Therapeutic Role of Anti-Inflammatory Cytokine to Treat Insulin Resistance in Obesity and Aging

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International Congress of Diabetes & Metabolism, Seoul, Sept. 29, 2017
✓ Dating back as far as 1,550 B.C., the Ebers papyrus testifies to the long history of the prevalence of diabetes.

✓ Diabetes mellitus is described as a polyuric state.

✓ The recommended treatment was a 4-day course of a decoction of bones, wheat, grain, grit, green lead, and earth.
Type 2 diabetes is a chronic and progressive disease with insulin resistance as an early event that leads to β-cell failure, hyperglycemia, and associated complications.

Obesity is a major cause of insulin resistance and type 2 diabetes!
High Fat Intake and Lipid Cause Type 2 Diabetes

Dietary factors that influence the dextrose tolerance test.

Arch. Intern. Med. 40:818-830 (1927)

High fat, low carbohydrate diet used in treating diabetic patients would cause diabetes in normal people. "If you want a quick case of diabetes, just eliminate the carbohydrate from your diet for a couple of days and then take the test. It will demonstrate diabetes."

Sir Philip Randle

The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus.

Lancet 1:785-789 (1963)

Edward W. Kraegen “Ted”

Fat feeding causes insulin resistance, decreased energy expenditure, and obesity in rats.

Obesity Is a Complex State of Abnormal Lipid Metabolism, Hormones, and Immune System

- Fat Cells
  - Ectopic fat accumulation
  - ER/oxidative stress (JNK)
  - Mitochondrial dysfunction
  - ↓ Insulin signaling and glucose metabolism

- Insulin Resistance

- Adiponectin ↓
- Resistin ↑
- Fatty Acids ↑
- IL-6 ↑
- TNF-α ↑

- RBP4
- Betatrophin
- FGF21

- Myokine
Inflammation and Type 2 Diabetes

NIDDM as a disease of the innate immune system: association of acute-phase reactants and interleukin-6 with metabolic syndrome X

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Diabetologia 40:1286 (1997)

“Circulating Levels of Inflammatory Cytokines are Elevated in Obese and Diabetic Subjects.”
Inflammation Develops in Multiple Organs Including Skeletal Muscle, Adipose Tissue, and Liver in Obesity

Recently I have endeavoured to ascertain whether sodium salicylate has any definite influence on the sugar excretion in glycosuria and diabetes.

The British Medical Journal, 1901
Interleukin-10 Is a Major Anti-Inflammatory Cytokine

- IL-10
- IL-10Rβ
- IL-10Rα
- TYK2
- JAK1
- JAK1
- P
- STAT3
- Anti-inflammatory Genes
Transgenic Mice with Muscle-Specific Expression of IL-10 (M^{IL10})

Interleukin-10 Prevents Diet-Induced Insulin Resistance by Attenuating Macrophage and Cytokine Response in Skeletal Muscle


Hong, E.-G. et al., *Diabetes* 58:2525-2535 (2009)
Altered Interleukin-10 Signaling in Skeletal Muscle Regulates Obesity-Mediated Inflammation and Insulin Resistance

Sezin Dagdeviren, Dae Young Jung, Eunjung Lee, Randall H. Friedline, Hye Lim Noh, Jong Hun Kim, Payal R. Patel, Nicholas Tsitsilanos, Andrew V. Tsitsilanos, Duy A. Tran, George H. Tsougraris, Caitlyn C. Kearns, Cecilia P. Uong, Jung Yeon Kwon, Werner Muller, Ki Won Lee, Jason K. Kim

Program in Molecular Medicine and Department of Medicine, Division of Endocrinology, Metabolism and Diabetes, University of Massachusetts Medical School, Worcester, Massachusetts, USA; World Class University Biomodulation Major, Department of Agricultural Biotechnology, Seoul National University, Seoul, Republic of Korea; Wellness Emergence Center, Advanced Institutes of Convergence Technology, Seoul National University, Suwon, Republic of Korea; Faculty of Biology, Medicine and Health, University of Manchester, Manchester, United Kingdom

