Obesity, Biomarkers and Management

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Metabolic Syndrome

- Deadly triad of **obesity, diabetes and CVD** - that increase the risk of developing micro- and macrovascular complications, *(Rizzo et al., 2013)*

- Several definitions
  - WHO criteria 1999
  - European Group for the Study of IR (1999)
  - US NCEP ATP III (2001)
  - American Heart Association/Updated NCEP – 2005
  - IDF consensus 2006

- Broadly defined as of BMI, physiological parameters such as BP, fasting plasma glucose, fasting plasma insulin, triglycerides and total cholesterol etc *(Rizzo et al., 2013)*
Obesity

Central obesity
- Men > 40 inches waist circumference
- Women >35 inches waist circumference

Fasting triglycerides ≥ 150 mg/dl

HDL cholesterol
- Men < 40 mg/dl
- Women < 50 mg/dl

Blood pressure ≥ 130/85 mm Hg

Fasting glucose ≥ 110 mg/dl

National Cholesterol Education Program.
Global Epidemic

- **2013 AHA statistics** - ~ 24 million children (overweight/obese)
- **2012 World health statistics** - 1 in 6 adults - obese,
- 1 in 3 – hypertensive; 1 in 10 – diabetic
- **Indian scenario** – NNMB, NIN 2012 – 51% ♂ & 64% ♀; diabetes 8.2% ♂ and 6.8% ♀
India has also seen a surge in obesity. In 1975, it had 0.4 million obese men, or 1.3% of the global obese population, but in 2014, it zoomed into the fifth position globally with 9.8 million obese men, or 3.7% of the global obese men's population.

As per the survey conducted by Ministry of Health and Family Welfare (MoHFW), people having Body Mass Index (BMI) more than 25 kilogram per metre square have been considered as obese.
Among women, India has jumped to the third rank with **20 million obese women or 5.3% of global population**.

As per the survey conducted by Ministry of Health and Family Welfare (MoHFW), people having Body Mass Index (BMI) more than 25 kilogram per metre square have been considered as obese.
Temporal changes in prevalence (%) of obesity (≥25 kg/m²) among urban and rural Asian Indians. (a) The data for urban population; (b) the data for rural population

Adapted from Karla et al (2012)
Childhood Obesity - Statistics

- Overweight and obesity rates in children and adolescents are increasing not just among the higher socio-economic groups but also in the lower income groups where underweight still remains a major concern. (RANJANI et al: Indian J Med Res 143, February 2016, pp 160-174)

- The maximum prevalence of overweight and obesity was observed in the urban private school (20%), while the minimum prevalence of overweight and obesity was observed in the rural government school (5.2%) Prasad RV, Int J Nutr Pharmacol Neurol Dis [serial online] 2016

- India sees progress in child health but rising obesity, shows health survey (BMJ 2016;352:i439)
The prevalence of obesity has nearly doubled between 1980 and 2008.
Transition Phases—Towards Obesity and Diabetes (India)

Demographic transition
Epidemiological transition
Rapid Urbanization
Industrialization
Increasing income levels
Changing lifestyles, values and culture

Prevalence of insufficient physical activity in persons aged 18 and over, 2011–12 (Australian study)
Confounding factors - Obesity

Genetic

Environmental [Diet / physical activity] factors?

Gene – Environment Interaction?

Imbalance (Overweight)

INTAKE

EXPENDITURE
Risk factors and complications

Metabolic syndrome
- Visceral Obesity
- Insulin Resistance
- Dyslipidemia
- Hypertension
- Glucose intolerance

Central Nervous system:
- Local Metabolic abnormalities
- Systemic Metabolic abnormality
- Systemic disease

Microvascular disease
- Eyes
- Kidneys
- Nerves

Macrovascular disease
- Ischaemic heart disease
- Strokes
- Peripheral vascular disease

Human disease
- Cardiovascular disease
- Liver disease
- Diabetes
- Pancreatitis
- Cancer
- Others

