immersed in the organ bath, while the upper side was rigidly attached to a force transducer (Letica TRI-201). Initially, arterial rings were stretched up to a force of 2g; thereafter, the rings were allowed to stabilize for 60 min and washed with fresh KRB every 20 min. VSM contraction was triggered in all these rings by 80 mM K+-induced depolarization for 20 min followed by relaxation induced by removal of the high K+ solution.

Signals for the force transducers were amplified and fed into an analog-digital board (DT16EZ, Data Translation, Inc. Marlboro, MA, USA). On-line recordings and files for subsequent processing were obtained with appropriate software (Labtech Note-book Pro, Laboratory Technology Corp., Wilmington, MA, USA).

To evaluate the potential modulatory role of adipose tissue we used the following protocol: a) intact aorta rings (IAR); b) aorta rings without the adherent PVAT (w/o/PVAT);c) aorta rings without endothelium (removed by carefully sliding the ring around a needle: w/o/E); and d) aorta rings with neither PVAT nor endothelium (VSM). To decrease data variability, in every experiment we simultaneously tested the contractile properties of the different rings (extracted from the same animal) under identical experimental conditions.

In another series of experiments and using the same protocol, PVAT from the aortic rings were removed and weighed, and thereafter the resulting VSM were incubated in the presence of different amounts of fresh minced non incubated thoracic aortic PVAT (33 and 66% of the total amount of PVAT removed).

Complementary, IAR were perifused with high K+ in the medium either with or without meclofenamic acid (MA), a cyclooxygenase inhibitor [16] in the perifusion media. In all cases, at the end of the incubation period, each ring was removed, the PVAT separated, and its dry weight recorded in order to express the contractile response in g/mg of VSM.

To measure relaxation properties we used the formula: y = A1*exp (-x/t1) + y0(OriginLab Program, Exponential decay 1), where T1= decay constant.

4.2. PVAT lipid composition analysis and in vitro fatty acid release: thin slices of isolated thoracic aorta PVAT were incubated in KRB with low and high K+ at 370 C, for 20 min. After incubation, tissue slices and incubation medium were processed independently by c-GLC to assess fatty acyl composition. Briefly, total lipids were extracted by the Folch method [22], and c-GLC of Fatty Acid Methyl Esters (FAME) was performed on extracts as previously reported [23], except that in this case we used a capillary column (Supelco, Avondale; PA, USA) mounted on a Hewlett Packard HP 6890 Series GC System Plus (Avondale, PA) equipped with a terminal computer integrator system. FAME were identified by comparison of individual relative retention times to authentic standards, and mass distribution was calculated electronically by quantification of peak areas.

4.3. Thromboxane release from isolated PVAT: to demonstrate the potential role of thromboxane in the mechanism by which PVAT modulates VSM contraction properties, we measured thromboxane A2 (TXA2) production in aliquots of PVAT pads incubated in Krebs medium with glucose 11mM and 4.7mM K+ (KRB medium) in the presence or absence of 1 μM MA. The incubation medium with or without MA was changed every 20 minutes for 1 h. Thereafter, we added: 1) only KRB medium; 2) KRB + MA; 3) KRB + 80 mM K+; and 4) KRB + 80 mM K+ + MA. Incubation was stopped after 20 minutes and an aliquot of incubation medium was held at -80°C for further measurement of TXB2 (a stable metabolite of TXA2)concentration. After lyophilization and appropriate dilution, TXB2 concentration was measured in the samples using the TXB2 EIA commercial assay kit following manufacturer's instructions (Cayman, MI, USA). The intra- and inter-assay variation coefficient was 15% and 12%, respectively. TXB2 content was

normalized to the amount of tissue in each incubation sample and expressed as pg of TXB2/mg of PVAT.

Table 1: Forward and reverse primer sequence for quantitative real-time PCR.

