Sex-dependent effects of forced exercise in the body composition of adolescent rats

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Aims

In this study [1], by using computerized tomography, we first aimed to define the baseline of the weight and body composition during adolescence (P30-60) and young adulthood (P60-90) of female and male Sprague-Dawley rats under standard laboratory chow diet. Then, during the early adolescence (P27-44), we determined the effect of a forced wheel training program on the weight and body composition.

Method

Animals and housing

The study was carried out in compliance with the ARRIVE and FORCED [2] guidelines. Thirty-six, 20-days-old Sprague-Dawley rats provided by the Animal Facilities of the University of Murcia were used in the experiment. From postnatal day 26, female and male animals of the experimental groups were exposed to an 8-days exercise habituation protocol in a forced running wheel system (Campden Instruments, 80805A) as in previous works (Fig. 1) [3, 4, 5]. The training program consisted of two sessions per day, on ZT14 and ZT20. The speed and/or total duration of the training are described in Fig. 1 B.

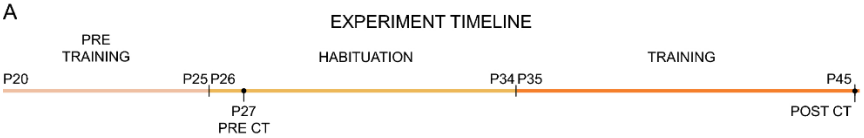
Computerized tomography

On postnatal day 30, 60 and 90 of the FB and MB groups and postnatal day 27 and 45 of the FT, FC, MT and MC groups, the rats were screened using an Albira trimodal preclinical-scanner and software (Bruker®, Billerica, MA, USA). The images were spatially reconstructed through the filtered back projection algorithm (Bruker, Albira reconstruction software).

Image analysis

The 3D image segmentation was performed using the pMod version 3.5. The thoracoabdominal (TA) region was analyzed and defined as the contents from the intervertebral space C7-T1 (cervical and thoracic) to the intervertebral space between S2-S3 (sacral) (Fig. 2 A-B). To split the visceral adipose tissue (VAT) from the subcutaneous (SAT), a contour was drawn manually with the software. Based on calibration standards that were validated previously in data from humans and rodents [6, 7], using the “segmentation” functionality of the software, three ranges of signal were obtained and considered in terms of adipose (-200 to -31 HU), lean (-30 HU to 189 HU) or bone tissue (≥190 HU). 3D images (Sup. 6) were created by loading the CT raw images in VolView 3.4 (Kitware). 3D videos (Vids. 1-14) were created by using VolView.

A



B

	PRE-TRAINING					
Day	1	2	3	4	5	6
Rat age (PND)	P20	P21	P22	P23	P24	P25
ZT14 (AM)	Caging		Handling			

	HABITUATION								
Day	7	8	9	10	11	12	13	14	15
Rat age (PND)	P26	P27	P28	P29	P30	P31	P32	P33	P34
ZT14 Speed (m/min)	0	0 |5	0 |7.2	7.2	8.1	8.1	9	9	Recovery
(AM) Duration (min)	5'	5'|2'	5'|5'	10'	2x10'/5'	3x10'/5'	2x10'/5'	3x10'/5'	
ZT20 Speed (m/min)	0	PRE CT	0	0	0	0	9	9	Recovery
(PM) Duration (min)	0'		0'	0'	0'	0'	2x10'/5'	3x10'/5'	

	TRAINING									
Day	16	17	18	19	20	21	22	23	24	25
Rat age (PND)	P35	P36	P37	P38	P39	P40	P41	P42	P43	P44
ZT14 Speed (m/min)	9	9.9	9.9	11.7	11.7	14.4	14.4	17.1	17.1	20.7
(AM) Duration (min)	6x10'/5'	4x10'/5'	6x10'/5'	4x10'/5'	6x10'/5'	4x10'/5'	6x10'/5'	4x10'/5'	6x10'/5'	4x10'/5'
ZT20 Speed (m/min)	9	9.9	9.9	11.7	11.7	14.4	14.4	17.1	17.1	20.7
(PM) Duration (min)	6x10'/5'	5x10'/5'	6x10'/5'	5x10'/5'	6x10'/5'	5x10'/5'	6x10'/5'	5x10'/5'	6x10'/5'	5x10'/5'

