Interscapular fat tissue morphology is altered in cystic fibrosis

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INTRODUCTION

- Cystic fibrosis (CF) is a disease caused by mutations in the gene CFTR
- Maintenance of a normal body mass index is a clinical goal for people with cystic fibrosis because it is associated with better pulmonary function
- Despite raised recommended caloric intake, the median BMI for patients borders the recommended level
- Low body mass index is typically attributed to lung disease and nutrient malabsorption. In the mouse model, however, lean body type is present despite lack of lung disease and nutrient malabsorption

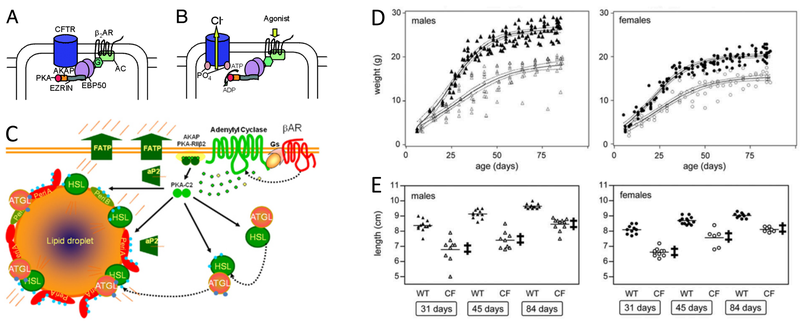
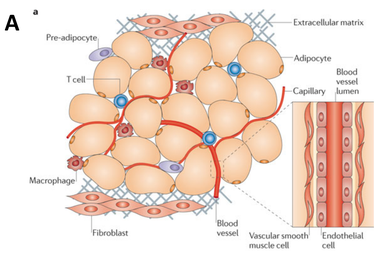
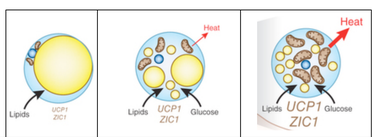


Figure 1: CFTR is an anion channel found at the plasma membrane. **(A,B)** CFTR's indirect interaction with the β adrenergic receptor (β-AR) provides a potential mechanism for a functional role of CFTR in adipocytes. **(C)** In adipocytes, glucagon and epinephrine stimulate the β3 adrenergic receptor to induce lipolysis. The cystic fibrosis mouse models the growth deficit observed in patients in both **(D)** weight and **(B)** length. Unlike patients, however, cystic fibrosis mice do not develop lung disease and are pancreatic sufficient. We hypothesize that lack of Cftr function in fat tissue contributes to the growth deficit.

(A, B) Image from: Naren AP et. al. PNAS (2003). (C) Image from: Collins S. Front. Endocrinol. (2012). (D,E) Figure from: Rosenberg, L. A., Schluchter, M. D., Parlow, A. F. & Drumm, M. L. Pediatr. Res. 59, 191–5 (2006)

BACKGROUND





B

Adipocyte Type	White	Beige/Brite	Brown
Energy-related Function	storage	Storage & thermogenesis	thermogenesis
Lipid droplets	one	intermediate	many
Mitochondria	few	intermediate	many
UCP1 expression	low	intermediate	high

Figure 2: (A) Cell types in adipose tissue include adipocytes, pre-adipocytes, white blood cells. Vascular cell types are also present. **(B)** Adipocyte range in morphological characteristics depending on their function. There is a growing appreciation for the role of beige adipocytes in classically called "white adipose" depots, such as the epididymal fat depot.

(A) Image from: *Nature Reviews Immunology* 11, 85-97 (February 2011)
(B) Images from: Kasper DL et. al. Harrison's Principles of Internal Medicine at accessmedicine.com

BACKGROUND

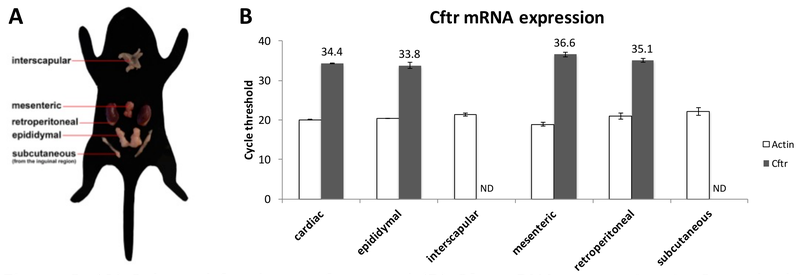


Figure 3: (A) Selected fat depots from and **(B)** Cftr mRNA expression in 6 week-old wild type mice (n = 3). Note that cycle threshold inversley correlates with expression level; epididymal fat has the highest expression and interscapular and subcutaneous fat, the lowest. ND = not detectable.

HYPOTHESIS

If Cftr in adipose tissue plays a role in tissue morphology, then the depot with the highest Cftr expression will have the largest morphological differences in Cftr-mutant mice.

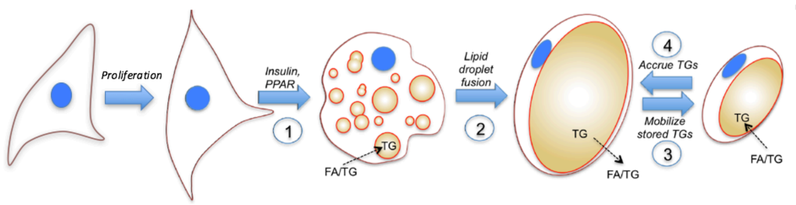


Figure 4: Steps in adipocyte differentiation and function that may be affected by Cftr. FA = fatty acids, TG = triglycerides.

