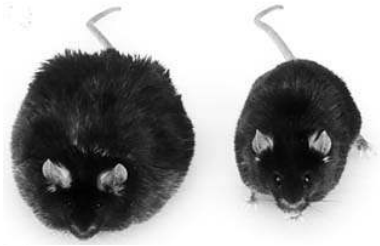
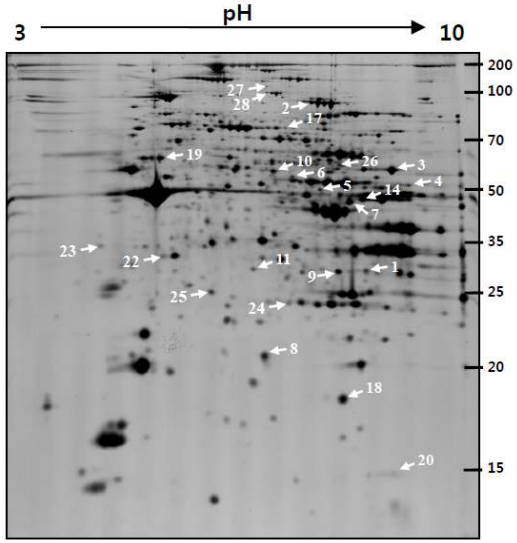
대한당뇨병학회 2012.5.10

Proteomic approach for mining biomarkers in diabetes and obesity



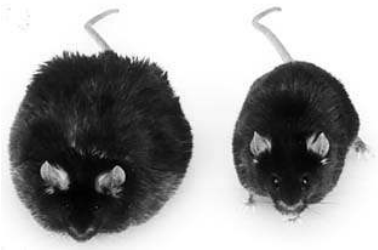


윤 종 원

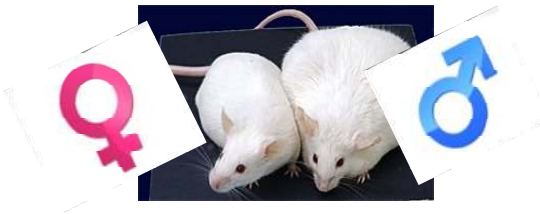
대구대학교 생명공학과

Contents

- Proteomics ?
- Proteomic approach for diabetes
- Proteomic approach for obesity
  - Obesity susceptibility and resistance in diet-induced obese rats



  - Gender differences in obesity development in diet-induced obese rats



Why proteome (protein+ome) ?

- **Genomics의 한계 : 염기서열만으로는 단백질의 기능을 알 수 없다.**

HUPO
Human Proteome Organisation

KHUPO

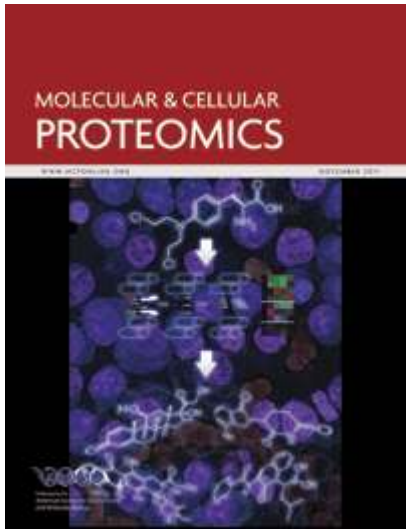
- **Proteomics 연구목표**

1. 다양한 단백질들이 세포 내외의 변화에 따라 어떻게 변화하는가?

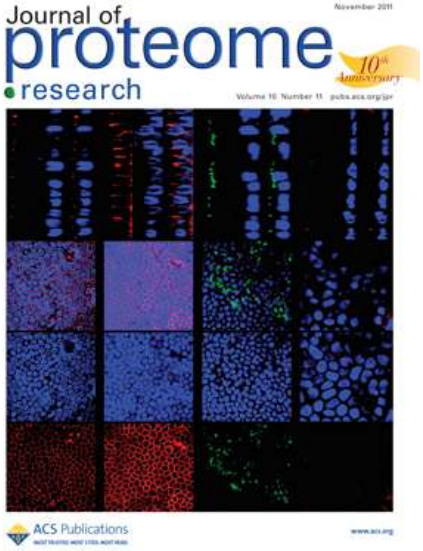
2. 단백질들의 발현이 어떤 양상으로 움직이는가?

3. 단백질간의 상호 작용이 어떻게 이루어지는가?

