FGF21 Resistance in Adipose Tissues as a Cause of Insulin Resistance

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Our research focus: Adipokines and hepatokines in obesity-related cardiometabolic syndrome

Adipokines (adiponectin, A-FABP, LCN2...)

Hepatokines (FGF21, MUP1)

White Adipose Tissue

Liver

Pancreas

Brain

Skeletal muscle

Blood vessel
Adipokines characterized in our laboratory

**A-FABP**
(Xu & Tso et al, Circulation, 2007)
(Tso & Xu et al, Diabetes Care, 2007)
(Yeung D et al, ATVB, 2007)
Yeung D et al, Euro Heart J, 2008)
Hoo R, J Hepatology, 2012,

**Adiponectin**
(Xu A et al, J. Clin. Invest, 2003,
Cancer Res, 2006, Chow WS et al, Hypertension, 2006;
Liu M, PNAS, 2008, Hepatology, 2008, Cell Metabolism,
Metabolism, 2011, PNAS, 2012

**Lipocalin-2**
(Law I, Diabetes, 2010)
JBC, 2012; Liu Y, BJP, 2012

**FGF21**
Zhang X, Diabetes, 2008; Diabetes,
2010; Chen C; Diabetes Care, 2011;
Yu H, Clin. Chem, 2011; Chen W,
JBC, 2011; Ge X, JBC, 2011; Xiang
Y, JECM, 2011 ; Li H, Diabetes,
2012; J Hepatology, 2012; Ong L,
JCEM, 2012; Lin ZF, Cell
Metabolism, 2013
FGF21 as a metabolic regulator

- It is secreted mainly from the liver.
- Its major target is adipose tissue.
- Administration of recombinant FGF21 acutely decreases blood glucose to a normal level in both rodents and monkeys with diabetes.
- It does not have mitogenic activities.
Multiple beneficial effects of recombinant FGF21 in animals

- Glucose Uptake
- Body Weight
- Insulin Sensitivity
- Blood Glucose
- Fat Utilization
- Triglyceride
- Energy Expenditure
- Blood Insulin
- HDL-c
- Glucagon
- LDL-c

Coskun et al, Endocrinology, 2008
Xu et al, Diabetes, 2009
Kharitonenoskov et al, JCI, 2005
Adipose tissue as a major action site of FGF21
Multiple effects of FGF21 in adipocytes

Adipocyte

FGFR β-Klotho

Glut1 transcription

GLUT1

Glucose uptake

Lipolysis

PPARγ

AMPK

SIRT1

PGC1α

UCP1

Heat dissipation

Chow WS, 2012
FGF21 induces glucose uptake by inducing the expression of GLUT1 in adipocytes

(Ge X, J Biol. Chem. 2011, 286:34533-41)
FGF21 fine-tunes growth hormone-induced lipolysis in adipocytes

FGF21 Regulates PGC-1α and Browning of White Adipose Tissues

Cold or β3-Adrenergic Stimuli

FGF21

FGF21 Regulates PGC-1α and Browning of White Adipose Tissues

Promoter

UCP1 Production

Thermogenesis

Transdifferentiation

WAT

BAT
Adipocytes play an obligatory role in mediating the metabolic actions of FGF21

How does FGF21 exert its profound effects on systemic insulin sensitivity and glucose homeostasis via its actions in adipocytes?

Adiponectin as a mediator?
Adiponectin, an insulin sensitizing adipokine predominantly produced from adipocytes


HMW: High molecular weight
Multiple protective effects of adiponectin against a cluster of obesity-related disorders

- Insulin sensitivity
- Hypertension
- Myocardial infarction
- Cardiomyopathy
- Atherosclerosis

- Fatty liver, NASH
- Fibrosis

- Inflammation

- Vascular protection
  - (Cheng K, Diabetes, 2007)
  - (Chang J, Diabetes, 2010)
  - (Wang Y, Diabetes, 2011)
  - (Wong J, Cell Metabolism, 2011)

- Dyslipidemia
  - (Xu A, Endocrinology, 2005)

- Dyslipidemia

- Obesity-related asthma
  - (Shore S, J Allergy Clin Immunol, 2006)

- Obesity-related cancers
  - (Liu J, Carcinogenesis, 2011)
FGF21 induces both expression and secretion of adiponectin in mouse adipocytes.