Primer Sequence(5' → 3')				
Gene	**Forward**	**Reverse**	**bp**	**GenBank**
UCP-1	CTGCCCTCCGAGCCAAGATG	CCAGCGGGAAGGTGATGATGTC	120	NM012682,2
UCP-2	GGCTGGCGGTGGTCGGAGATAC	CATTTCGGGCAACATTGGGAGAGG	112	AB010743,1
CIDEA	CAGCCTGCGGGAACTTATCA	TTGCCCGGTGTCCATFTCTG	176	NM001170467
Wdnm1-like	TGTCCCTCGTCACCCTCAG	GGCCCACAGTCCCAGTC	111	EF122001
TMEM26	GGGACGGCGGCAGACATACT	TCACTGAGGCGGGGCATACC	168	NM001107623
COX-1	GGTTGCTCCCGGGTCTGATG	AAGGATGAGGCGAGTGGTCTGG	131	NM017043
COX-2	CGGAGGAGAAGTGGGTFITAGGA	GTCTTGGTAGGCTGCGGGTCTTG	121	NM017232
B-actin	AGAGGGAAATCGTGCGTGAC	CGATAGTGATGACCTGACCGT	138	NM031144

Table 2: Contractile aortic ring response to different experimental conditions.

Aortic rings	**Maximum force (g/mg PVAT)**	**T1 (seconds)**
IAR	0,865 ± 0,11	694,6 ± 69,3
wo/E	0,869 ± 0,08	585,8 ± 103,6
wo/PVAT	0,495 ± 0,04†	144,9 ± 41,1*
VSM	0,443 ± 0,07†	203,9 ± 39,2*

Results are expressed as the mean ± SEM of 7 animals.

IAR vs. wo/PVAT and vs. VSM: * p <0.001; †p <0.01 (ANOVA one way and Dunnet 's multiple comparison test).

Table 3: Contractile aortic ring response to different amounts of PVAT.

				PVAT
	IAR	wo/PVAT	33%*	66%*
Max. Force (g/mg PVAT)	0,81 ± 0,08	0,53 ± 0,05*	0,69 ± 0,05	0,61 ± 0,05
T1 (seconds)	5,61 ± 0,24	4,55 ± 0,15†	4,70 ± 0,22†	4,70 ± 0,22†

Results are expressed as the mean ± SEM of 8 animals.

Max. force: IAR vs. wo/PVAT: * p<0,05 (ANOVA one way and Dunnett's test).

Tl: these values were converted to their natural logarithm to perform statistical analysis. IAR vs. wo/PVAT, 33% and 66%: †p< 0,02 (ANOVA one way and Kruskal-Wallis test).

*Represents the percentage of total PVAT removed from the IAR (see material and methods).

Table 4: Aortic rings response to Meclofenamic acid.

	IAR	**Meclofenamic acid 1µM**
Max. Force (g/mg PVAT)	0,850±0,01	0,872±0,06
T1 (seconds)	676,9±81,0	469,1±32,3*

Results are expressed as the mean ± SEM of 8 animals.

IAR vs. Meclofenamic acid: * p <0.05 (Student's t-test).

4.4. Statistical analysis: results are expressed as means ± SEM. Statistical data analysis used SPSS (Statistical Package for Social Sciences) version 15.0 for Windows (SPSS Inc, Chicago, IL, US). This analysis included one-way ANOVA followed by Dunnett´s, Bonferroni´s, or Student´s t-test to establish statistically significant differences between groups. We also used the non-parametric Kruskal-Wallis test when the pattern of data distribution was not normal. The test used is included in tables and figures. Results were expressed as means ± SEM; in all cases, differences were considered significant when P was < 0.05.

Results & Discussions

1. PVAT profile of different gene expressions

Analysis of mRNA from adipose tissue samples WAT, BAT, and PVAT of the thoracic aorta by qPCR, showed that UCP1 expression (characteristic of BAT tissue) was significantly higher in BAT and in PVAT than in WAT (Figure 1A). Conversely, UCP2 mRNA was similar in WAT and PVAT whereas it was significantly lower in BAT (Figure 1B).