Life style changes
- Genetic factors

Coagulopathy
- Smoking

Hyperglycaemia
Hypertension
Dyslipidaemia
Risk of Obesity

Psychosocial problems
* Low self-esteem

Lungs and respiratory system
* Breathing problems at night
* Asthma

Stomach and digestive tract
* Gallstones
* Fatty Liver

Kidneys
* Kidney insufficiency (Diabetes)

Skeleton and musculature
* Burning foot
* Flat feet
* Knock knee deformity
* Bowleg
* Lack of movement
* Osteoarthritis

Neurologic and Psychiatric Disorders
* Depression
* Headache (increase in the normal brain pressure)
* Sight problems
* Eating disorders

The Heart and the Circulatory System
* Increased cholesterol
* High Blood Pressure
* Thrombosis
* Chronic inflammation
* Heart disease
* Stroke

Hormonal system
* Type 2 diabetes
* Premature puberty
* Cancer
* Diminishing sex drive

www.JoeDiAngelo.com
Diabetes is a state of inflammation.
Animal models of Obesity and Diabetes

- Bridging the gap in understanding the mechanism of metabolic syndrome towards extrapolation to human scenario.

Animal models

- In vivo
  - Diet induced
  - Hypothalamic

- In vitro
  - Genetic
    - Monogenic
    - Polygenic

- Cell lines
  - Among illustrious list of genetic animal models ob/ob, db/db mice, Zucker and Koletsky rats
Animal models of obesity

Experimental, genetic and spontaneously developed exist and have helped in **Bridging the gap** in the advancement of the knowledge on pathophysiology and treatment regimes for obesity.
Biomarkers

A **biomarker**, a **substance** used as an **indicator** of a biological state. It is a "measurable and quantifiable biological parameters (eg, specific enzyme, specific hormone, specific gene phenotype, presence of biological substances) which serve as indices for health- and physiology-related assessments."

**Classification of Biomarkers**

**Type 0 biomarker**: A marker of the **natural history** of a **disease** and correlates longitudinally with known clinical indices.

**Type 1 biomarker**: A marker that **captures the effects** of a **therapeutic intervention** in accordance with its mechanism of action.

**Surrogate end point (type 2 biomarker)**: A marker that is intended to substitute for a **clinical end point**; a surrogate end point is expected to predict clinical benefit (or harm or lack of benefit or harm) on the basis of epidemiological, therapeutic, pathophysiological, or other scientific evidence.

**Risk marker**: A risk marker is **associated with the disease** (statistically) but need not be causally linked; it **may be a measure of the disease process itself**.

**Clinical end point**: A characteristic or variable that **reflects how a patient feels**, functions, **or survives**.
Obesity is a state of oxidant stress. Obesity may involve some or all of these contributors to systemic oxidative stress. Depending on the status of the obese individual, one contributor may exert a greater oxidative stress effect than the others.
Biomarkers used in Obesity

- **Childhood obesity** –
  - **Adipocytokines**
    - \(\uparrow\) increased - leptin, resistin, gherlin:
    - \(\downarrow\) decreased adiponectin, visfatin

- **Adult onset obesity** –
  - insulin, proinflammatory cytokines,
  - adipokines such as adiponectin, leptin, and resistin
Leptin, adiponectin, and leptin:adiponectin ratios - informative biomarkers for obesity, central obesity.

Journal of Obesity, 2010

interleukin (IL)-1-beta (1), IL-6 (6), adiponectin (A), leptin (L), or resistin (R) human adipocytes in response to obesity.

J Am Coll Cardiol, 2005
WNIN RAT COLONY - >80 YEARS OLD

**UNIQUE FEATURES**
- FIRST INBRED RAT MUTANT
- KINKY TAIL
- AUTO. INCOMP. DOMIN. MUT.
- AGES FASTER & DEVELOPS DEGENERATIVE DISEASES LIKE TUMOURS AND CATARACT ETC.
- INFERTILITY REVERSIBLE BY DIET MANIPULATIONS

**COMMON TRAITS**
- DEVELOPS OBESITY BY 35 DAYS
- HYPERPHAGIA, HYPERINSULINIMIA, HIGH CHOLESTEROL AND TRIGLYCERIDES
- LEPTIN RESISTANT