FGF21 enhances adiponectin secretion in mouse adipocytes

**A**

**Pulse-chase experiment with $^{35}$S methionine**

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vehicle</strong></td>
<td>CL</td>
<td>CM</td>
<td>CL</td>
<td>CM</td>
</tr>
<tr>
<td><strong>FGF21</strong></td>
<td>CL</td>
<td>CM</td>
<td>CL</td>
<td>CM</td>
</tr>
</tbody>
</table>

**B**

**Percentage of adiponectin released**

- Vehicle
- FGF21

- CL: Cell lysates; CM: Conditioned medium
PPARγ agonists increase adiponectin expression and secretion

Suppression of PPARγ attenuates FGF21-induced expression and secretion of adiponectin

GW9662: PPARγ antagonist
FGF21 induces the expression of molecular chaperones involved in adiponectin secretion

**Ero1-Lα**

- Vehicle
- FGF21
- GW9662
- FGF21+GW9662

**DsbA-L**

- Vehicle
- FGF21
- GW9662
- FGF21+GW9662

**β-actin**

- Vehicle
- FGF21
- GW9662
- FGF21+GW9662

**PPARγ**

- Vehicle
- FGF21
- GW9662
- FGF21+GW9662

* p<0.05; ** p<0.01. n=5-6
FGF21 acts in an autocrine manner to induce adiponectin production in adipocytes

**A**

![Bar chart comparing Adiponectin mRNA levels in WT and FGF21KO mice under vehicle and Rosiglitazone treatment.](chart1.png)

**B**

![Bar chart comparing Adiponectin levels in WT and FGF21KO mice under vehicle and Rosiglitazone treatment.](chart2.png)

**C**

![Bar chart comparing Adiponectin levels in WT and FGF21KO mice under Rosi and anti-Fgf21 IgG treatment.](chart3.png)

*p<0.05; **p<0.01. n=5 in each group.
FGF21 induces adiponectin production in mice

A

Adiponectin concentration in mice fed Chow or HFD for 4 weeks or 35 weeks.

B

Change in FGF21 concentration over time in mice fed Vehicle or FGF21 (0.5 mg/kg/day).

C

Adiponectin concentration over time in mice receiving Vehicle or FGF21.

D

Absorbance at 280nm for HMW, Hexamer, and Trimer in Vehicle and FGF21 treatments.
The acute metabolic benefits of FGF21 are abrogated in adiponectin-deficient mice with dietary obesity.

A

B

C

D

**Figure A**: Glucose levels over time in WT+Vehicle, WT+FGF21, ADN(-/-)+Vehicle, and ADN(-/-)+FGF21 groups. *p < 0.05, **p < 0.01, ***p < 0.001.

**Figure B**: Insulin levels over time in WT+Vehicle, WT+FGF21, ADN(-/-)+Vehicle, and ADN(-/-)+FGF21 groups. *p < 0.05, **p < 0.01, ***p < 0.001.

**Figure C**: Triglycerides levels over time in WT+Vehicle, WT+FGF21, ADN(-/-)+Vehicle, and ADN(-/-)+FGF21 groups. *p < 0.05, **p < 0.01, ***p < 0.001.

**Figure D**: Western blot analysis showing phosphorylated Erk1/2 (p-Erk1/2) and total Erk1/2 (Erk1/2) in WT and ADN(-/-) with and without FGF21 treatment.
The beneficial Effects of FGF21 on glucose metabolism and insulin sensitivity are impaired in adiponectin KO mice.