Whole Body Fat Mass (g; measured using $^1$H-MRS)

Weeks of High-Fat Diet

Wild-type

$^{IL10}$

Sezin Dagdeviren, Ph.D.
Hyperinsulinemic-Euglycemic Clamp to Assess Insulin Sensitivity

- Insulin (2.5 mU/kg/min)
- Variable 20% Glucose
- 2-[1-14C]-deoxyglucose (10 mCi)
- [3-3H]glucose (0.1 mCi/min)

Basal Period: 0 - 75 min
Clamp Period: 0 - 120 min

CMA microdialysis pump
Jugular vein cannulation
Tail blood sample
Obese Wild-type Mice Develop Insulin Resistance After 16 Weeks of High-Fat Diet

Whole Body Glucose Metabolism
(\(\mu\)mol/kg/min)


\*P<0.05 vs. Chow Diet
Obese $M^{IL10}$ Mice Are More Insulin Sensitive Due to Increased Glucose Uptake in Skeletal Muscle

Whole Body Glucose Metabolism
($\mu$mol/kg/min)

Muscle Glucose Uptake
(nmol/g/min)


*$P$*<0.05 vs. HFD-Fed WT mice
Increased Inflammatory Cytokines in Skeletal Muscle of Wild-type Mice After 16 Weeks of High-Fat Diet


*P<0.05 vs. Chow Diet
IL-10 Completely Suppresses Obesity-Mediated Inflammation in Skeletal Muscle


*P* < 0.05 vs. WT mice
Skeletal Muscle IL-10 Expression Improves Glucose Metabolism in Leptin-Deficient Ob/Ob Mice

Body Weight

Glucose Metabolism


*P<0.05 vs. Ob/Ob mice
Muscle-Specific Deletion of IL-10 Receptor (M-IL10R1−/−)

C57BL/6 background

MCK-Cre mouse  ×  IL-10R1^fl/fl mouse

Provided by Dr. Werner Muller (University of Manchester)

Loss of Muscle IL-10 Signaling Causes Hyperglycemia and Insulin Resistance After 6 Weeks of High-Fat Diet

Plasma Glucose

Whole Body Glucose Turnover


*P<0.05 vs. WT mice
Loss of IL-10 Signaling Exacerbates Diet-Induced Inflammation in Skeletal Muscle

Muscle Macrophage

Muscle TNFα


*P<0.05 vs. Chow, **P<0.01 vs. WT mice
Interleukin-10 Regulates Muscle Glucose Metabolism

IL-10Rα

IL-10Rβ

TYK2

JAK1

STAT3

STAT3

P

P

Reduces local inflammation

Increases glucose metabolism

Skeletal Muscle
Insulin Resistance Is a Major Characteristic of Aging and Affects Our Quality of Life and Life Span
Adipose Tissue Inflammation Develops with Aging in Mice at 16 Months of Age

Essential role of protein tyrosine phosphatase 1B in obesity-induced inflammation and peripheral insulin resistance during aging

Águeda González-Rodríguez,¹,² Jose A. Más-Gutierrez,³ Mercedes Mirasierra,¹,² Antonio Fernandez-Pérez,¹,² Yong J. Lee,⁴ Hwi J. Ko,⁴ Jason K. Kim,⁴,⁵ Eduardo Romanos,⁶ Jose M. Carrascosa,⁷ Manuel Ros,³ Mario Vallejo,¹,² Cristina M. Rondinone⁸ and Ángela M. Valverde¹,²

IL-10 prevents aging-associated inflammation and insulin resistance in skeletal muscle