Hyperinsulinemia, Hypertriglyceridemia, Hypercholesterolemia, Frank diabetic upon challenge, Insulin resistance

- Unilocus mutation, upstream of leptin receptor (Kalashikam et al., 2013)
- Hypermethylation of leptin promoter (Kalashikam et al., 2014)

Pancreatic tissue

- Pancreatic tissue --- exocrine component and endocrine component.
- Exocrine glands (85-90 %)
  - It secretes different digestive enzymes such as Amylase, Lipase, Trypsinogen, chymotrypsin, carboxypeptidase, nucleases
- Endocrine component helps in maintaining glucose homeostasis
  - Islets of Langerhans: 4 cell types
    - α cells: secrete glucagon (15–20% of total islet cells)
    - β cells: secrete insulin (65–80%)
    - δ cells: secrete somatostatin (3–10%)
    - F cells: secrete pancreatic polypeptide (3–5%)
Insulin resistance

HOMA-IR: (Fasting glucose in mg/dl \times \text{Fasting plasma insulin in mU/ml})/2430

↑ HOMA-IR
↑ Plasma insulin
A. Insulin positive cells

Age (months) 1 6 12

(%)

100 80 60 40 20 0

* WNIN Lean Mutant

B. Glucagon positive cells

Age (months) 1 months 6 months 12 months

(%)

25 20 15 10 5 0

* WNIN Lean Mutant

C. Somatostatin positive cells

Age (months) 1 6 12

(%)

15 10 5 0

* WNIN Lean Mutant

Singh et al 2012
primary Islet cell cultures-Functional assay

Insulin Secretion Assay

TEM/SEM

Pancreatic insulin content
Pancreatic tissue
Hypertrophy/Oxidative Stress in Pancreas

H&E  Insulin/Glucagon  Insulin / HSP

35 days

6 months

12 months

Hypertrophy and increased stress levels (HSP) demonstrated
TNFα expression (6 months) in Islet cells
Inflammatory markers as Biomarkers from animal Studies

Zucker Rats

a. Relative amount of TNF-α mRNA

b. % TUNEL positive nuclei

+/- indicates control

TNFα mRNA and protein

Nisoli E et al. PNAS 2000

Obese mice

A. Serum FFAs Levels

B. Serum TNF level

C. Serum cytokine levels

MCP1 expression - mRNA and circulation

Takahashi K et al. J. Biol. Chem. 2003

Obese mice

D. MCP-1 (pg/mL)

Zhou Q et al. PNAS 2011

serum cytokines II-6, II-12p70, MCP-1, TNFα
Oxidative stress

An imbalance between the ROS and a biological system's ability to readily detoxify the reactive intermediates

Islet have weak antioxidative system making them highly vulnerable to deleterious effect of antioxidative stress

Venkatesan et al 2014

**WNIN/GR-Ob**  ↑ plasma TBARS levels  ↑ tissue TBARS ↑ Reactive Oxygen Species
Insulin resistance and hyperglycemia drive the atherosclerotic process.
RNA integrity was assayed using the Agilent bioanalyzer 2100 and 260/280 ratio range of 2.0 to 2.2 and RNA integrity Number greater than 8 was accepted.

500 ng of total RNA from each pool

T7-polymerase and labeled, using the Agilent Low RNA Input Fluorescent Linear Amplification Kit

Agilent Rat whole genome 4 X44 array (#G4131F, Agilent Technologies)
Macrophage differentiation
Response to unfolded protein
Regulation of transcription
Chromatin
Retinol Metabolism
<table>
<thead>
<tr>
<th>Gene Ontology</th>
<th>Description</th>
<th>GO ID</th>
<th>P-value</th>
</tr>
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<tbody>
<tr>
<td>Macrophage differentiation</td>
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<td>GO:0030225</td>
<td>0.031</td>
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<tr>
<td>Response to unfolded protein</td>
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<td>GO:0006986</td>
<td>0.015</td>
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<tr>
<td>Transcription</td>
<td></td>
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<td>0.013</td>
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<tr>
<td>Chromatin</td>
<td></td>
<td>GO:000785</td>
<td>0.0003178</td>
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<tr>
<td>Retinol Metabolism</td>
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<td>rno00830</td>
<td>0.005</td>
</tr>
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</table>
Network of inflammatory genes.