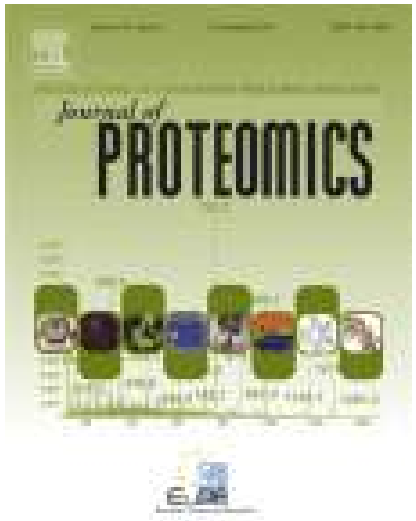
Proteomics journals



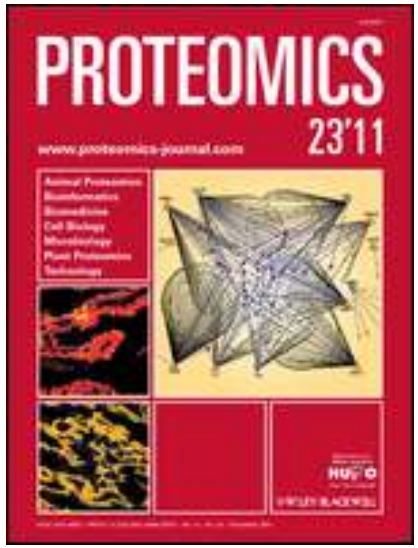
IF 9.392



IF 5.617



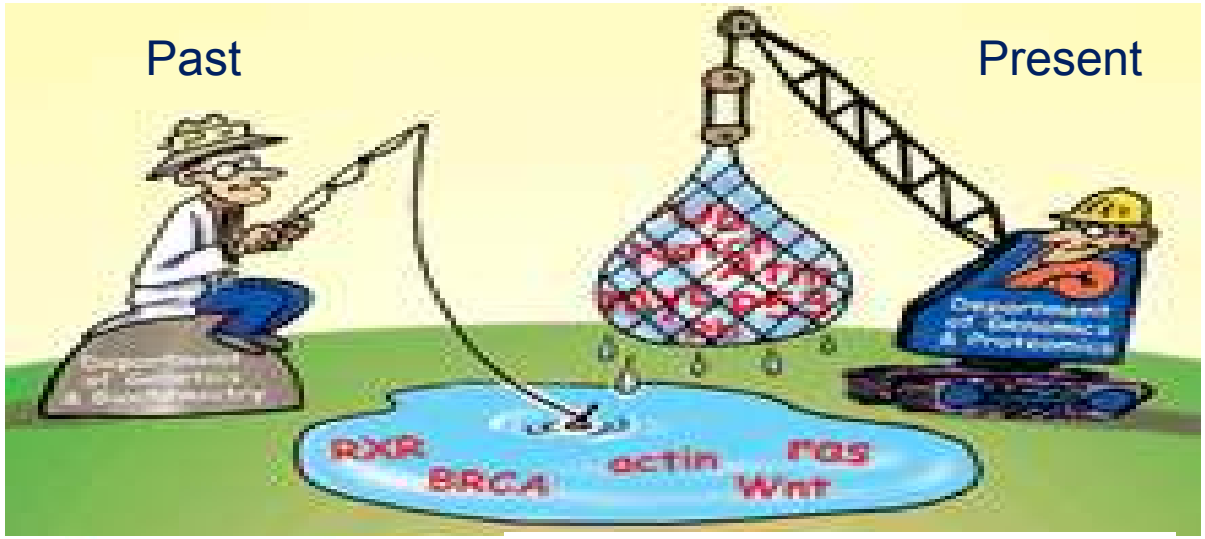
IF 5.080

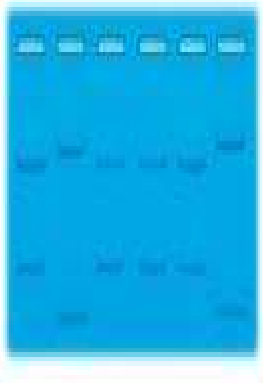


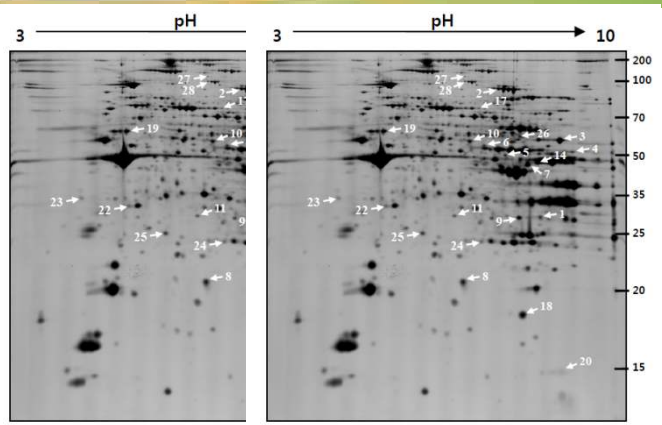
IF 4.940

IF: 5 years' average

What is proteomics ?





