The Role of AEBP1 in Sex-Specific Diet-Induced Obesity

Adipogenesis

Fat mass ↑
Adipocyte hyperplasia
Adipocyte hypertrophy

Adipocyte Enhancer-Binding Protein 1 (AEBP1)

- Transcriptional factor
  - carboxypeptidase (CP) activity
- Transcriptional repression activity
  (He G-P et al., 1995)

AEBP1 gene
AEBP1(748 aa)
AORTIC CARBOXYPEPTIDASE-LIKE PROTEIN

ACLP(1128 aa)
Preadipocyte Differentiation & Proliferation
Vascular Smooth Muscle Cell Differentiation

Obesity

Energy intake (food)

Metabolism

Energy storage (fat)

Energy expenditure

Adipocyte Enhancer-Binding Protein 1 (AEBP1)

MEK 1/2
ERK 1/2
MKP-3

RTKs

c-Raf

Transcription

Kim et al., 2001
Phosphatase & Tensin homolog (PTEN)

- Tumor suppressor gene
- Specific lipid-phosphatase activity
- Inhibitor of pAKT pathway

Role of PTEN in the PI 3-kinase/Akt pathway

AEBP1 promotes cell survival & growth

AEBP1 Modulates In Vivo Adiposity

Material & Method

Transgenic AEBP1
**Transgenic AEBP1**

F1 mice

**RNA Analysis**

- Northern blot
  - 20 μg of Total RNA
  - Formaldehyde denaturing
  - 1% agarose gel
  - Nylon membrane
  - 32P-labeled AEBP1 cDNA
  - X-ray film

- 20 μg of Total RNA
  - Reverse transcriptase
  - cDNA
  - Primer leptin
  - 1.4% agarose gel
  - UV light

**Animal Experiments**

- **AEBP1 transgenic**
  - SD
  - HPD (45% fat)

- **Nontransgenic control mice**
  - SD
  - HFD (45% fat)

- Free access to food & water ad libitum

- Weighed q wk

**Animal Experiments**

- **Glucose levels measurement**
  - Fed & fasted glucose levels q 3 wks (mmol/l)

- 32-week later:
  - Insulin level (ng/ml) & leptin levels (ng/g)
  - Total serum cholesterol (mg/dl)
  - Triacylglycerides (mg/dl)

**Insulin Tolerance Test**

- Fast for 16 hr

- Insulin 0.75 units/kg

- Fasted & fed blood glucose levels: LifeScan Sure-Step Glucometer

**Glucose Tolerance Test**

- Fast for 16 hr

- Glucose 1 mg/g

- Fasted & fed blood glucose levels: LifeScan Sure-Step Glucometer
Western Blot Analysis

**RESULTS & DISCUSSION**

**Western Blot Analysis from TG and control**

- **WAT**
  - AEBP1 protein
    - TG > NT (4.5-fold)
  - PTEN protein
    - TG < NT

In TG, AEBP1 increase only in WAT

**Growth patterns of TG & NT mice on HFD**

- **Total food consumption**
  - Food consumption
    - TG female > NT female
      - 1.3 fold

- **Energy Intake**
  - Energy intake
    - TG female ~ NT females
massive obesity

Hyperphagia

feed efficiency ↑
(BW/kcal eaten)

Average feed efficiency

TG females

• WAT↑1.5-fold

Average values of total WAT

The AEBP1 Modulates In Vivo

• AEBP1 gene & diet interact to influence fat mass in a sex-specific manner
  Only female AEBP1 TG → Fat↑

• Leptin ↔
  Leptin expression is not regulated by AEBP1

• Obesity associates with adipocyte hypertrophy
  - blood glucose↑
  - Insulin ↑
  - FFA↑
  - Leptin ↑

This study

Expanding fat mass may not be associated with adipocyte hypertrophy

• This study
  • Blood glucose ↔
  • Insulin ↔
  • Cholesterol & triacylglyceride ↔
  • Leptin ↔

AEBP1 expression is modulated by aging in a sex-specific manner

NT female mice

AEBP1 expression is not sufficient to explain for massive obesity in female TG mice

NT female mice

AEBP1 expression is modulated by aging in a sex-specific manner

In NT female mice:
  Age ↑ → AEBP1 ↓ → weight ↑

In NT male mice:
  Age ↑ → AEBP1 ↑ → weight ↑
AEBP1 expression is modulated by diet in a sex-specific manner

Female mice

AEBP1 expression is modulated by diet in a sex-specific manner

NT Male mice

AEBP1 expression is modulated by diet in a sex-specific manner

Hyperactivation of Akt with Adipocyte Hyperplasia in WAT of TG Mice

To evaluate the mechanisms involved in AEBP1 action on PTEN and PI3K activation in TG mice