CIDEA (a gene involved in up regulation of UCP1 expression and in activation of brown adipocytes) [24], it also showed uneven expression in the tissues studied: higher in BAT, lower in WAT, and intermediate expression in PVAT (Figure 1C). TMEM26 (a marker for beige adipocyte precursors *in vivo* and *in vitro*) [25] was expressed in all three tissues studied, showing comparable values in WAT and BAT but significantly lower expression in PVAT (Figure 1D). Wdnm1-like (a differentiation-dependent adipogenesis gene expressed mainly in white adipose depots) [26] was largely expressed in WAT, in smaller proportion in PVAT, and very little in BAT tissue (Figure 1E). These data suggest that in the thoracic aorta PVAT marker profiles share WAT and BAT tissue characteristics. It has a gene enzyme profile characteristic of WAT (UCP2, Wdnm1-like differentiation-dependent adipogenesis gene expressed 500 times more in white adipose depots than in brown- [26]) and BAT (UCP1 and CIDEA, involved in upregulation of UCP1 expression and in activation of brown adipocytes [24]) as described previously in rodents [27-29]. The presence of TMEM26 gene currently shown, a marker for beige adipocyte precursors in both in vivo and in vitro conditions [25], adds a new component of this tissue. These mixed gene profile of PVAT - shared by rodent and human thoracic aorta –demonstrates that it possess both energy storage and thermoregulatory properties [4,30]. Regarding the importance of the latter, Chang et al using a mouse model lacking PVAT demonstrated that intravascular temperature was regulated by PVAT [30]. It is also known that a blood temperature gradient exists in humans, vasculature closest to the heart having the highest temperatures; therefore, PVAT thermogenesis could help to maintain the normal blood temperature gradient [27].

2. Different profile and magnitude of aortic ring response to K+ stimulus

After the equilibrating period (3 cycles of 20 min each), 80 mM K+ stimulus induced a contraction in IAR followed by a relaxation period when K+ concentration in the bath returned to 5 mM (Figure 2a). The height of the wave represents the maximum force developed by the ring under stimulus, and the time needed to return to basal tension corresponds to the relaxation period (T1).

Removal of PVAT significantly decreased both the maximal force contraction and T1 values (Figure 2b), whereas removal of the endothelium induced little change in these two parameters (Figure 2c). Finally, the profile and magnitude of high K+-induced response in VSM closely resembles that recorded after removal of PVAT alone (Figure 2d). Absolute values for maximal force and T1 for each experimental condition are shown in (Table 2).

3. Effect of addition of minced PVAT to incubation medium on SMVT response

Addition of different amounts of minced isolated PVAT to fat-deprived rings (w/o PVAT) tended to restore contraction force and T1 values to those measured in IAR (Table 3).

The fact that removal of PVAT significantly decreased both contractile force and relaxation period and addition of PVAT pieces to perfused isolated aortic VSM partially restored, though not in a dose-response

manner, its contractile response to K+, reinforces the assumption that some PVAT compound/s mainly modulate this response.

4. Effect of MA on K+-induced IAR contractility

Addition of MA to the perifusion medium only affect K+-induced T1 values without affecting significantly the maximum force response (Table 4)

Changes induced by K+ on PVAT fatty acid composition and release

The FA composition of total lipids extracted from PVAT and incubation medium was significantly affected by 80 mM K+ stimulus, as was their release pattern into the incubation medium (Table 5). Although in both cases these changes selectively affected the concentration of some unsaturated fatty acids, the one affecting arachidonic acid (20:4 n-6) was the largest and most consistent: it decreased its concentration at tissue level with a concomitant increase in the incubation media.

Table 5: Fatty acid composition of total lipids of PVAT and of the incubation medium.