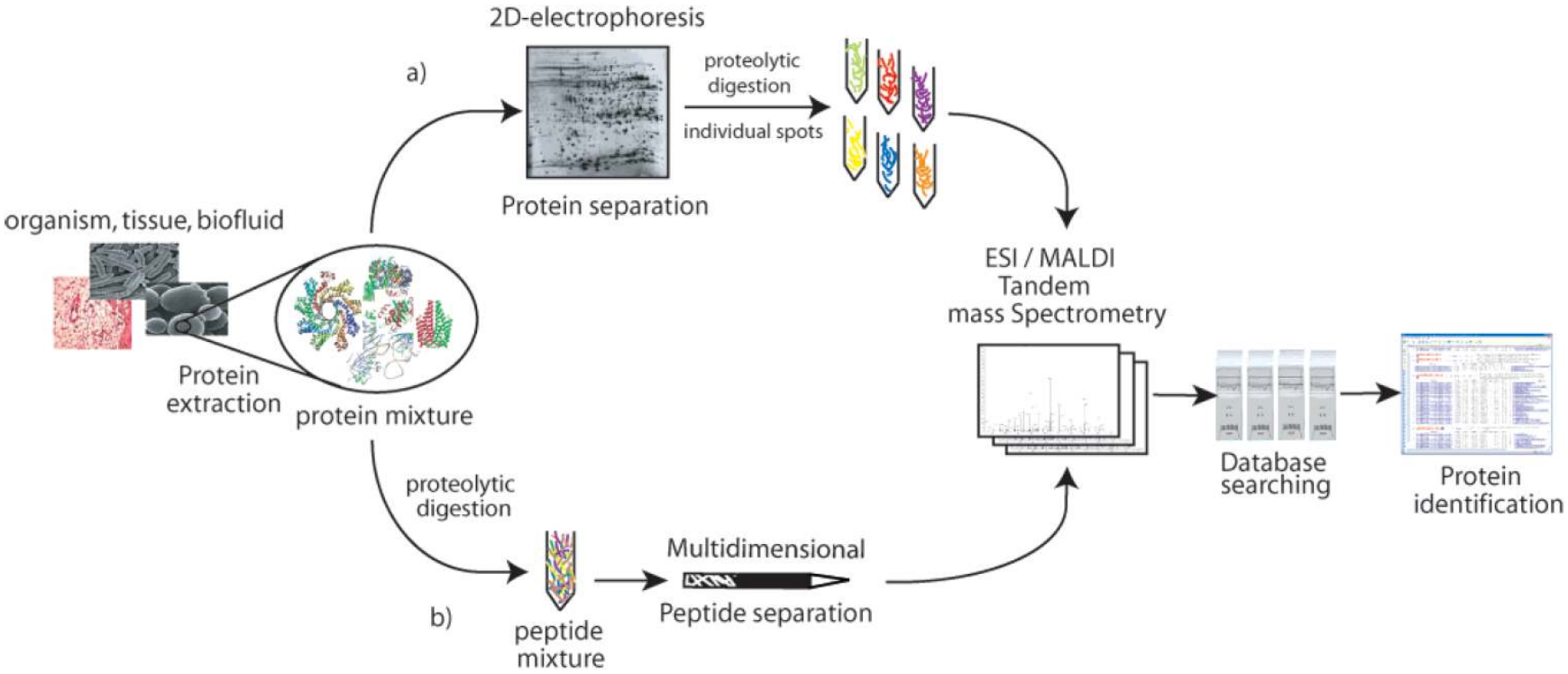


Control vs. Disease state

How to analyze proteome ?

1 Gel-based proteomics

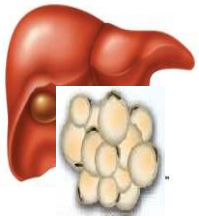
combination of two-dimensional electrophoresis combined with mass spectrometry



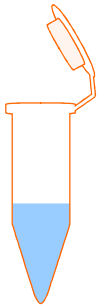
2 Gel-free proteomics (shotgun)

combination of high performance liquid chromatography combined with mass spectrometry