Interaction among inflammatory genes was analyzed, using STRING 9.1 (http://string-db.org).

STRING infers functional relationship (edges) between genes (nodes) by various direct and indirect methodologies.

Network representation of functionally associated genes **visualize, interpret and prioritize genes**

Singh et 2014
Hypertrophy/Oxidative Stress in Adipose Tissue

Adipocyte Hypertrophy

35 days

Obese

Lean

6 months

Obese

Lean

12 months

Obese

Lean

Oxidative Stress

Plasma TBARS

35 days

6 months

12 months

WNIN

Ob/L

Ob/Ob

SCAT Tissue TBARS

35 days

6 months

12 months

WNIN

Ob/L

Ob/Ob

ER stress

57kDa

Adipocyte hypertrophy and increased oxidative stress levels demonstrated
Hypertrophy/Oxidative Stress in Adipose Tissue

Adipocyte Hypertrophy

35 days
Obese  Lean
6 months
Obese  Lean
12 months
Obese  Lean

Oxidative Stress

Plasma TBARS

SCAT Tissue TBARS

ER stress

Adipocyte hypertrophy and increased oxidative stress levels demonstrated
Phased gene expression in Adipose tissue development

Embryonic phase
- Embryonic stem cell
  - Nanog, Oct-4
  - WNT, BMP4, FGF

Mesenchymal phase
- Mesenchymal stem cell
  - KLF4, NR3C1, C/EBPβ

Pre-adipocyte phase
- Proliferation
  - KLF4, NR3C1, C/EBPβ
- Growth arrest
  - PPARγ, C/EBPβ/δ
- Limited growth resumption
  - ADD-1, PPARγ2, C/EBPα
- Immature adipocyte
  - IGF-1
- Mature Adipocyte phase
  - Lipogenesis
    - FAT/CD36, ALBP, ACBP, ACC, FAS, GLUT-4, GPDH, GPAT, LPAT, DGAT, PEPCK
  - Lipolysis
    - β2/β1AR, α2-AR, perilipin, low Km PDE, HSL
  - Emergence of very late markers, further lipid accumulation
- Mature adipocyte
  - Leptin, Adipsin, Angiotensin, PAI-1, PGAR/FIAF, Adiponectin, Resistin
Embryonic

Oct-4, Sox-2, nanog

Adipose tissue
SCAT/RPAT

Mesenchymal/Transcriptional Factors
Dact-1, PPARγ2, SREBP-1C, C/EBPα

Stress/Inflammatory markers
IL-6, TNFα

Preadipocyte and Adipocyte
Pref-1, Leptin, Adiponectin
GLUT-4, LPL, αP2, IRS-1

Madira et al., 2012
Our findings - Hypothetical Model for Adipocyte Recruitment and Commitment in Obesity from WNIN/Ob Obese Mutant Rats

Candidate genes - Sox-2, Pref-1, PPARγ2, LPL, IRS-1, GLUT-4, IL-6, TNFα

Madhira et al., Molecular and cellular biochemistry 2011 (In Press)
Pancreatic tissue

Phased gene expression in Pancreas development
Multilineage Differentiation potential of BM-MSCs

1 month
WNIN  GR/L  GR/Ob

6 months
WNIN  GR/L  GR/Ob

12 months
WNIN  GR/L  GR/Ob

✓ Up islet neogenic potential
✓ Similar to AD-MSCs
✓ Down with age
✓ Impaired response to glucose challenge

Modified from Chandra, Nair and Bhonde. Stem Cells, 2009
MSCs from mutant obese rats demonstrate ↑ stress levels in cytokines - TNFα and IL-6 similar to AD Stromal cells.
Overall Summary

WNIN Mutant Rats

35 days 6 months 12 months

Blood/Plasma Profile

Hyperinsulinemia
Hypertriglyceridemia
Insulin resistance
Oxidative stress (TBARS)