	PVAT		Incubation Medium	
FA	IAR (baseline)	K+ 80mM	IAR (baseline)	K+ 80mM
14:0	0,90 ± 0,10	0,90 ± 0,05	2,90 ± 0,10	0,50 ± 0,01*
16:0	29,2 ± 1,70	34,6 ± 1,1	28,1 ± 1,90	36,8 ± 1,40§
16:1	1,00 ± 0,10	2,30 ± 0,10*	5,00 ± 0,20	1,30 ± 0,10*
18:0	10,1 ± 0,30	15,3 ± 030*	21,0 ± 1,10	35,5 ± 1,20*
18:1	37,7 ± 1,60	32,1 ± 1,20	19,8 ± 1,40	7,10 ± 1,20*
18:2n6	13,6 ± 0,40	9,00 ± 0,10*	11,3 ± 0,20	3,00 ± 0,10*
18:3n3	0,10 ± 0,03	0,10 ± 0,02	0,10 ± 0,02	0,10 ± 0,03
20:3n6	0,20 ± 0,04	0,20 ± 0,01	1,40 ± 0,10	1,20 ± 0,10
20:4n6	**7,70 ± 0,40**	**5,1 ± 0,20†**	**5,80 ± 0,10**	**10,3 ± 0,10***
22:5n6	0,20 ± 0,01	0,20 ± 0,04	0,20 ± 0,04	0,20 ± 0,10
22:5n3	0,10 ± 0,02	0,10 ± 0,03	1,3 ± 0,10	1,00 ± 0,20
22:6n3	0,20 ± 0,03	0,20 ± 0,02	3,10 ± 0,10	3,00 ± 0,10
∑Sat/∑Mono	**1,07 ± 0,11**	**1,48 ± 0,10‡**	**2,10 ± 0,26**	**8,99 ± 1,77§**
∑Pura	22,1 ± 0,93	14,9 ± 0,42*	23,2 ± 0,64	18,8 ± 0,73†
∑Sat/∑Pufa	**1,82 ± 0,17**	**3,41 ± 0,19***	**2,24 ± 0,19**	**3,87 ± 0,29†**

The values were represented the mean ± SEM; n = 4 different experiments.
FA are expressed as mol%. Sat saturated; Mono: monounsaturated; PUFA: Polyunsaturated Fatty Acids. IAR vs. K+80mM *p< 0,001; † 0,005; ‡ 0,025; § 0,01; 1 0,05 (Student's t-test).

These changes were associated with a significant modification of the saturated/ Mono and PUFA ratio at both tissue and incubation media levels. In both cases K+ stimulus induced a significant switch towards the saturated fatty acid fraction.

In our model, K+ stimulus simultaneously induces a rapid and significant change in PVAT fatty acid composition and release as well as the saturated/unsaturated ratio towards the former. The exact meaning of this change is not yet clear, and merits further investigation. Because dietary-induced change in PVAT fatty acid composition significantly affects aortic ring contractility, we should in the meantime consider that this composition is dynamic rather that static, and it is significantly modified under acute circumstances –such as the high K+stimulus in this study- but also as an adaptative reaction to dietary changes [17]. These evidences suggest that these changes might play an important modulatory role on VSM contractile properties.

5. Effect of Cyclooxygenase inhibitor on gene expression and contractile properties of aorta rings

In incubated PVAT tissue pads, high K+ affected neither COX-1 nor COX-2 mRNA level (Figure 3). Addition of MA to the perfusion media, anon-specific inhibitor of both cyclooxygenase pathways of arachidonic acid metabolism [27], significantly enhanced both basal and K+-stimulated COX-1 mRNA level to similar values (Figure 3a). Conversely, COX-2 mRNA concentration was affected by neither high K+nor MA treatment (Figure 3b).

6. Tromboxane B2 release by PVAT

To assess the possible role of thromboxane production/release in the mechanism by which PVAT mediates its enhancing effect on vascular smooth muscle, we measured K+-stimulated thromboxane B2 release by isolated PVAT in the presence/absence of MA. High K+ stimulus decreasedTxB2 basal release though not significantly; MA significantly decreased this release under control conditions, but enhanced TxB2 release in the presence of high K+ (Table 6).