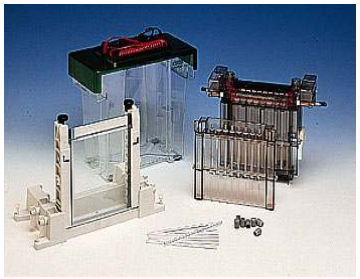
Gel-based proteomics

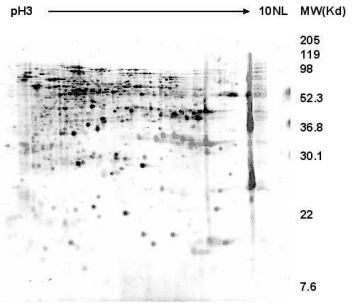


Target tissue

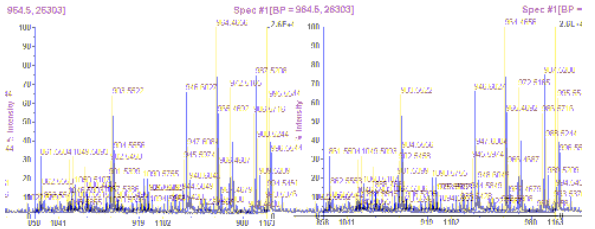


Protein isolation

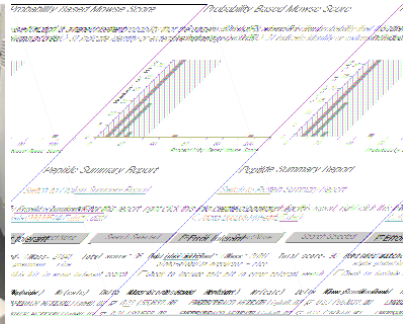




Proteome mapping by 2-dimensional electrophoresis



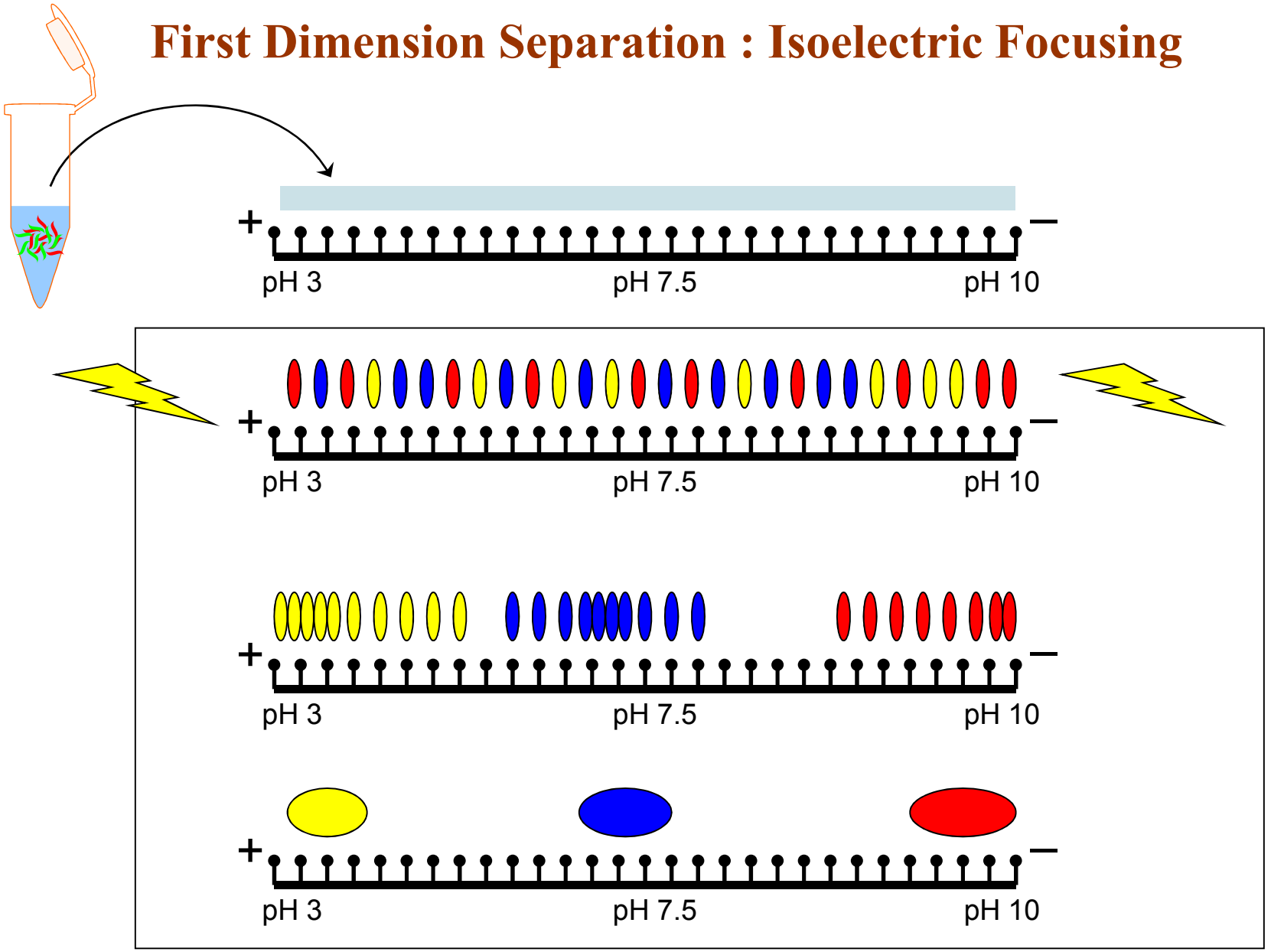
Peptide separation by Mass spectrometry