	POST-TRAINING
Day	26
Rat age (PND)	P45
ZT14 (AM)	Recovery
ZT20 (PM)	POST CT

C

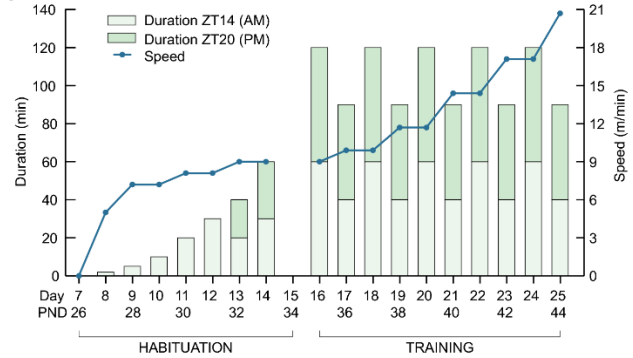


Fig. 1. Forced wheel exercise schedule and protocol. A) Experiment timeline. **B)** Forced exercise schedule and protocol. The training program was divided in 4 steps, namely Pre-training, Habituation, Training and Post-training. The duration of the exercise was split in bouts of 10 minutes, with a resting period of 5 minutes between bouts. **C)** Representation of the duration (bars) and speed (line) of the training program. Figure from Kutsenko et al., 2021 [1].

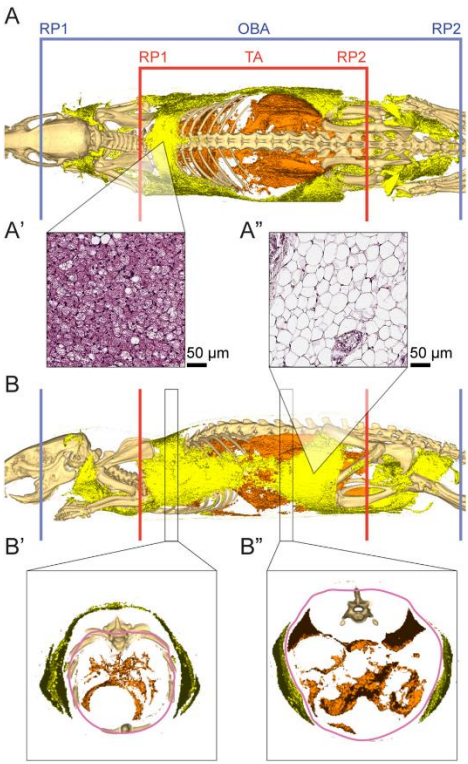
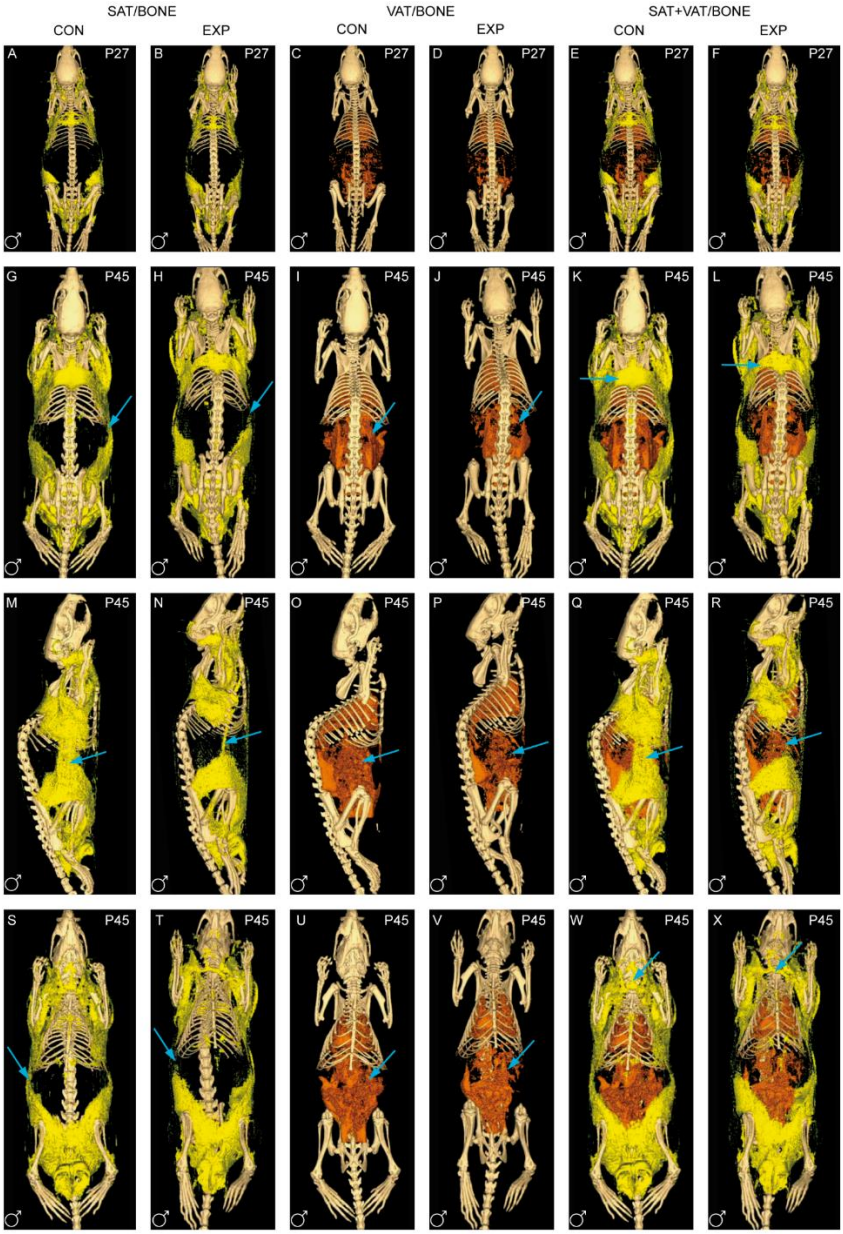