Concerning the importance of PVAT metabolism in vascular contractility regulation, Meyer et al. [16]

reported that in obese mice, aortic PVAT produces an Adipose-Derived Contracting Factor (ADCF) that potentiates vascular contractile response, an activity fully prevented by Cyclooxygenase (COX) inhibition [16,31]. In view of these data and considering the close association between aortic ring contractility and arachidonic release just described, we assume that ADCF could arguably form a link between these two phenomena. In our model, however, COX1 and 2 inhibitions only partially affected both aortic ring contractility and tromboxane B2 release from PVAT. Therefore, we might accept that in our experimental conditions COX metabolites were not the main mediator of PVAT on smooth muscle contractility. In this regard, we could assume that the previously reported peptide chemerin could play such role in our model through a receptor commonly described to function in immune cells [32]. At this time we cannot discard that arachidonic acid itself might have this modulatory role. Experiments are in progress to test these possibilities.

It is not clear whether this PVAT modulation on aortic VSM could play any role in blood pressure control. But since the aortic is an elastic artery that directs and controls systemic blood distribution, alteration of its contractile properties could affect its wall stress and thereafter blood availability to certain body sector/s [33].

Table 6: Thromboxane B2 released by PVAT into the incubation medium.

IAR (baseline)	IAR High K^+	IAR + MA	IAR High K^++MA
8,48 ± 2,54	5,72 ± 1,41	1,22 ± 073*	8,87 ± 0,27†

Results are expressed in pg / mg tissue and as the mean ± SEM of 16 animals.
IAR *vs.* Meclofenamic acid * p <0.01; High K^+ vs. High K^++MA†p< 0,05 (ANOVA one way and Bonferroni's multiple comparison test).

In brief, we have demonstrated that PVAT in thoracic aorta exerts a physiological role as an active modulator of both contractility components: maximum force and relaxation period. This effect was independent of endothelial presence/activity as shown by the scanty effect induced by its removal. Conversely, changes in contractility properties were closely associated with PVAT fatty acid composition and release, mainly affecting arachidonic acid; however in our experimental conditions, blocking its enzymatic processing by COX only partially affected smooth muscle contractility. Therefore, it remains for us to demonstrate the main mediator and intrinsic mechanism by which PVAT metabolism enhances aortic VSM contractility.

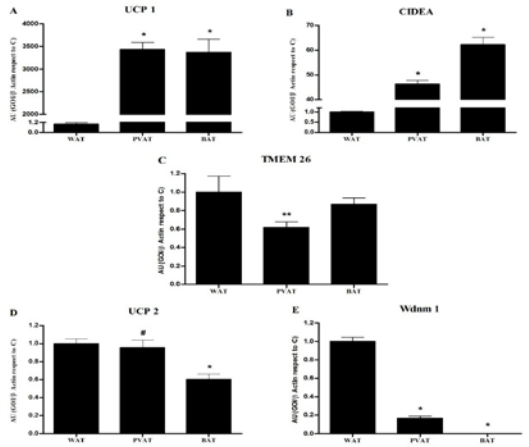

Figure 1: PVAT profile of different gene expressions.

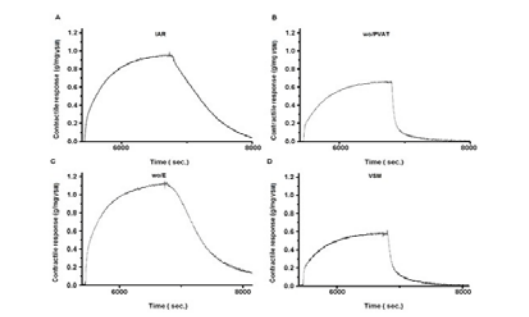

Figure 2: Different profile and magnitude of aortic ring response.

