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Hyperinsulinaemia-induced structural remodeling of visceral and subcutaneous white adipose depots in rats

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Insulin has a central role in the regulation of anabolic responses in fat through the stimulation of glucose and free fatty acids uptake, inhibition of lipolysis and stimulation of *de novo* fatty acids synthesis in adipocytes. In addition, insulin regulates adipose tissue growth and differentiation. Recent studies demonstrated morphological and functional differences among visceral and subcutaneous fat, including differences in the responsiveness to insulin [1]. To investigate effects of hyperinsulinaemia on different white adipose tissue (WAT) depots, male Wistar rats were treated acutely (1 day) or chronically (3 days) with low (0.4 IU/kg bm) or high (4 IU/kg bm) dose of insulin. Saline-treated animals served as controls. The portions of retroperitoneal, epididymal and subcutaneous WAT (rWAT, eWAT, sWAT, respectively) were removed and prepared for the microscopic examinations. In order to determine the ratio of WAT components (adipocytes, blood vessels, stroma) in the overall tissue volume, stereological analyses of their volume densities were performed (Vv_{AC}, Vv_{BV}, Vv_{ST}, respectively). Also, the number of adipocytes per 10^6 μm^3 of WAT was counted and their mean volume was estimated. To reveal differences among WAT depots in the insulin-induced activation of its receptor, immunofluorescence detection of phosphorylated insulin receptor (IR-p) was performed. Morphometric and stereological analyzes revealed differences among sWAT and visceral WAT depots in their response to hyperinsulinaemia since the most pronounced structural changes were demonstrated in rWAT, especially after acute insulin treatments which lead to the increase of Vv_{BV} followed by the increase of adipocyte number per the volume unit of WAT and the decrease of their mean size. The microscopic analysis confirmed this finding, demonstrating higher abundance of preadipocytes in these groups. Chronic insulin treatments return adipocyte number and size to the control level probably through the lipogenic mechanisms and the maturation of preadipocytes. The most prominent effect in rWAT of the animals which were treated chronically with insulin (especially in the group treated with the lower dose) was the increase of Vv_{BV}. In eWAT significant increase of Vv_{BV} occurs only in the group treated acutely with the lower dose of insulin and is followed by the decrease of Vv_{AC}, since no signs of hypertrophy and hyperplasia of adipocytes are visible (no changes in their number and size, no microscopic evidence of higher abundance of preadipocytes). On contrary, in sWAT no significant changes in Vv_{BV} were demonstrated in the hyperinsulinaemic groups. Acute insulin treatments lead to the increase of Vv_{AC}, but since no changes in their number and size are visible, this could be a consequence of Vv_{ST} decrease in these groups. Immunofluorescence detection of IR-p revealed its weakest immunoexpression in sWAT in comparison with the visceral depots. This is in line with the absence of the hyperinsulinaemia-induced structural alterations in this depot.

Hyperinsulinaemia-induced structural remodeling and higher responsiveness to insulin in visceral WAT depots, especially in rWAT, may contribute to the visceral obesity associated with metabolic and cardiovascular risks.

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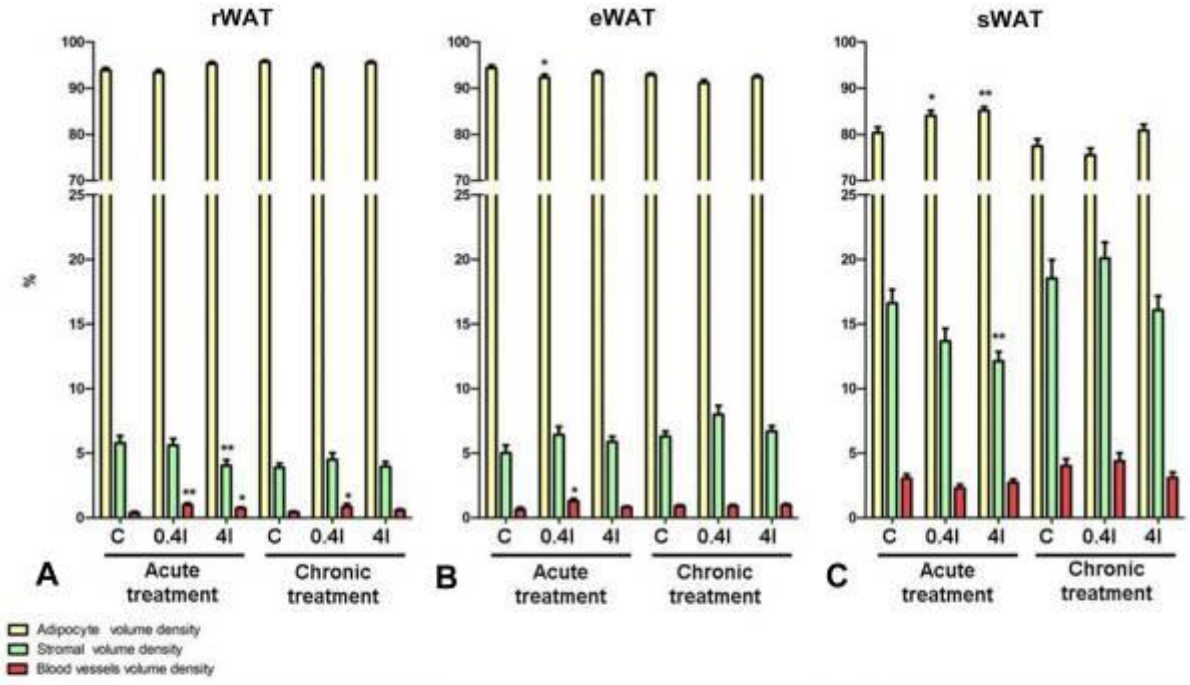
2. Laviola, L., et al., *Insulin signaling in human visceral and subcutaneous adipose tissue in vivo.* Diabetes, 2006. 55(4): p. 952-61.



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	rWAT						eWAT						sWAT					
	Acute treatment			Chronic treatment			Acute treatment			Chronic treatment			Acute treatment			Chronic treatment		
	C	0.4l	4l	C	0.4l	4l	C	0.4l	4l	C	0.4l	4l	C	0.4l	4l	C	0.4l	4l
Adipocytes number per 10^6 µm^3	4.08	5.98**	5.87**	4.31	4.55	4.47	9.34	10.62	8.76	6.65	7.18	8.31*	14.05	13.16	14.44	19.25	20.32	15.7
±SEM	0.19	0.28	0.37	0.14	0.29	0.40	0.44	0.58	0.35	0.21	0.45	0.53	0.98	0.53	0.75	1.40	1.64	1.13
Mean adipocyte volume (µm^3)	266165	184897***	213351**	250232	270786	282362	115563	116133	129063	165679	183806	160747	89818	84375	80572	80405	67323	84313*
±SEM	13160	8003	11997	9036	15988	15312	4578	5249	3417	6455	9870	7032	4839	3342	3970	4587	6453	6868

Figure 1. Volume density of adipocytes, stroma and blood vessels in (A) retroperitoneal (rWAT), (B) epididymal (eWAT) and (C) subcutaneous white adipose tissue depots. (D) Adipocytes number per unit volume (10^6 µm^3) of three WAT depots and mean volume of adipocytes (µm^3).