Fig. 2. Reference points of the image analysis. A: Dorsal view of a scanned rat. The thoracoabdominal region was comprised by the reference points TA RP1 and TA RP2 (**red lines**). The OBA region is delimited between the posterior boundary of the olfactory bulb and the anus, by the reference points OBA RP1 and OBA RP2 (**blue lines**) The VAT is represented in orange, and the SAT in yellow. **A' and A") ** Histological view of BAT (A') and WAT (A"). **B)** Lateral view of a scanned rat. **B' and B") ** Transversal view of two 3.7mm-sections from B. The manual contour (pink) was drawn inside the muscle/bone plane of the thoracoabdominal wall. Figure from Kutsenko et al., 2021 [1].

Results

A group of female and male SD rats was analyzed on P30, P60 and P90 to define a baseline of body weight and body composition. These key ages defined two life stages, namely adolescence (P30-60) and young adulthood (P60-90). Next, we determined the effect of a forced wheel training during the early adolescence period of female and male rats. Baseline female and male SD rats experimented the highest increment in weight, volume adipose tissue content during adolescence. On the other hand, similar increments between adolescence and young adulthood were observed in the LEAN% and BONE%. Forced running during the early adolescence decreased the weight, TA volume and body fat in male (Sup. 6) but not female SD rats. On the contrary, forced running increased LEAN% in both males and females.



Sup. 6. Reconstruction of tomography images in 3D of the male control and experimental rats. A-X) subcutaneous (SAT, yellow), visceral (VAT, orange), and total (SAT and VAT) adipose tissue contents of male rats. Blue arrows highlight visible differences between control

and experimental rats. The blue arrows draw the attention to areas of interest that are visually different between control and experimental rats. Figure from Kutsenko et al., 2021 [1].

Conclusion

In summary, our data indicate that the most drastic changes in the body composition of SD rats occur during the adolescence when compared with the young adulthood, and that adolescent male rats showed higher susceptibility to body weight and body composition changes than females when subjected to forced wheel exercise. Also, this susceptibility seems to occur mainly in the adolescence. Computerized tomography is revealed as an appropriate in-vivo method of body composition quantification that enables to perform a precise analysis of the tissue distribution across the body throughout the rodents' life. This method can be of especial relevance in the field of physical exercise science to further unravel the impact of exercise on the body composition, and potentially open new windows in the field of obesity research. Future studies should analyze the molecular mechanisms which mediate the decrease of adipose tissue under physical activity in males but not females during the early adolescence.

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