Skin from obese (*Lep^{ob}/Lep^{ob}*) mice shows compromised dermal fibroblast physiology and disorganised collagen structure

THE UNIVERSITY OF BUCKINGHAM

Mohamed S. Zaibi, Amgad Al-Habian, Claire J. Stocker and Kenneth Langlands

Clore Laboratory, University of Buckingham, Buckingham MK18 1EG, UK

Background and Aims

Obese individuals have impaired skin function and integrity, while cutaneous fragility is found in obese mice. Increased adiposity in obese states may contribute to compromised dermal fibroblast physiology and promote damage by altering collagen deposition and integrity through the release of inflammatory cytokines.

Skin architecture and collagen organisation were compared in 3-month-old lean and *Lep^{ob}/Lep^{ob}* C57BL/6 mice. Growth, senescence and collagen synthesis were investigated in primary dermal fibroblasts established from the same animals. Cytokine levels in plasma and subcutaneous adipose tissue incubated media from our models were also profiled.

Methods

Histology. 4 μ M paraffin sections were prepared from shaved dorsal skin and stained with H&E or PicroSirius as standard. Images were captured under brightfield, fluorescence or cross-polar optics.

Cell culture. Cells were isolated by collagenase digestion and maintained in DMEM with 10% FCS. To assess growth, cells were fixed in 10% formaldehyde solution and stained with 1% methylene blue. Stain was eluted in HCl before the OD at 630nm was measured.

Collagen synthesis. Cells were fixed with Bouin's solution and stained for one hour in PicroSirius solution. Incorporated dye was eluted in 0.1N NaOH and the OD at 550nm was determined.

Senescence assay. Cells were fixed with 0.2% gluteraldehyde and incubated overnight at 37° C with 1mg/ml X-gal before washing and imaging.

Cytokine assays. Cytokine profiles in plasma and Ham F12 medium exposed to adipose tissue for 90min were determined using a MesoScale -Ultrasensitive Kit.

Results

Fig. 1 shows an increase in bodyweight in *ob/ob* mice due to the accumulation of adipose tissue (AT).

Histological analysis revealed an increase in sub-dermal AT, a reduction in dermal compartment size **(Fig. 2 a, d)** and loss of collagen integrity compared to lean controls **(Fig. 2 b, c, e, f).**

Primary dermal fibroblasts prepared from *Lep^{ob}/Lep^{ob}* mice showed a reduced proliferative capacity (**Fig. 3a**), a reduction in collagen deposition **(Fig. 3b and Fig. 4e and 4f)** and a loss of normal fibroblast morphology **(Fig. 4a-4f)** compared to controls.

Very few β-galactosidase positive fibroblasts were observed in wild-type dermal fibroblasts, whereas *Lep^{ob}/Lep^{ob}* fibroblasts were highly senescent (**Fig. 5**).

Cytokine profiles revealed increased MCP1 and RANTES in ob/ob plasma **(Fig. 6a)**, whereas adipose tissue from these animals showed elevated IL10, IL6, mKC and MCP1 **(Fig. 6b).**

Conclusion: **The cutaneous phenotype In *Lep^{ob}/Lep^{ob}* mice may arise as a consequence of the accumulation of sub-dermal adipose tissue and, consequently, increased pro-inflammatory cytokine release. This may lead to reduced fibroblast growth, accelerated senescence and decreased collagen secretion. The effect of subcutaneous adipokines on human primary dermal fibroblast physiology is currently under investigation in our Lab.**

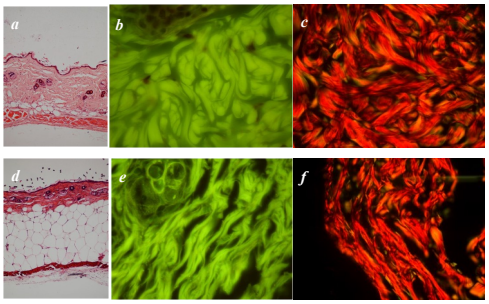

Fig. 2. Representative H&E and PicroSirius stained sections of dorsal mouse skin. 3-month-old lean C57BL/6 **(a-c)** and age-matched C57BL/6 *Lep^{ob}/Lep^{ob}* (*ob/ob*) mice **(d-f)** are shown. Brightfield H&E sections **(a, d)** are shown at 10x original magnification. Collagen structure was assessed using eosin auto-fluorescence **(b, e)** and cross-polar imaging of PicroSirius stained sections **(c, f)** all at 100x original magnification).