Protein identification by database search

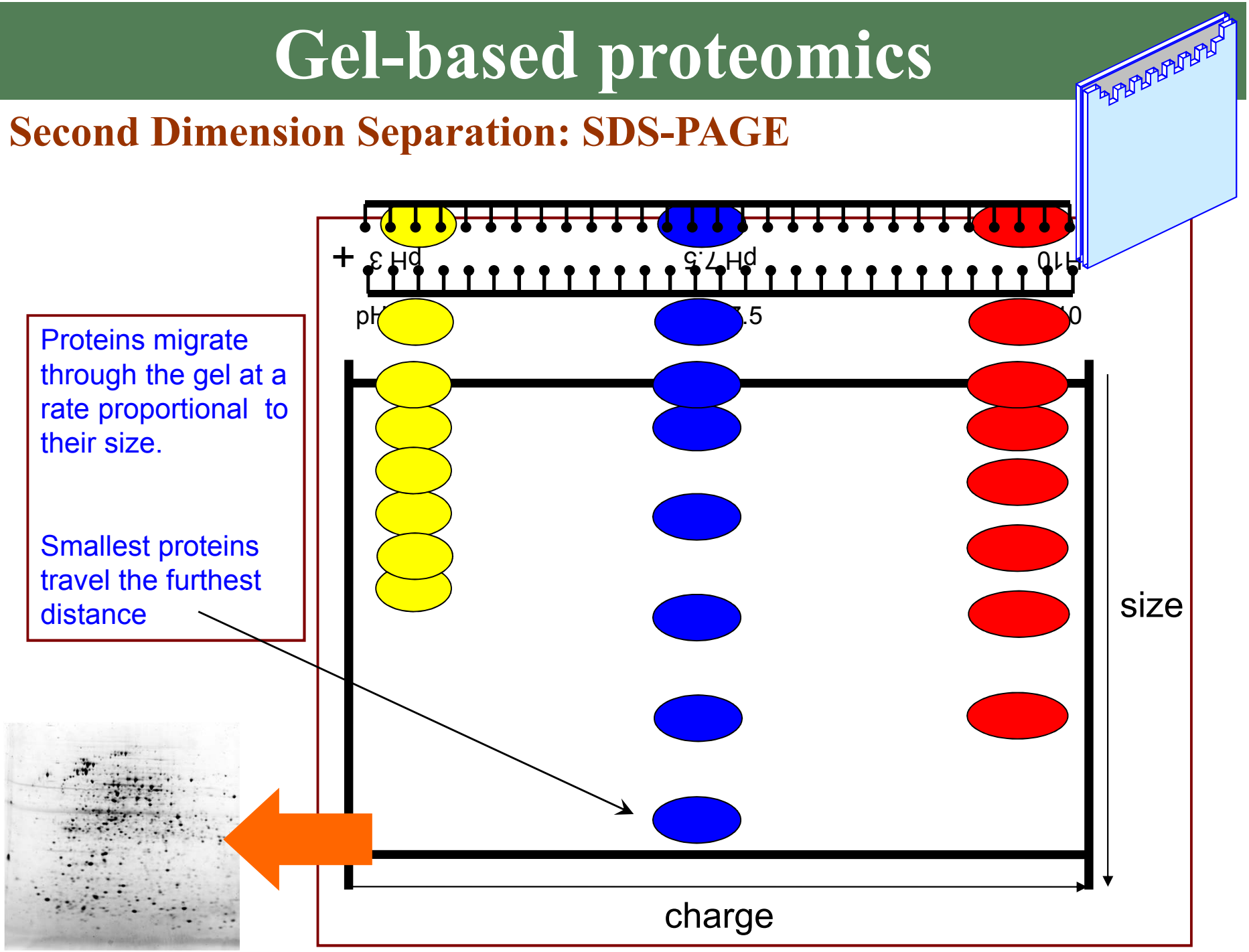
Gel-based proteomics

First Dimension Separation : Isoelectric Focusing



Gel-based proteomics

Second Dimension Separation: SDS-PAGE

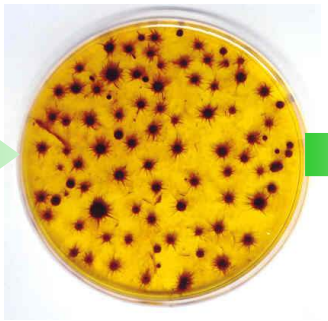


Part I

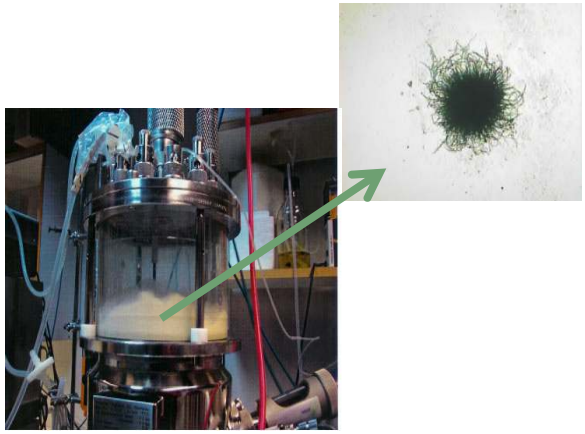
Proteomic approach for diabetes

Anti-diabetic mushroom exopolysaccharides (EPS)