The insulin-sensitizing effects of FGF21 in the liver are mediated by adiponectin

A

WT

FGF21
-
-
+
+
Insulin
-
+
-
+

p-Akt
Akt
p-GSK3β
GSK3β

Phosphorylation levels
0
2
4
6
8
10
*
**
*p<0.05; **p<0.01. n=5 in each group

B

ADN(-/-)

FGF21
-
+
+
+
Insulin
-
+
-
+

p-Akt
Akt
p-GSK3β
GSK3β

Phosphorylation levels
0.0
0.5
1.0
1.5
2.0
2.5
*
***
Adiponectin is required for FGF21-mediated alleviation of fatty liver disease in obese mice

A

WT

ADN(-/-)

B

C

*p<0.05; **p<0.01. n=6 in each group
Adiponectin is obligatory for FGF21-mediated reduction of HFD-induced lipid accumulation in skeletal muscle.
The insulin-sensitizing effects of FGF21 in skeletal muscle are dependent on adiponectin.
Adiponectin confers the metabolic actions of FGF21 in the liver and skeletal muscle

Serum FGF21 levels are significantly elevated in overweight/obese subjects

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**Serum FGF21 levels**

- **Lean**: N=105
- **Overweight/obese**: N=127

**r² = 0.229, p<0.001**

**BMI (kg/m²)**

**Log Serum FGF21 levels**

**Log Visceral fat (%)**

- **Lean**
- **Obese HFF<5.5%**
- **Obese HFF≥5.5%**

**N=217**

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**Zhang, X., et al. Diabetes 2008**

**Giannini C et al., J Clin Endocrinol Metab. 2013**
Elevated circulating FGF21 is associated with a cluster of obesity-related complications.

**Coronary heart disease**

**Atherosclerosis**
Chow WS, et al. ATVB. 2013

**Diabetes**

**NAFLD**
Li, H., et al. J Hepatol 2010
Dushay, J., et al. Gastroenterology 2010

**Obesity**
Reinehr, T. et al. JCEM 2012

**Metabolic syndrome**
Reinehr, T. et al. JCEM 2012

**Diabetic Nephropathy**

**Serum FGF21**
Elevated FGF21 production in obese animals

**Genetic-induced obesity**

- **Tissue**
  - Liver

- **Serum**
  - FGF21 Protein (ng/mL)

**Diet-induced obesity**

- **Liver**
- **Epid**

**FGF21 Resistance**

Impaired actions of FGF21 in ob/ob obese mice

Fasting glucose and free fatty acid

Glucose clamping

The glucose-lowering effects of FGF21 are progressively decreased in High Fat High Cholesterol (HFHC) diet-induced obese mice.
A progress development of FGF21 resistance during diet-induced obesity

Serum triglyceride levels (fold changes)

Time after FGF21 injection

0 min, 30 mins, 60 mins, 120 mins

24 weeks, 20 weeks, 12 weeks, 8 weeks, 4 weeks, Baseline

HFHC diet induction

FGF21: 1 mg/kg; n=5
The ability of recombinant FGF21 (rmFGF21) to increase circulating adiponectin is progressively impaired in diet-induced obesity.
FGF21-induced signal transduction pathways in adipose tissues are impaired in obesity

Mechanisms of FGF21 resistance?

- Adipocyte
- ERK1/2
- SRF
- Elk-1
- Glut1 transcription
- GLUT1
- Glucose uptake
- PPARγ
- FGF21
- AMPK
- SIRT1
- PGC1α
- UCP1
- Heat dissipation

Chow WS, 2012
A marked down-regulation of β-klotho and FGFR1 in different fat depots in obese mice

**HFHC**: High fat high cholesterol diet
A marked down-regulation of β-klotho and FGFR1 in different fat depots in obese mice
How does obesity cause reduced \(\beta\)-klotho and FGFR1 expression?
Involvement of TNFα-JNK pathway in modulating β-klotho expression???

FGF21 resistance as a cause of systemic insulin resistance

Adipocytes

FGF21

βklotho

FGFR1c

Liver

Muscle

Blood vessel

Autocrine

Endocrine

Insulin Sensitivity

APN

Obesity

Systemic Insulin resistance
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