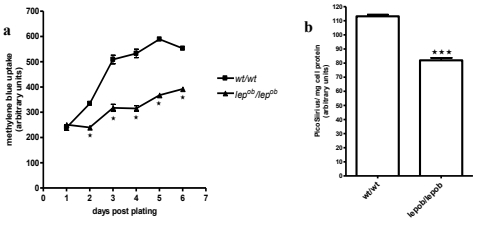

Fig. 3. *In vitro* assessment of insulin-resistant dermal fibroblast physiology. a) growth curves were generated for wild-type (*wt/wt*) and C57BL/6 *Lep^{ob}/Lep^{ob}* cell lines. Experiments were performed on cells from 3 different mice per group.* indicates P<0.001. **b)** Quantitative measures of collagen deposition normalised to total cellular protein using a PicroSirius assay (*** indicates P<0.0001).

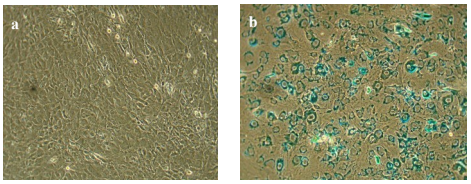

Fig. 5. Senescence assays. Typical fields of β-galactosidase stained wild-type C57BL/6 **(a)** and C57BL/6 *Lep^{ob}/Lep^{ob}* primary dermal fibroblasts **(b)** are shown at 10x original magnification. Cells with blue staining cytoplasm prevalent in **b** are senescent.

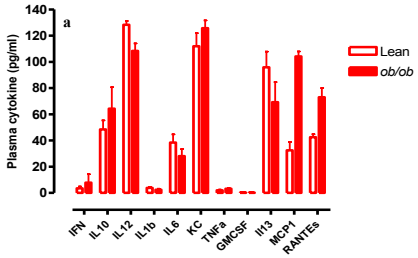

Fig. 6. Cytokine profiles. Cytokine release from **(a)** lean and *ob/ob* mouse plasma and **(b)** subcutaneous fat incubated media from the same animals is shown.

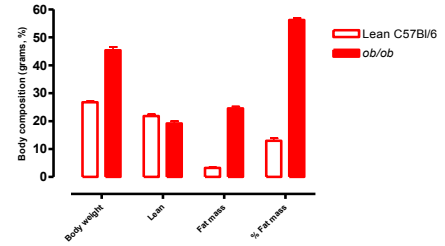

Fig. 1. Body composition. Dual-Energy X-ray Absorptiometry (DEXA) measurements of 3-month-old lean C57BL/6 controls and age-matched *Lep^{ob}/Lep^{ob}* (*ob/ob*) mice are shown.

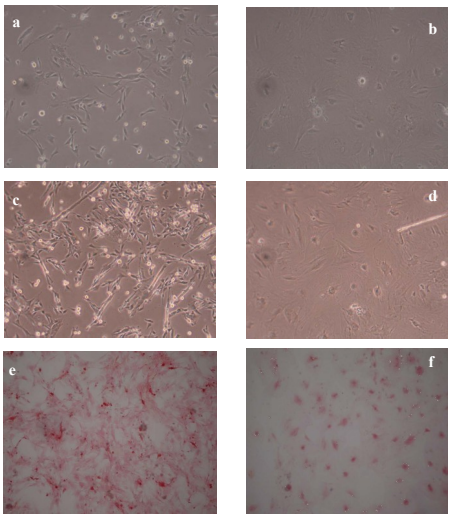

Fig. 4. Primary mouse dermal fibroblast morphology. Wild-type **(a, c and e)** and C57BL/6 *Lep^{ob}/Lep^{ob}* **(b, d and f)** fibroblasts at 2 **(a, b),** 4 **(c, d)** and 6 days **(e, f)** post-plating are shown. Cells in **e** and **f,** are stained with PicroSirius for collagen determination (x10 original magnification).