Hypertrophy
TG Content
SC recruitment to adipocyte lineage
Lipolysis
Oxidative stress (TBARS)
Inflammatory stress
Yield of MSCs
Adipocyte lineage commitment &
Adipogenic index
Memory for obesity, HI, IR and IGT

Adipose tissue (SCAT & VAT) and their resident Progenitors (AD-MSCs)

Bone Marrow

SC Recruitment to adipocyte lineage
Adipocyte lineage commitment &
Adipogenic Index
Oxidative stress (TBARS)
Inflammatory stress
Memory for Obesity, HI, IGT and IR
Schematic representation of integration of metabolic pathways in obesity

- **Non-adipogenic sources**
  - **Bone marrow**

- **Adipocytes**
  - Pretend to be ischemic, recruit with TNF-α
  - Adipogenic Transcription factors
    - PPARγ2

- **Preadipocytes**
  - Pref-1
  - PPARγ

- **MSCs**
  - Dact-1

- **ESCs**
  - Oct-4, Sox-2, Nanog

- **Normal AT**
  - Obese AT

- **ROS, DNA Damage, Telomerase dysfunction**

- **Genes Environment Disease**
  - **Obesity**
  - **Hypertrophy, macrophage infiltration**

- **FFAs**
  - Adipocytes

- **Disease**
  - **Environment**
  - **Genes**

- **ER Stress**
  - JNK
  - IRE-1

- **Cellular Stress**
  - (Mitochondria) - ROS

- **Cellular Senescence**
  - Telomerase
Obesity interventions / Weight management

Strategies

Weight loss

Weight Management Triangle

Measure body composition
Modify energy balance
Monitor diet & activity

Weight Loss Surgery

Liposuction Process

Before

After

Fat Cells
Subcutaneous Layer
Deep Dermis
Epiidermis

Medindia.net

Medication:

DISOXYN

Adrenergic Tents, SR-1
5mg
Inflammatory Biomarkers in Weight Management

Calorie restriction

<table>
<thead>
<tr>
<th>Inflammatory biomarker</th>
<th>Very-low-carbohydrate</th>
<th>Low-fat</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsCRP</td>
<td>0.30 ± 0.35</td>
<td>0.18 ± 0.81</td>
<td>0.534</td>
</tr>
<tr>
<td>hSTNF-α</td>
<td>0.30 ± 0.43</td>
<td>0.58 ± 2.02</td>
<td>0.334</td>
</tr>
<tr>
<td>hSIL-6</td>
<td>0.38 ± 0.27</td>
<td>0.49 ± 0.84</td>
<td>0.596</td>
</tr>
<tr>
<td>sICAM-1</td>
<td>13.90 ± 15.75</td>
<td>33.67 ± 71.07</td>
<td>0.240</td>
</tr>
<tr>
<td>sP-selectin</td>
<td>3.13 ± 6.84</td>
<td>0.78 ± 0.793</td>
<td>0.031</td>
</tr>
</tbody>
</table>

Surgical intervention

- N-terminal propeptide of type IIA collagen (PIIAPN),
- type II collagen helical peptide (Helix-II),
- cartilage-oligomeric matrix protein (COMP)
- hyaluronan

CRP, TNFα, IL-6, ICAM-1, P-selectin

Clin Sci (Lond). 2004

Ann Rheum Dis. 2011
Why Adult Stem cells in the management Of Obesity
**Plasticity**: Plasticity is the ability of a stem cell from one tissue to generate the specialized cell type(s) of another tissue.