Mushroom in field



in agar plate



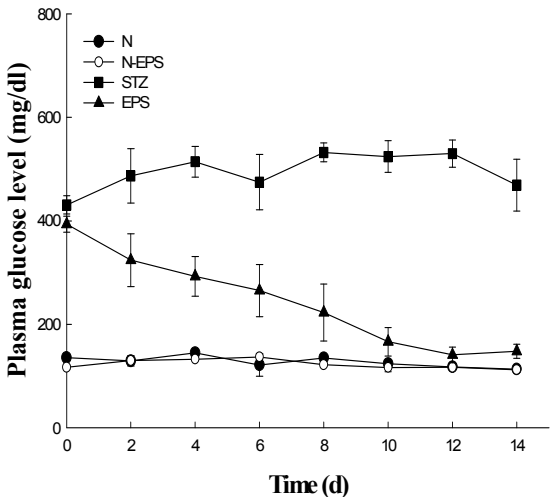
in bioreactor

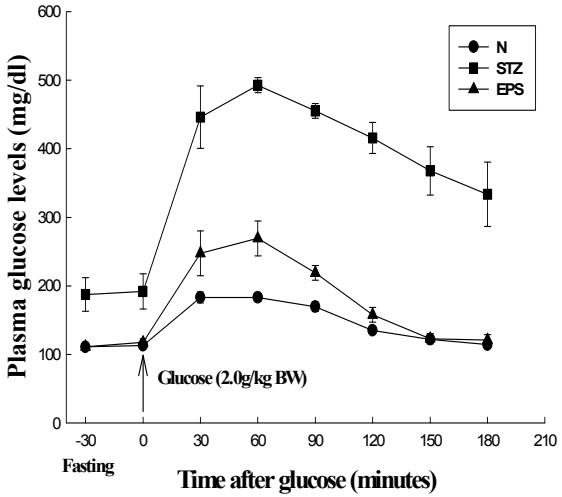
EPS production

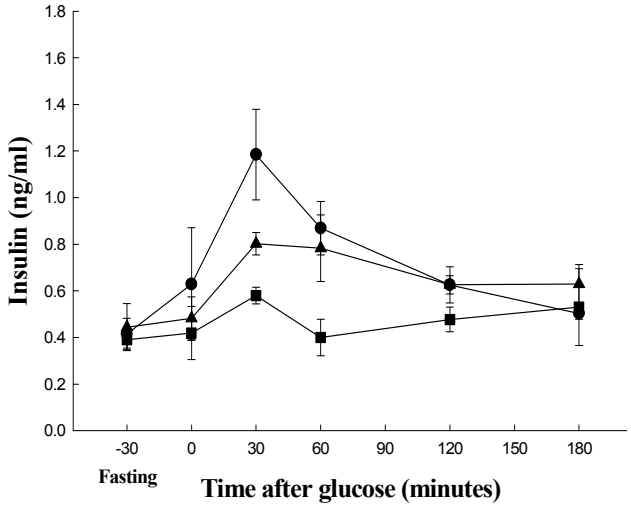
Anti-diabetic activity of EPS

STZ-induced diabetic rat model