RESULTS

We examined fat tissue morphology in two fat depots: epididymal (highest Cftr expression) and interscapular (lowest Cftr expression). Cystic fibrosis mice have the F508del/F508del mutation in Cftr. This mutation was chosen because 86.5% of patients with cystic fibrosis carry at least one F508del mutation in CFTR.

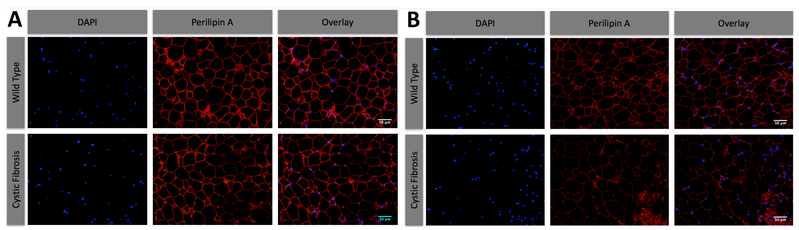
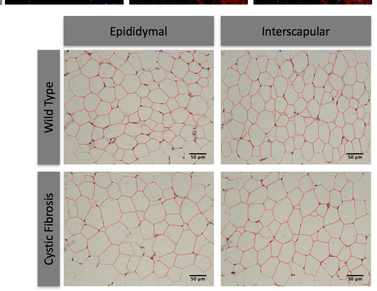
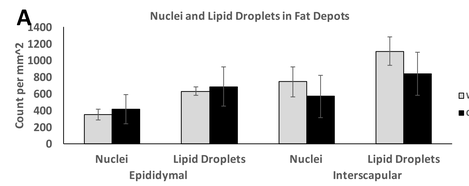


Figure 5: Histological analysis of adipose tissue from 6 week-old mice. Immunostaining of **(A)** epididymal and **(B)** interscapular for nuclei (DAPI stain) and perilipin A. Perilipin A surrounds lipid droplets. **(C)** Hematoxylin and eosin (H&E) staining is an alternative way to image fat tissue. On these images, lipid droplet perimeters are traced in red.



RESULTS



B

Mouse #	1	2	3	4	5	6
Avg. Epidid. Fat weight (mg)	76.6	78.3	93.5	52	65.4	65.1
Age (days)	48	48	45	54	54	53

Figure 6: (A) Nuclei and lipid droplet density in immunostained fat tissue. (B) Fat pad weight and mouse age for mice in this study.

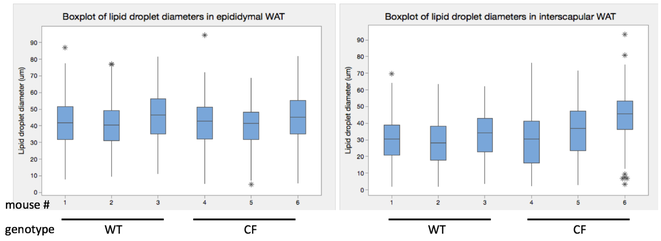


Figure 7: Lipid droplet diameter is normally distributed in epididymal and interscapular fat depots (Anderson-Darling $p < 0.03$ in all cases). In epididymal fat, the average lipid droplet size is the same in WT and CF mice (T-test $p = 0.24$). In interscapular fat, the average lipid droplet size is larger in CF mice ($p < 0.0001$). Note: Diameter of the equivalent circle was calculated from lipid droplet areas, which were manually measured from H&E images (e.g.. Fig. 5c).

Epididymal Fat		
Measurement	WT	CF
Nuclei/mm^2 (DAPI)	349 ± 68	411 ± 175
Lipid droplets/mm^2 (H&E)	628 ± 53	685 ± 234
Predicted lipid droplet diameter* (um)	45 ± 2	43 ± 6
Lipid droplet diameter (um)	42 ± 14	43 ± 13

Interscapular Fat		
Measurement	WT	CF
Nuclei/mm^2 (DAPI)	774 ± 179	568 ± 255
Lipid droplets/mm^2 (H&E)	1109 ± 172	840 ± 257
Predicted lipid droplet diameter* (um)	34 ± 2	38 ± 5
Lipid droplet diameter (um)	30 ± 13	35 ± 16

Figure 8: Summary of histological quantification

*Predicted from lipid droplet/mm^2 (H&E) value with the assumptions that all lipid droplets are equal in size and completely fill the field of view (this is an overestimate of diameter)

CONCLUSIONS

- The average area of lipid droplets in interscapular fat is smaller than that in epididymal fat
- In 6 week-old male mice, there is no difference in the nuclear density or lipid droplet area between (cystic fibrosis and wild type mice
- There is not a change in inflammatory state as indicated by macrophages in crown-like structures) in cystic fibrosis fat tissue [not quantified]

FUTURE DIRECTIONS

Determine whether morphological differences exist in 3-week old mice

- de-novo lipogenesis is decreased in 3 week-old but not 6 week-old cystic fibrosis mice. Bederman, I. R. et al. Am. J. Physiol. Gastrointest. Liver Physiol. (2012)
- The growth of adipocyte diameter and number throughout childhood plateaus at puberty. Perhaps there is a delay in adipocyte diameter in cysitc fibrosis mice that can be seen at 3 weeks-old but not 6 weeks-old

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