To validate that isolated primary preadipocytes retain their properties ex vivo, we will investigate the insulin signalling pathways in primary preadipocytes from white adipose tissue of CD248 WT and KO mice by quantifying insulin-triggered phosphorylation of AKT by Western blot.

Delineating the mechanisms by which CD248 functions in glucometabolism would be aided by having primary preadipocytes from the genetically modified mice. There are different methods for isolating preadipocytes:

1. **Explant culture**
   - This technique allows small fragments of tissue to adhere to the growth surface, which may give rise to the cells but the yield may be limiting.

2. **Enzymatic digestion**
   - This is a common method for isolating cells with collagenase, but the process may damage the natural microenvironment of adipose tissue, and may affect viability, phenotype, and differentiation potential.

3. **Magnetic labelling isolation**
   - In this method adipose tissue derived progenitor cells are isolated by depletion of non-target cells followed by positive selection of the target cells.

**OBJECTIVE**

To validate that isolated primary preadipocytes retain their properties ex vivo, we will investigate the insulin signalling pathways in primary preadipocytes from white adipose tissue of CD248 WT and KO mice by quantifying insulin-triggered phosphorylation of AKT by Western blot.

**RESULTS**

- **CD248 WT Preadipocytes**
  - Pref-1 (Preadipocyte factor 1)
  - mRNA Level for Pref-1
  - CD248 KO Preadipocytes

- **CD248 KO Preadipocytes**
  - Pref-1 (Preadipocyte factor 1)
  - CD248 WT
  - ~150 KD
  - ~62 KD
  - ~42 KD

**CONCLUSIONS**

1. Purified preadipocytes isolated from the CD248 WT & KO mice revealed increased sensitivity of the KO cells to insulin triggered activation of the PI3K/AKT pathway. The findings are in line with in vivo observations of increased resistance of CD248 KO mice to high fat diet induced diabetes and obesity.

2. Having a validated technique to isolate and culture pure preadipocytes that reflects in vivo findings, will facilitate investigations of the molecular mechanisms and functional interactions between CD248 and the insulin signalling pathway components.

**FUTURE DIRECTIONS**

Isolate and expand preadipocytes from other fat depots of CD248 WT and KO mice to investigate the mechanisms by which CD248 modulates glucometabolism.

**REFERENCES**


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**METHODS**

- Excise adipose tissue from euthanized mice and cut into small pieces with 2 sterile sharp blades
- Digest with collagenase for 30-40 min 37°C shaking water bath
- Filter through 100 and 70um nylon mesh
- Remove the supernatant and resuspend the pellet of SVF cells in MACS buffer
- Add the Non-Adipocytes Progenitor Depletion Cocktail to the resuspended cells
- Centrifuge 300xg/5min
- Add adipocytes Progenitor Isolation Cocktail to the resuspended cells
- LS column
- MS column
- Add the Non-Adipocytes Progenitor Depletion Cocktail to the resuspended cells
- Centrifuge 300xg/5min

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