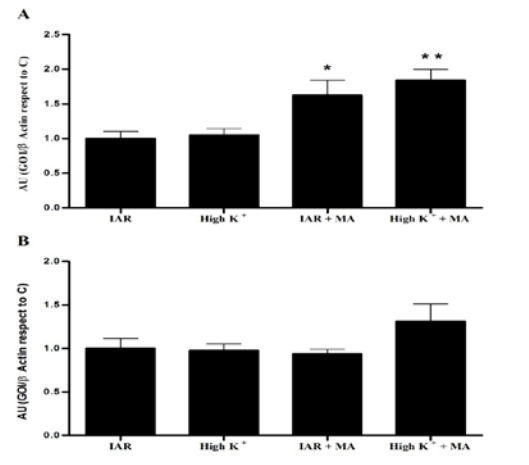

Figure 3: Effect of ciclooxigenase inhibitor on gene expression and contractile properties of aorta rings.

Conclusions

PVAT in the thoracic aorta has a gene enzyme profile that comprises WAT, BAT, and beige adipocyte types, thus possessing both energy storage and thermoregulatory properties. The close association of PVAT fatty acid composition and release in thoracic aorta rings and changes occurring in its VSM contractile properties strongly suggest that the former actively modulates the latter. A compound derivative of arachidonic acid processed by COX would not be the main mediator of this effect, which remains to be identified. Altogether, our data could open new avenues to identify the main contractility enhancer of VSM, and thereafter to develop potential therapeutic approaches to control aortic vascular contractility by modifying PVAT fatty acid composition/release.

References

1. Mohamed-Ali V, PinkeyJH, CoppakSW. (1998). Adipose tissue as an endocrine and paracrine organ. lntJ ObesRelatMetabDisorders. 22: 1145-1158.
2. Trayhum P, Wood IS. (2004). Adipokines: inflammation and the pleiotropic role of white adipose tissue. Br J Nutr. 92: 347-355.
3. Wozniak SE, Gee LL, Watche MS, Frezza EE. (2009). Adipose tissue: the new endocrine organ? A review article. Dig Dis Sci 54: 1847-1856.
4. Brown NK, Zhou Z, Zhang J, Zeng R, Wu J, et al. (2014). Perivascular adipose tissue in vascular function and disease: a review of current research and animal models. Arterioscler Thromb Vasc Biol. 34: 21-1630.
5. Ouwens DM, Sell H, Greulich S, Ecke J. (2010). The role of epicardial and perivascular adipose tissue in the pathophysiology of cardiovascular disease. J Cell Mol Med. 14: 2223-2234.
6. Maenhaut N,Van de Voorde J. (2011). Regulation of vascular tone by adipocytes. BMC Med. 9: 25-36.
7. Gollash M. (2012). Vasodilator signals from perivascular adipose tissue. Br J Pharmacol. 165: 633-642.
8. Eringa EC, Bakker W, van Hinsbergh VW. (2012). Paracrine regulation of vascular tone, inflammation and insulin sensitivity by perivascular adipose tissue. Vascul Pharmacol. 56: 204-209.
9. GaoYJ, Zeng ZH, Teoh K, Sharma AM, Abouzahr L,et al. (2005). Perivascular adipose tissue modulates vascular function in the human internal thoracic artery. J Thorac Cardiovasc Surg. 130: 1130-1136.
10. Soltis EE, Cassis LA. (1991). Influence of perivascular adipose tissue on rat aortic smooth muscle responsiveness. Clin Exp Hypertens A. 13: 277-296.
11. Löhn M, Dubrovska G, Lauterbach B, Luft FC, GollashMet al. (2002). Periadventitial fat releases a vascular relaxing factor. FASEB J. 16: 1057-1063.
12. Fésüs G, Dubrovska G, Gorzelniak K, Kluge R, Huang Y et al. (2007). Adiponectin is a novel humoral vasodilator. Cardiovasc Res. 75: 719-727.
13. Greenstein AS, Khavandi K, Withers SB, Sonoyama K, Clancy O et al. (2009). Local inflammation and hypoxia abolish the protective anticontractile properties of perivascular fat in obese patients. Circulation 119: 1661-1670.
14. Ma L, Ma S, He H, Yang D, Chen X, et al. (2010). Perivascular fat-mediated vascular dysfunction and remodeling through the AMPK/mTOR pathway in high-fat diet-induced obese rats. Hypertens Res. 33: 446-453.
15. Gao YJ. (2007). Dual modulation of vascular function by perivascular adipose tissue and its potential correlation with adiposity/lipoatrophy-related vascular dysfunction. Curr Pharm Des 13: 2185-2192.
16. Meyer MR, Fredette NC, Barton M,Prossnitz ER. (2013). Regulation of vascular smooth muscle tone by adipose-derived contracting factor. PLOS one. 8:e79245.
17. Rebolledo A, Rebolledo OR, Marra CA, García ME, Roldán Palomo AR, et al.(2010). Early alterations in vascular contractility associated to changes in fatty acid composition and oxidative stress markers in perivascular adipose tissue. Cardiovasc Diabetol 9: 65-73.