**MSCs – Bone Marrow stem cells**

**Embryonic Stem Cells**

**Hematopoietic stem cells (HSCs)**
Sources of MSCs

- Foetal Liver
- Amniotic fluid
- Placenta
- Muscle
- Adipose Tissue
- Cord Blood
- Peripheral Blood
- Synovium
- Bone Marrow
Immunomodulatory properties of MSCs

**Inflammation:**
- Reduce T-lymphocyte activation
- Reduce macrophage infiltration & microglia activation

**Neurogenesis:**
- Increase neuronal growth & differentiation

**Apoptosis:**
- Reduce apoptotic cell death

**Angiogenesis:**
- Increase cerebral blood vessels

**Myelination:**
- Increase axonal remyelination

**Synaptogenesis:**
- Increase synaptic connections

**Trophic Factors:**
- Secretion of neurotrophic & angiogenic factors

**Increase reactive astrocytosis:**
- Astrocyte proliferation & activation
Management Of Obesity: Feasibility of Mesenchymal Stem Cells of human perinatal origin to ameliorate Type 2 Diabetes with Insulin resistance in WNIN/GR-Ob mutant rat model system
WNIN and WNIN-Gr/Ob Rats
human placental mesenchymal stem cells (hPDMS’C)

Experimental condition

human placental mesenchymal stem cells (hPDMS’C) with Conditional medium

WNIN/Grob Rat

hPDMS’c + Conditional medium

WNIN-Rat

(hPDMS’C) with Conditional medium

Biochemical analysis:
- FBS, TAG, LDL, VLDL
- Estimation of Plasma glucose and insulin
- Measurement of HOMA-IR
## Body weight of GR-Ob & WNIN Pre and post injections of hPDMSC's

### Table 1: Body Weight

<table>
<thead>
<tr>
<th></th>
<th>1st week Pre injection</th>
<th>1st week Post injection</th>
<th>2nd week Pre injection</th>
<th>2nd week Post injection</th>
<th>3rd week Pre injection</th>
<th>3rd week Post injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>WNIN/GR-OB PdMSC</td>
<td>533</td>
<td>556</td>
<td>533</td>
<td>547</td>
<td>535</td>
<td>507</td>
</tr>
<tr>
<td>WNIN/GR-OB Control</td>
<td>524</td>
<td>524</td>
<td>522</td>
<td>531</td>
<td>523</td>
<td>532</td>
</tr>
<tr>
<td>WNIN/GR-OB C.M</td>
<td>745</td>
<td>756</td>
<td>750</td>
<td>782</td>
<td>760</td>
<td>700</td>
</tr>
</tbody>
</table>

### Graph 1: Comparison of Body Weight

![Graph showing comparison of body weight across groups](image)

### Graph 2: Comparison of Body Weight

![Graph showing comparison of body weight across groups](image)
Oral Glucose tolerance test (OGTT) before of hPDMSC’s injection

<table>
<thead>
<tr>
<th>OGTT</th>
<th>0 MIN</th>
<th>30 MIN</th>
<th>60 MIN</th>
<th>90 MIN</th>
<th>120 MIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>WNIN Pre injection</td>
<td>95</td>
<td>130</td>
<td>94</td>
<td>89</td>
<td>100</td>
</tr>
<tr>
<td>WNIN Post injection</td>
<td>95</td>
<td>100</td>
<td>114</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>WNIN Control</td>
<td>99</td>
<td>111</td>
<td>139</td>
<td>125</td>
<td>100</td>
</tr>
<tr>
<td>WNIN Control</td>
<td>97</td>
<td>121</td>
<td>138</td>
<td></td>
<td>121</td>
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<tr>
<td>WNIN Pre injection C.M</td>
<td>100</td>
<td>125</td>
<td>132</td>
<td>141</td>
<td>100</td>
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<tr>
<td>WNIN Post injection C.M</td>
<td>88</td>
<td>108</td>
<td>116</td>
<td></td>
<td>122</td>
</tr>
</tbody>
</table>

The diagram shows glucose levels over time for different conditions.
Oral Glucose tolerance test (OGTT) after 3 weeks of PdMSC’s injection