In Vivo Insulin Stimulation

- Somnotol 40-50mg/kg (intraperitoneal injection)
- Laparotomy

- Insulin 5 units inferior vena cava
- remove Mammary gland & WAT (control)
- remove Mammary gland & WAT (experiment)
- protein lysates
- Western blot analysis

Hyperactive Akt with Adipocyte Hyperplasia in WAT of TG Mice

Massive obesity → only in the female TG mice was due to

- Age and Sex-specific HFD-mediated induction of endogenous AEBP1 expression
Protein Analysis

HRP + Luminol ECL

HRP-conjugated secondary antibody

Specific primary antibody

Antigen of interest

Non-specific protein

Nitrocellulose membrane

Detected by film

Fat Pad Weight, Cellularity, and Histology

Visceral fat pads

weighed

flash-frozen in liquid nitrogen

• Fat cell size & number determined by method of Hirsch & Gallian
• Tissue stained with hematoxylin & eosin → histological analysis

RESULTS & DISCUSSION

TG NT

p-Akt normalized by Akt

p-Gsk normalized by Akt

- before insulin injection

+ after insulin injection

Hyperactivation of Akt with Adipocyte Hyperplasia in WAT of TG Mice

↑ AEBP1

↓ PTEN function

↑ PI3K/Akt

↑ Cell proliferation (Hyperplasia)

↓ Adiposity
Hyperactivation of Akt with Adipocyte Hyperplasia in WAT of TG Mice

♀ TG +HFD → adiposity ↑

↑ proliferative preadipocytes

PTEN-specific KO in adipocyte
• glucose tolerance ↔
• insulin sensitivity ↔
• adiposity ↑

This study
AEBP\(^{TG}\) mice - PTEN↓
• glucose tolerance ↔
• insulin sensitivity ↔
• adiposity ↑

aP2 promoter & upstream regulatory sequence in BAT, WAT, macrophage

↓ PTEN in BAT and Macrophage

↓ adiposity & insulin sensitivity

AEBP\(^{TG}\) mice - PTEN↓
• glucose tolerance ↔
• insulin sensitivity ↔
• adiposity ↑

FIRKO mice
Insulin resistance only in adipose tissue
But whole-body glucose metabolism ↔
70-90% glucose metabolism in skeletal muscle
(Weisberg SP et al. 2003)

Adipocyte size ↑ → insulin sensitivity ↓

Estrogen modulates AEBP1 expression

E plays an important role in WAT regulation

OVX mice → WAT ↑
OVX mice + E\(_2\) → WAT↑
= postmenopausal → WAT↑
postmenopausal + E\(_2\) → WAT↑

To examine AEBP1 expression in OVX mice and OVX+E\(_2\) mice

Adipocyte size ↑ → insulin sensitivity ↓ ??
Animal Experiments

Ovariectomy (OVX mice)  Ovariectomy (OVX mice)  Sham Operation (Control mice)

AEBP1 expression in WAT

Approved by: Institutional Animal Care Committee

Luciferase reporter assay

AEBP1 promoter  Luciferase gene

Transfection in CHO cell

ERα  ERβ  ERα + ERβ

Luciferase assay

RESULTS & DISCUSSION

Estrogen modulates AEBP1 expression

E → AEBP↓
E → AEBP↑

AEBP1 expression is regulated by estrogen

• OVX mice → Adipocyte hypertrophy
• AEBP1↑ mice → Adipocyte hyperplasia

AEBP↓ in OVX mice

Adipocyte hypertrophy

Obesity

Estrogen receptor (ER): ER α & ER β

ERα↑ → AEBP↓
Estrogen modulates AEBP1 expression

WAT ↑ in ERα–KO, AEBP1^TG

Both hyperplasia & hypertrophy

ERα↓ AEBP1↑ Adipocyte Hyperplasia

Estrogen modulates AEBP1 expression

WAT ↑ in ERα-KO mice

Adipocyte hypertrophy

ERβ ↑ AEBP1↑

ERα: ERβ ↓ AEBP1↑

Estrogen modulates AEBP1 expression

E_2 → human preadipocyte proliferation ↑

Cowley SM et al. (1997), Anderson LA et al. (2001)

AEBP1 ↑ in 3T3-L1 preadipocytes → proliferation rate ↑

Kim S et al. (2001)

ERβ ↑ AEBP1↑

Conclusion

- Female: AEBP1 ↑ → feed efficiency
- AEBP1 ↑ → PTEN↓ → adipocyte hyperplasia
- ERα → AEBP1↓
- ERβ → AEBP1↑
- AEBP1 may be a novel effector of estrogen action that is specific for adipose tissue & pathways related to lipid metabolism on the regulation of central obesity

Future Study

- The role AEBP1 will lead to the identification of potential molecular targets → lead to novel methods of anti-obesity therapies
Thank you