Primary and Stem Cell Applications for Diabetes and Obesity Research

Webinar
23 September 2014 / Speaker: Andrew Winner
24 September 2014 / Speaker: Dr. Elke Lorbach
Agenda

- Introduction
  - Diabetes and obesity
  - Adipose Tissue
  - Preadipocytes and ADSCs

- Cell Applications
  - ADSC Applications
  - Preadipocyte Applications

- Lonza Products

- References
What is Diabetes?

- Diabetes mellitus is a group of diseases characterized by high levels of blood glucose resulting from defects in insulin production, insulin action, or both
  - Type 1 Diabetes
    - Type 1 diabetes develops when the body’s immune system destroys pancreatic beta cells; strong genetic component, 5-10% of all diabetes
  - Type 2 Diabetes
    - Previously called non-insulin-dependent diabetes mellitus, 90-95% of diagnosed diabetes cases
  - Other types:
    - LADA (latent autoimmune diabetes of adults)
    - MODY (maturity-onset diabetes of youth)
    - Secondary diabetes mellitus
    - Gestational diabetes
Type 1 vs. Type 2 Diabetes

Type 1 Diabetes: Insufficient Insulin

Type 2 Diabetes: Insulin Resistance

Images Courtesy of the University of Pittsburgh
Type 2 Diabetes (Cont.)

- Usually begins as insulin resistance
- Associated with older age, obesity, impaired glucose metabolism and physical inactivity
- Well known association with lifestyle choices
- Increasingly being linked to visceral adipose tissue

Image Courtesy of The Mayo Clinic
Obesity

- Obesity: having a very high amount of body fat in relation to lean body mass, or Body Mass Index (BMI) of 30 or higher
  - Body Mass Index (BMI): a measure of an adult’s weight in relation to his or her height, specifically the adult’s weight in kilograms/pounds divided by the square of his or her height in meters/feet

- Increasingly tied to genetics and various metabolic syndromes
  - Environmental factors such as endocrine disruptors are just now starting to be linked to obesity
  - Main causes still seen to be sedentary lifestyle and diet.
Diseases Associated with Obesity

- Diabetes: 80% related to obesity
- Hypertension: prevalence is >40% in obesity
- Arteriosclerosis: blood vessel damage
- Cancer: obesity accounts for 15-20% of cancer-related deaths
- Death: obese individuals have a 50-100% increased risk of death from all causes compared to lean individuals

Image Courtesy of adameducation.com
Not All Fat is Created Equal: Subcutaneous Adipose Tissue

- Found just below the skin
- Not related to many of the classic obesity-related pathologies, such as heart disease, cancer, and stroke
- Regulated by insulin
- More complicated than initially thought
  - Location plays a factor in health risks
  - Shown to actually lower insulin resistance

Image Courtesy of Cleveland Clinic Foundation
Not All Fat is Created Equal: Visceral Adipose Tissue

- Found surrounding the internal organs
- Visceral fat cells continually mobilize FFA (no regulator)
- Contributes to a variety of disorders
  - Insulin resistance
  - Type II diabetes
  - Cardiovascular disorders
  - Dementia

Image Courtesy of Cleveland Clinic Foundation
Adipose Derived Stem Cells

- Adult stem cells isolated from adipose tissue that have very similar phenotypic and functional characteristics to that of bone marrow-derived Mesenchymal stem cells
- Multipotent
  - Reported to differentiate down chondrogenic, osteogenic, adipogenic, myogenic, neural, and endothelial pathways
- Adherent in culture
Preadipocytes are undifferentiated fibroblasts that can be stimulated to form an adipocyte. They can be isolated from both subcutaneous and visceral sources.
Subcutaneous Preadipocytes

- These cells are isolated from subcutaneous adipose tissue via enzymatic digestion and selective culturing techniques, usually from the CD34+/CD31- cell fraction
- Able to fully differentiate in 10 days
- Can be used to produce large amounts of platelets in culture
- Differentiate more readily in response to thiazolidinediones
Visceral Preadipocytes

- Cells are isolated from adipose around internal organs tissue via enzymatic digestion and selective culturing techniques.
- They can usually be expanded one passage prior to differentiation.
- Strong evidence supports genetic determinants, such as sex and race influence visceral fat distribution.
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ADSC Applications: Diabetes