(●) Normal, (■) STZ-diabetic, (▲) EPS-treated diabetic group







Glucose-lowering effect

Glucose tolerance

Insulin release

Kim et al. *Proteomics* 2006, 6, 5291–5302

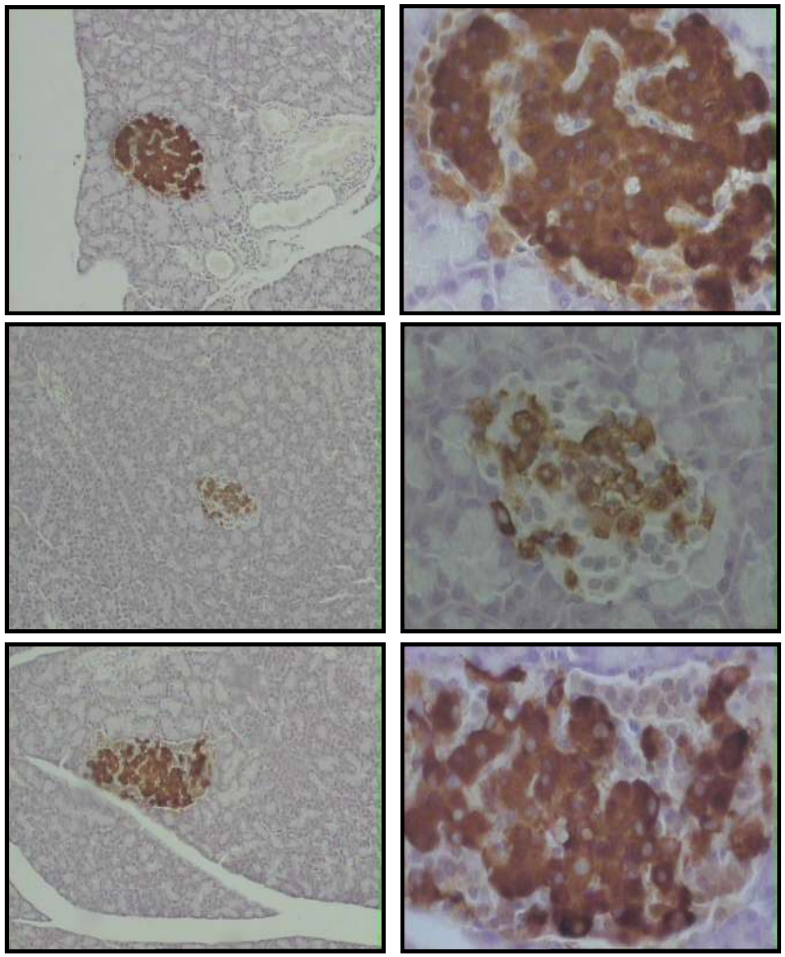
Anti-diabetic activity of EPS

Insulin immunohistochemical staining