Sezin Dagdeviren,* Dae Young Jung,* Randall H. Friedline,* Hye Lim Noh,* Jong Hun Kim,*† Payal R. Patel,* Nicholas Tsitsilianos,* Kunikazu Inashima,* Duy A. Tran,* Xiaodi Hu,* Marilia M. Loubato,* Siobhan M. Craig,* Jung Yeon Kwon,*† Ki Won Lee,*† and Jason K. Kim,*†,§,¶

*Department of Molecular Medicine, †Division of Endocrinology, Metabolism, and Diabetes, Department of Medicine, University of Massachusetts Medical School, Worcester, Massachusetts, USA; ‡Wellness Emergence Center, Advanced Institutes of Convergence Technology, Seoul National University, Suwon, South Korea; and §Department of Agricultural Biotechnology, Seoul National University, Seoul, South Korea


Body Weight

Sezin Dagdeviren, Ph.D.
IL-10 prevents aging-associated inflammation and insulin resistance in skeletal muscle

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*Department of Molecular Medicine, ‡Division of Cardiovascular Medicine, and †Division of Endocrinology, Metabolism, and Diabetes, Department of Medicine, University of Massachusetts Medical School, Worcester, Massachusetts, USA; ×Wellness Emergence Center, Advanced Institutes of Convergence Technology, Seoul National University, Suwon, South Korea; and §Department of Agricultural Biotechnology, Seoul National University, Seoul, South Korea


Whole Body Fat Mass

Sezin Dagdeviren, Ph.D.
Aging $M^{IL10}$ Mice Maintain Higher Energy Expenditure and Physical Activity than Aging Wild-type Mice

**Energy Expenditure**

(kcal/kg/hr using Metabolic Cages)

**Physical Activity**

(counts per day using Metabolic Cages)

*Dagdeviren, S. et al., FASEB J. 31:701-710 (2017)*

*P<0.05 vs. WT mice*
Aging M^{IL10} Mice Are More Insulin Sensitive Than Aging Wild-type Mice at 18 Months of Age

Whole Body Glucose Metabolism


*P<0.05 vs. Aging WT mice
Increased Glucose Metabolism and Insulin Signaling in Skeletal Muscle of Aging M$^{IL10}$ Mice

Muscle Glucose Uptake


*P<0.05 vs. Aging WT mice
Reduced Inflammation (Macrophages & Cytokines) in Skeletal Muscle of Aging M\textsuperscript{IL10} Mice

Muscle Inflammation

![Graph showing mRNA levels of CD68 and IL-6 for Aging Wild-type and Aging M\textsuperscript{IL10} mice. CD68 and IL-6 levels are significantly reduced in Aging M\textsuperscript{IL10} mice.]

Muscle Cytokines

![Graph showing protein levels using Luminex for IL-1\beta and MCP-1. IL-1\beta and MCP-1 levels are significantly increased in Aging Wild-type mice compared to Aging M\textsuperscript{IL10} mice.]


*P<0.05 vs. Aging WT mice
Obesity and aging are physiological states of low-grade systemic inflammation, and IL-10 may be used to treat skeletal muscle inflammation and insulin resistance.
Macrophage Is the Orchestrator of Obesity-Mediated Inflammation and Insulin Resistance

IL-10 Secretion by Alternatively-Activated Macrophage

• First discovered as a glucose regulated protein induced by glucose starvation

• GRP78  50% Homology  HSP70

• A major chaperone in the ER with ATPase activity
  a) Protein folding
  b) Degradation of misfolded protein
  c) Prevent protein aggregation

• Ca$^{2+}$-binding protein

• Regulator of ER stress signaling

• Potent anti-apoptotic protein

• Regulator of PI3K/AKT signaling
GRP78: A Master Regulator of the Unfolded Protein Response (UPR) and ER Homeostasis
Mice with Myeloid Cell-Specific Deletion of *Grp78* (Lyz-Grp78 KO)

C57BL/6 background

| LysM-Cre mouse | × | *Grp78* fl/fl mouse |

Myeloid Cell-Specific *Grp78* KO mouse (Lyz-Grp78 KO)