Recent study by Rennert et al, May 2014

The authors examined the impact of diabetes on the ADSC

They found that diabetes impairs the growth and differentiation potential of adipose-derived stem cells

- Diabetes alters the ADSC niche *in situ*
- Diabetic ADSCs are compromised in their ability to establish a vascular network both *in vitro* and *in vivo*
- Diabetic cells are ineffective in promoting soft tissue neovascularization and wound healing

Rennert et al. Stem Cell Research & Therapy 2014 5:79
Wild Type ADSC vs. Type 2 Diabetes ADSC

Adipose-derived mesenchymal stem cell (ASC) adipogenic and osteogenic differentiation.

(A) Representative images of Oil red O and Alizarin red staining following adipogenic and osteogenic differentiation of WT and DM2 ASCs. Scale bar = 50 μm. **P <0.01.
Wild Type ADSC vs. Type 2 Diabetes ADSC (Cont.)

Adipose-derived Mesenchymal stem cell (ASC) adipogenic and osteogenic differentiation.

(B) quantification of Oil red O and Alizarin red staining following adipogenic and osteogenic differentiation of WT and DM2 ASCs. Scale bar = 50 μm. **P <0.01.
Preadipocyte Applications: Obesity

2009 study by Isakson et al published in *Diabetes*

- The authors examined preadipocyte differentiation in obese and non-obese individuals.

- They found that preadipocyte’s ability to differentiate to adipose cells was negatively correlated with both BMI and the adipocyte cell size of the donors.

  - The number of CD133-positive cells was positively correlated with BMI, suggesting an impaired differentiation of preadipocytes in obesity.

  - Preadipocytes from obese individuals had an increased expression of mitogen-activated protein 4 kinase 4 (MAP4K4), which is known to inhibit peroxisome proliferator-activated receptor-γ induction.

Impaired differentiation of human preadipocytes from obese donors. C: BMI of the preadipocyte donor is significantly and inversely correlated to the degree of preadipocyte differentiation ($R^2 = 0.59$, $P = 0.006$). D: Correlation between preadipocyte differentiation and mean adipocyte size of the donors ($R^2 = 0.69$, $P < 0.001$).

Impaired differentiation of human preadipocytes from obese donors. E: The relative number of CD133-positive cells in the stromal vascular fraction is positively correlated with BMI of the donor (R2 = 0.51, P < 0.01). F: mRNA levels of MAP4K4 in cultured preadipocytes in relation to BMI of the donors (R2 = 0.42, P < 0.05)).

Impaired differentiation of human preadipocytes from obese donors. G and H: Correlation between MAP4K4 mRNA levels in isolated mature adipose cells and BMI (R² = 0.41, P < 0.04) (G) or waist-to-hip ratio (WHR) (H) of the donors (R² = 0.67, P = 0.002). Abs, absorbance; RQ, relative quantification.

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Lonza’s Normal and Diseased Cells – Diabetes

- Cells from normal, type 1 and 2 diabetes donors
  - Adipose-derived stem cells (ADSCs)
  - Pre-adipocytes (visceral and subcutaneous) (PrAd)
  - Endothelial (large vessel and microvascular)
  - Epidermal keratinocytes (NHEKs) Renal cells
  - Smooth muscle cells

- Media systems designed to work with Lonza’s primary and stem cells
  - Expansion, maintenance and differentiation media/kits for all of Lonza’s cells
Lonza’s Functional Fluorescent Assays

- Bone Assays
  - OsteoImage™ Mineralization Assay
  - OsteoLyse™ Assay Kit
  - OsteoAssay™ Human Bone Plate

- Fat Assays
  - AdipoRed™ Assay Reagent
  - AdipoLyze™ Lipolysis Assay
References


- Bray GA. Medical Consequences of Obesity. *J Clin Endocrinol Metab* 2004; 89: 2583-2589

- Faust IM Adipose Tissue Regeneration Following Lipectomy. *Science* 22 July 1977; Vol. 197 No. 4301 391-393
Support Tools and Contact Details

Our online databases

- Citations: [http://www.lonza.com/citations](http://www.lonza.com/citations)

Primary cell culture experts

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Interested in Learning More?

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    Register at: http://www.lonza.com/webinar-genome-editing-2014

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