<table>
<thead>
<tr>
<th>OGGT</th>
<th>0 MIN</th>
<th>30 MIN</th>
<th>60 MIN</th>
<th>90 MIN</th>
<th>120 MIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>WNIN/GR-OB Pre injection</td>
<td>102</td>
<td>145</td>
<td>159</td>
<td>102</td>
<td>82</td>
</tr>
<tr>
<td>WNIN/GR-OB Post injection</td>
<td>95</td>
<td>116</td>
<td>118</td>
<td>87</td>
<td>99</td>
</tr>
<tr>
<td>WNIN/GR-OB Control</td>
<td>100</td>
<td>128</td>
<td>189</td>
<td>156</td>
<td>132</td>
</tr>
<tr>
<td>WNIN/GR-OB Control</td>
<td>97</td>
<td>167</td>
<td>177</td>
<td>154</td>
<td>132</td>
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<tr>
<td>WNIN/GR-OB Pre injection C.M</td>
<td>106</td>
<td>121</td>
<td>116</td>
<td></td>
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<tr>
<td>WNIN/GR-OB Post injection C.M</td>
<td>88</td>
<td>87</td>
<td>91</td>
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<td>97</td>
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![Graph showing OGTT results](image-url)
<table>
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<tr>
<th></th>
<th>WNIN/GR-OB Pre PdMSC injection</th>
<th>WNIN/GR-OB control</th>
<th>WNIN/GR-OB Pre CM Injection</th>
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<tbody>
<tr>
<td>Fasting blood glucose</td>
<td>98.9 ± 3.78</td>
<td>98 ± 3.2</td>
<td>98 ± 3.45</td>
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<tr>
<td>Total cholesterol (mg/dL)</td>
<td>245.19 ± 4</td>
<td>270 ± 4.3</td>
<td>233 ± 5.5</td>
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<tr>
<td>Triglycerides (mg/dL)</td>
<td>236.19 ± 6.5</td>
<td>233 ± 11.2</td>
<td>199 ± 10.3</td>
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<tr>
<td>Low density lipoprotein (mg/dL)</td>
<td>150 ± 3.44</td>
<td>138 ± 2.9</td>
<td>130 ± 3.3</td>
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<tr>
<td>High density Lipoprotein (mg/dL)</td>
<td>98 ± 8.9</td>
<td>93 ± 8.6</td>
<td>97 ± 8.6</td>
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<tr>
<td>Very Low density lipoprotein (mg/dL)</td>
<td>48 ± 4.5</td>
<td>45.5 ± 6.7</td>
<td>45.5 ± 8.7</td>
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<table>
<thead>
<tr>
<th></th>
<th>WNIN Pre PdMSC injection</th>
<th>WNIN control</th>
<th>WNIN Pre CM Injection</th>
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<tbody>
<tr>
<td>Fasting blood glucose</td>
<td>89 ± 7.7</td>
<td>89 ± 3.5</td>
<td>89 ± 3.0</td>
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<tr>
<td>Total cholesterol (mg/dL)</td>
<td>120 ± 3.8</td>
<td>89 ± 0.8</td>
<td>110 ± 2.2</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>97 ± 4.4</td>
<td>100 ± 7</td>
<td>110 ± 4.4</td>
</tr>
<tr>
<td>Low density lipoprotein (mg/dL)</td>
<td>69 ± 8</td>
<td>56 ± 7</td>
<td>87 ± 2</td>
</tr>
<tr>
<td>High density Lipoprotein (mg/dL)</td>
<td>46 ± 4</td>
<td>29 ± 0.9</td>
<td>55 ± 7.9</td>
</tr>
<tr>
<td>Very Low density lipoprotein (mg/dL)</td>
<td>19.5 ± 4.3</td>
<td>28 ± 3.67</td>
<td>22 ± 2.34</td>
</tr>
</tbody>
</table>

Effect of hPDMSC’s on TAG, LDL, HDL, VLDL levels
Plasma insulin levels

<table>
<thead>
<tr>
<th></th>
<th>Insulin (µU/mL) Pre PdMSC injection</th>
<th>Insulin µU/mL Post PdMSC injection</th>
<th>HOMA-IR Pre PdMSC injection</th>
<th>HOMA-IR Post PdMSC injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>WNIN PdMSC</td>
<td>19.27</td>
<td>14.09</td>
<td>0.7612839506 17284</td>
<td>0.568238683127 572</td>
</tr>
<tr>
<td>WNIN Control</td>
<td>23.28</td>
<td>22.58</td>
<td>0.9197037037 03704</td>
<td>0.910633744855 967</td>
</tr>
<tr>
<td>WNIN C.M</td>
<td>15.045</td>
<td>11.49</td>
<td>0.5943703703 7037</td>
<td>0.463382716049 383</td>
</tr>
</tbody>
</table>

