

Supplementary material

Table S1. Growth rate, viability and size of cell cultures. Growth rate was determined as days from passage to confluent, upon passage from P(n) to P(n+1). Viability and live cell sizes were measured on an Automated Cell Counter upon passage from P(n-1) to P(n). P-values were calculated by student T-test (2-tail, homoscedastic).

Growth rate (days)	P-value		Viability (%)	P-value		Live cell size (µm)	P-value		
	Lean	Obese		Lean	Obese		Lean	Obese	
P1	8	3	P1	93.0	66.5	P1	16.97	11.05	
	7	-		86.5	80.5		18.79	13.01	
	4	4		84.0	90.5		20.20	15.26	
Average	6.33	3.5	0.174	87.8	79.2	0.310	18.65	13.10	0.022
P2	7	5	P2	93.0	92.5	P2	16.05	19.84	
	10	-		91.5	-		17.04	-	
	4	7		87.5	93.0		23.01	19.26	
Average	7	6	0.700	90.7	92.8	0.400	18.70	19.55	0.782
P3	5	7	P3	91.0	82.5	P3	17.51	15.93	
	7	-		88.0	-		19.10	-	
	5	5		92.5	91.0		19.37	17.37	
Average	5.67	6	0.789	90.5	86.8	0.374	18.66	16.65	0.117

Table S2. Primers used for gene expression profiling. Ampliqon size in bp.

Gene name	Forward primer	Reverse primer	Ampliqon size
<i>ADIPOQ</i>	CGAGAAGGGTGAGAAAGGAG	TAGGCGCTTTCTCCAGGT	123
<i>LPL</i>	CCCTGGCTTTGCTATTGAGA	ACTTGTCGTGGCATTTCACA	139
<i>PPARG</i>	GCCGTGTCTGTGGGGATAAA	CCGACAGTTAAGATCGCACCT	128
<i>KLB</i>	TGGTTCACAGACAGTCACGT	TGCCATTCAAAGCCATCCAG	152
<i>HPRT1</i>	GGACTTGAATCATGTTTGTG	CAGATGTTTCCAAACTCAAC	91
<i>YWHAZ</i>	TGATGATAAGAAAGGGATTGTGG	GTTCAGCAATGGCTTCATCA	203

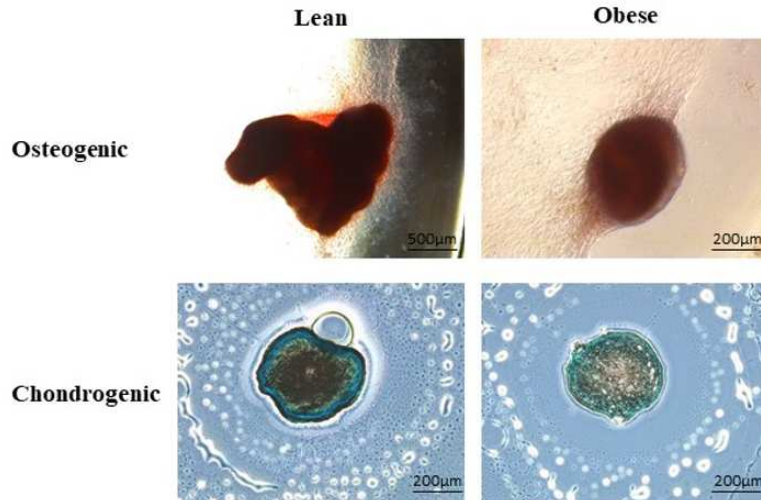


Figure S1. Representative images of osteogenic and chondrogenic differentiation in lean and obese cultures. Osteogenic cultures stained with Alizarin S Red. Osteogenic, lean: 4x magnification, bar indicate 500µm. Osteogenic, obese: 10x magnification, bar indicate 200µm. Chondrogenic cultures stained with Alcian Blue: 10x magnification, bars indicate 200µm.

Supplementary text. Differentiation media:

Adipogenic media: DMEM/F-12 (Sigma-Aldrich) +10% FBS (Gibco) + 1% Penicillin-Streptomycin (Thermo Fisher Scientific), 1 μ M dexamethasone (Sigma-Aldrich), 0.5 μ M 3-isobutyl-1-methylxanthine (IBMX)(Sigma-Aldrich), 60 μ M indomethacin (Sigma-Aldrich), 10 μ g/ml insulin solution from bovine pancreas (Sigma-Aldrich).

Osteogenic media: DMEM/F-12 (Sigma-Aldrich) + 10% FBS (Gibco) + 1% Penicillin-Streptomycin (Thermo Fisher Scientific), 10nM dexamethasone (Sigma-Aldrich), 10mM β -glycerophosphate (Sigma-Aldrich), 50 μ g/ml ascorbic acid (Sigma-Aldrich).

Chondrogenic media: DMEM/F-12 (Sigma-Aldrich) w. 1.35mg/ml glucose + 1% Penicillin-Streptomycin (Thermo Fisher Scientific), 100nM dexamethasone (Sigma-Aldrich), 10 μ l/ml insulin-transferrin-sodium selenite media supplement (ITS)(Sigma-Aldrich), 1mM sodium pyruvate (Gibco), 50 μ g/ml ascorbic acid (Sigma-Aldrich), 5ng/ml transforming growth factor β 1 (TGF- β 1) from porcine platelets (Sigma-Aldrich)(100x solution used = 1 μ g TGF- β 1 dissolved in 1ml TGF- β solvent (4 nM HCl + 100 μ l bovine serum albumin (BSA)(Sigma-Aldrich) in 1ml PBS)).

Prior to adipogenic differentiation in porcine cells, we tested 2 different adipogenic media, as described by Kim *et al.* (2016) and Zimmerlin *et al.* (2010). After 21 days of adipogenic induction, the media from Zimmerlin *et al.* showed more cells with lipid accumulation, in addition to higher cell viability and was used for our differentiation procedure.

Two of the lean cell cultures were subjected to an additional passage before differentiation, due to insufficient cell numbers. No visible difference between P3 and P4 lean cultures could be determined in differentiation procedures.

References

- Kim, B., et al., *Gene expression profiles of human subcutaneous and visceral adipose-derived stem cells*. Cell Biochemistry and Function, 2016. **34**(8): p. 563-571.
- Zimmerlin, L., et al., *Stromal vascular progenitors in adult human adipose tissue*. Cytometry Part A, 2010. **77A**(1): p. 22-30.