18. Marra CA, Alaniz MJT de. (1999). Acyl-CoA synthetase activity in liver microsomes from calcium-deficient rats. Lipids 34: 343-354.

19. Reeves PG, Nielsen FH,FahleyJr GC. (1993). AIN-93 Purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr 123: 1939-1951.

20. Sue Machado. (1996). Revised guide for the care and use of laboratory animals. National Institutes of Health.

21. Chomczynski P, Sacchi N. (1987). Single-step method of RNA isolation by acid guanidiniumisothiocyanate-phenol-chloroform extraction. Analytical Biochemistry 162: 156-159.

22. Folch J, Lees M, Sloane Stanley GH. (1957). A simple method for the isolation and purification of total lipids from animal tissues. J BiolChem 226: 479-509.

23. MarraCA, AlanizMJ de. (1992). Incorporation and metabolic conversion of saturated and unsaturated fatty acids in SK-Hep1 human hepatoma cells in culture. MolCell Biochem 117: 107-118.

24. Seale P, Kajimura S, Yang W, Chin S, Rohas LM, et al. (2007). Transcriptional control of brown fat determination by PRDM16. Cell Metab. 6: 38–54.

25. Lee MW, Odegaard JI, Mukundan L, Qiu Y, Molofsky AB, et al. (2015). Activated type 2 innate lymphoid cells regulate beige fat biogenesis. Cell 160: 74-87.

26. Wu Y, Smas CM. (2008). Wdnm1-like, a new adipokine with a role in MMP-2 activation. Am J Physiol Endocrinol Metab. 295: E205-215.

27. Robinson S. (1952). Physiological effects of heat and cold. Annu Rev Physiol 14: 73-96.

28. Fitzgibbons TP, Kogan S, Aouadi M, Hendricks GM, Straubhaar J, et al. (2011). Similarity of mouse perivascular and brown adipose tissues and their resistance to diet-induced inflammation.Am J Physiol Heart CircPhysiol 301: H1425–1437.

29. Padilla J, Jenkins NT, Vieira-Potter VJ, Laughlin MH. (2013). Divergent phenotype of rat thoracic and abdominal perivascular adipose tissues. Am J PhysiolRegulIntegr Comp Physiol 304: R543-552.

30. Chang L, Villacorta L, Li R, Hamblin M, Xu W, et al. (2012). Loss of perivascular adipose tissue on peroxisome proliferator- activated receptor- deletion in smooth muscle cells impairs intravascular thermoregulation and enhances atherosclerosis. Circulation 126: 1067-1078.

31. Stadler I, Kapui Z, Ambrus JL. (1994). Study of the mechanisms of action of sodium meclofenamic acid (Meclomen) a “double inhibitor” of the arachidonic acid cascade. J Med. 25: 371-382.

32. Watts SW, Dorrance AM, Penfold ME, Rourke JL, Sinal CJ, et al. (2013). Chemerin connects fat to arterial contraction. Arterioscler Thromb VascBiol 33: 1320–1328.

33. Murtada SI, Lewin S, Arner A, Humphrey JD. (2016). Adaptation of active tone in the mouse descending thoracic aorta under acute changes in loading. Biomech Model Mechanobiol. 15: 579-592.