Jong Hun Kim, Ph.D.
Research Scientist
Seoul National University

Eunjung Lee, Ph.D.
Principal Scientist, KFRI

Manuscript in Review
GRP78 Is Reduced by ~70% in Peritoneal and Bone Marrow-Derived Macrophages of Lyz-Grp78−/− Mice

C57BL/6 background

LysM-Cre mouse × Grp78fl/fl mouse

**P<0.01, ***P<0.001 vs. WT mice
Monocyte and Macrophage Population in Spleen and Lymph Nodes Are Not Affected in Lyz-Grp78 KO Mice

Manuscript in Review
Lyz-Grp78 KO and Wild-type Mice Develop Obesity During 9 Weeks of High-Fat Diet

Manuscript in Review
Despite Similar Obesity, Lyz-*Grp78 KO* Mice Are More Insulin Sensitive with Increased Whole Body Glucose Turnover

**Fat Mass**
(measured using $^1$H-MRS)

Despite Similar Obesity, Lyz-Grp78 KO Mice Are More Insulin Sensitive with Increased Whole Body Glucose Turnover

**Whole Body Glucose Turnover**

*P<0.05 vs. Chow Diet, **P<0.05 vs. WT mice*
**Lyz-Grp78 KO Mice Have Increased Skeletal Muscle Glucose Metabolism After High-Fat Diet**

**Fat Mass**
(measured using $^1$H-MRS)

- WT
- Lyz-Grp78 KO

**Skeletal Muscle Glucose Metabolism**

- WT
- Lyz-Grp78 KO

*P*<0.05 vs. Chow Diet, **P*<0.05 vs. WT mice
Adipose Tissue Inflammation Develops in Obesity

**Obesity is associated with macrophage accumulation in adipose tissue**

Stuart P. Weisberg,¹ Daniel McCann,¹ Manisha Desai,² Michael Rosenbaum,¹ Rudolph L. Leibel,¹,³,⁴ and Anthony W. Ferrante, Jr.³,⁴


Hypertrophy & Hyperplasia of Adipocytes in Obesity

↑ IL-6 ↑ TNF-α
Obesity-Mediated Adipose Infiltration of Macrophages Is Reduced in Lyz-Grp78 KO Mice

Macrophages in White Adipose Tissue

**HFD-Fed WT**  **HFD-Fed Lyz-Grp78 KO**

![Bar charts showing relative mRNA expression of various markers in different macrophage subtypes.](image)

- **Macrophage** markers: CD68, F4/80
- **M1** markers: CD11c, NOS2
- **M2** markers: ARG1, IL-10, MGL1, MGL2, MRC2

*P<0.05, **P<0.01, ***P<0.001 vs. HFD-fed WT mice*

Manuscript in Review
Loss of GRP78 Induces Adaptive UPR with Increased Expression of \textit{ATF4}, \textit{ATF6}, \textit{sXBP1}, and \textit{Grp94} in Macrophages

Unfolded Protein Response in Bone Marrow-Derived Macrophages

- \textit{ATF4}
- \textit{ATF6A}
- \textit{sXBP1}
- \textit{Grp94}

* \(P<0.05\), ** \(P<0.01\) vs. HFD-fed WT mice
GRP78-Deficient Macrophages Are Polarized to Alternatively-Activated State in Skeletal Muscle

**Macrophage Polarization in Skeletal Muscle**

- **HFD-Fed WT**
- **HFD-Fed Lyz-Grp78 KO**

**Graphs:**
- **CD68**, **F4/80**, **CD11c**, **NOS2** (Macrophage)
- **ARG1**, **IL-10**, **MGL1**, **MGL2**, **MRC2** (M2)

*P* = 0.06

*P*<0.05 vs. HFD-fed WT mice
Anti-Inflammatory Cytokine IL-10 Is Increased in HFD-Fed Lyz-Grp78 KO Mice