* Insulin (µU/mL) Pre PdMSC injection

Insulin µU/mL Post PdMSC injection

HOMA-IR Pre PdMSC injection

HOMA-IR Post PdMSC injection
Homeostatic model assessment for IR (HOMA-IR)

<table>
<thead>
<tr>
<th></th>
<th>Insulin (µU/mL) Pre PdMSC injection</th>
<th>Insulin µU/mL Post PdMSC injection</th>
<th>HOMA-IR Pre PdMSC injection</th>
<th>HOMA-IR Post PdMSC injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>WNIN/GR-OB PdMSC</td>
<td>184.5</td>
<td>113.174</td>
<td>7.288888888888889</td>
<td>4.56421893004115</td>
</tr>
<tr>
<td>WNIN/GR-OB Control</td>
<td>332</td>
<td>363</td>
<td>13.116049382716</td>
<td>14.6395061728395</td>
</tr>
<tr>
<td>WNIN/GR-OB C.M</td>
<td>234</td>
<td>223.1</td>
<td>9.2444444444444</td>
<td>8.99744855967078</td>
</tr>
</tbody>
</table>

* indicates statistical significance.
### TBARS in plasma sample

#### Table: TBARS in Plasma Sample

<table>
<thead>
<tr>
<th></th>
<th>µmol/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>WNIN Pre injection</td>
<td>2.51</td>
</tr>
<tr>
<td>WNIN Post injection</td>
<td>1.51</td>
</tr>
<tr>
<td>WNIN Control</td>
<td>2.4</td>
</tr>
<tr>
<td>WNIN Control</td>
<td>2.5</td>
</tr>
<tr>
<td>WNIN Pre injection C.M</td>
<td>3.0</td>
</tr>
<tr>
<td>WNIN Post injection C.M</td>
<td>2.37</td>
</tr>
<tr>
<td>WNIN/GR-OB Pre injection</td>
<td>3.5</td>
</tr>
<tr>
<td>WNIN/GR-OB Post injection</td>
<td>2.07</td>
</tr>
<tr>
<td>WNIN/GR-OB Control</td>
<td>2.40</td>
</tr>
<tr>
<td>WNIN/GR-OB Control</td>
<td>2.69</td>
</tr>
<tr>
<td>WNIN/GR-OB Pre injection C.M</td>
<td>2.15</td>
</tr>
<tr>
<td>WNIN/GR-OB Post injection C.M</td>
<td>2.05</td>
</tr>
</tbody>
</table>

#### Graph: TBARS µmol/mL

- **WNIN Pre injection**: 2.51 µmol/mL
- **WNIN Post injection**: 1.51 µmol/mL
- **WNIN Control**: 2.4 µmol/mL
- **WNIN Control**: 2.5 µmol/mL
- **WNIN Pre injection C.M**: 3.0 µmol/mL
- **WNIN Post injection C.M**: 2.37 µmol/mL
- **WNIN/GR-OB Pre injection**: 3.5 µmol/mL
- **WNIN/GR-OB Post injection**: 2.07 µmol/mL
- **WNIN/GR-OB Control**: 2.40 µmol/mL
- **WNIN/GR-OB Control**: 2.69 µmol/mL
- **WNIN/GR-OB Pre injection C.M**: 2.15 µmol/mL
- **WNIN/GR-OB Post injection C.M**: 2.05 µmol/mL
Conclusion

• Our present data advocate for the beneficial effects of hPdMSCs as an acute phase response to reduce and negate IR by end of three weeks of treatment, appreciable more with Mutants as compared to Controls.

• Keeping in view of the fact that IR forms an important predisposing factor for the development of metabolic syndrome (diabetes, CV, etc) treatment with hPdMSCs open up new avenues in the management of obesity with IR.

• However, further studies are required to understand the interplay between the systemic and progenitor pool to assess the efficacy of hPdMSCs under obesity.
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