Plasma IL-10 Levels

Chow Diet | WT | Lyz-Grp78 KO
---|---|---
High-Fat Diet | WT | Lyz-Grp78 KO

**IL-10 (pg/ml)**

**Plasma IL-10 Levels**

**P = 0.06**

**M2**

**ARG1** | **IL-10** | **MGL1** | **MGL2** | **MRC2**

**P<0.01 vs. HFD-fed WT mice**
Increased IL-6 Expression and Secretion by GRP78-Deficient Macrophages

**Plasma IL-6 Levels**

<table>
<thead>
<tr>
<th>Chow Diet</th>
<th>WT</th>
<th>Lyz-Grp78 KO</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-Fat Diet</td>
<td>WT</td>
<td>Lyz-Grp78 KO</td>
</tr>
</tbody>
</table>

**IL-6 mRNA (BMDM)**

**IL-6 Protein (BMDM Supernatant)**

*P<0.05 vs. HFD-fed WT mice*
Circulating IL-13 Level Is Increased by 7-fold in HFD-Fed Lyz-Grp78 KO Mice

Plasma IL-13 Levels

Spleen

Lymph Nodes

***P<0.001 vs. HFD-fed WT mice
Increased *IL-13* and *IL-13RA1* Expression in Skeletal Muscle of HFD-Fed Lyz-Grp78 KO Mice

**Skeletal Muscle**

<table>
<thead>
<tr>
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<th>HFD-Fed WT</th>
<th>HFD-Fed Lyz-Grp78 KO</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-13 mRNA</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>IL-13RA1 mRNA</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>IL-4RA mRNA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**C2C12 Cells**

<table>
<thead>
<tr>
<th></th>
<th>IL-6</th>
<th>IL-6+</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-13 mRNA</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>IL-13RA1 mRNA</td>
<td>**</td>
<td></td>
</tr>
</tbody>
</table>

*P<0.05 vs. HFD-fed WT mice

Manuscript in Review
Acute IL-13 Infusion Increases Insulin Action and Glucose Metabolism in Chow-Fed Lyz-Grp78 KO Mice

Glucose Infusion Rate

Whole Body Glucose Turnover

Muscle Glucose Uptake

Adipose Tissue Glucose Uptake

**P<0.05 vs. WT mice
Activated UPR stimulates IL-10 and IL-6 secretion by M2-macrophages, and increases myocyte expression of IL-13 signaling to increase glucose metabolism.
UMass Medical School in Worcester
Our Research is Funded by ....

NIH Grants: 2U2C DK-093000, R01 DK-080756, and R24 DK-090963
Welcome to the National Mouse Metabolic Phenotyping Centers

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New Publications

Adipocyte JAK2 mediated growth hormone-induced hepatic insulin resistance

Integrin-Linked Kinase 1 Is Necessary for the Development of Diet-Induced Hepatic Insulin Resistance

Triglyceride-rich lipoprotein lipolysis products increase blood-brain barrier transfer coefficient and induce astrocyte lipid droplets and cell stress
Authors: Lee LL, Aung HH, Wilson DW, Anderson SE, Rutledge JC, Rutkowski JM

A role for Gsa1, a regulator of DEAD-box RNA helicases, at centrosomes and basal bodies
Authors: Jao LE, Akef A, Wente SR

Contribution of Organic Anion-Transporting Polypeptides 1A1/1B to Dextrorubin Uptake and Clearance
Authors: Lee HJ, Leake BF, Kim RB, Ho RH

All Publications

Video Publications

Hyperinsulinemic-euglycemic Clamps in Conorous, Unrestrained Mice
Authors: Julio E. Ayala, Deanna P. Bracy, Carlo Malabanan, Freyja D. James, Tasneem Ansan, Patrick T. Fuegen, Owen P. McGuinness, David H. Wasserman
Working to Cure Diabetes