before and after EPS therapy

Normal
healthy group

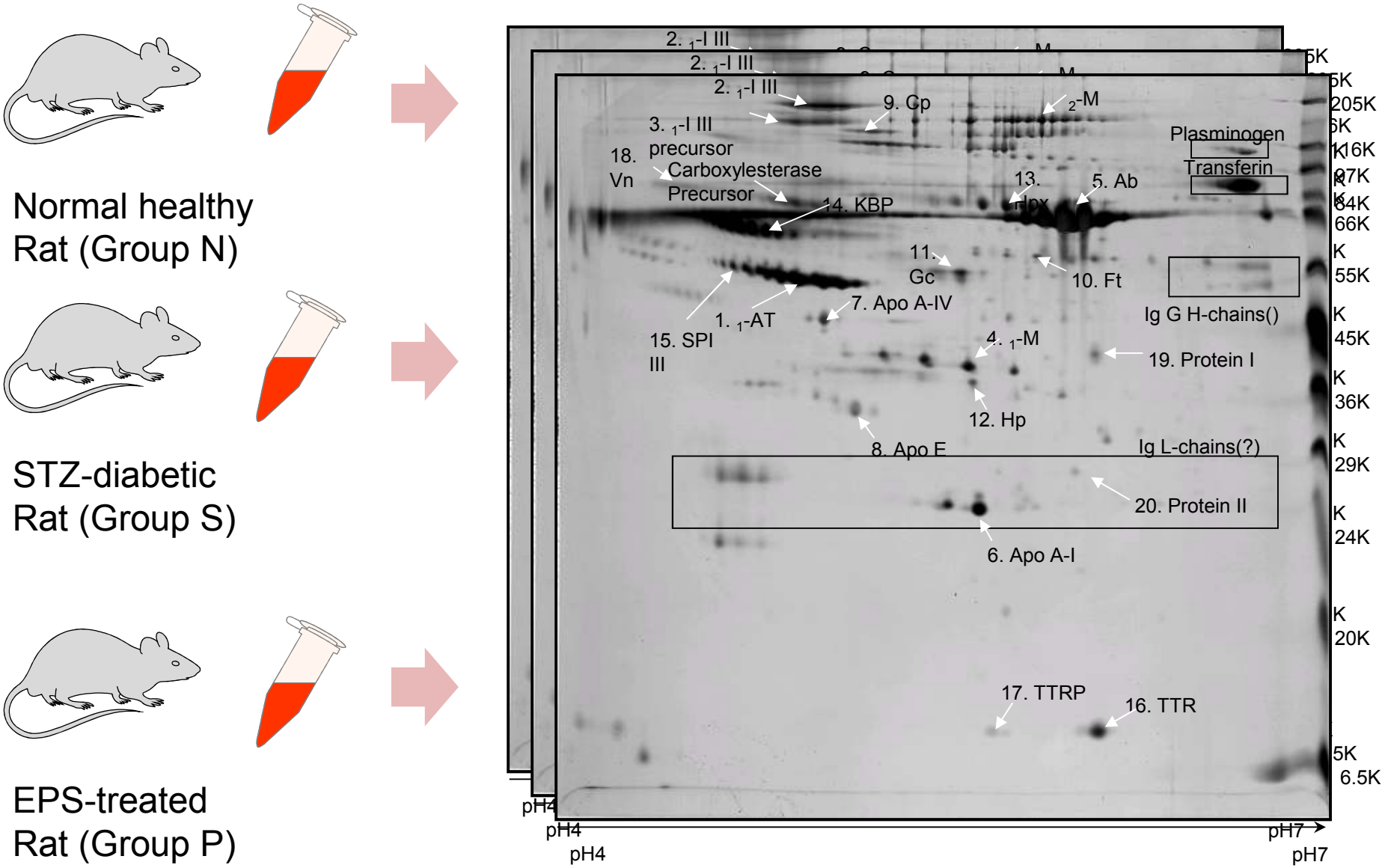
STZ-induced
diabetic group

EPS-treated
diabetic group

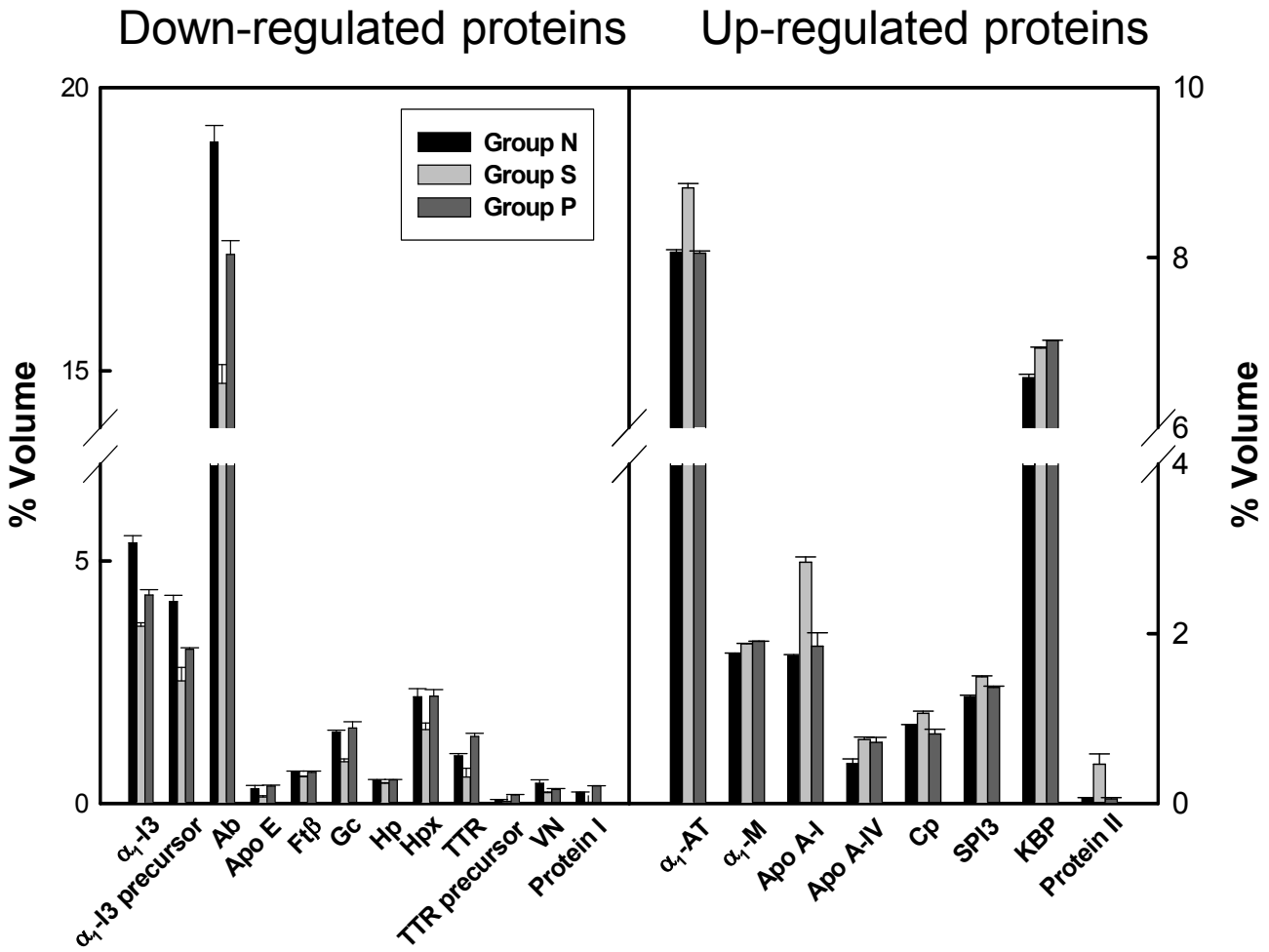


Kim et al. *Proteomics* 2006, 6, 5291–5302

Proteome analysis of plasma by 2-DE

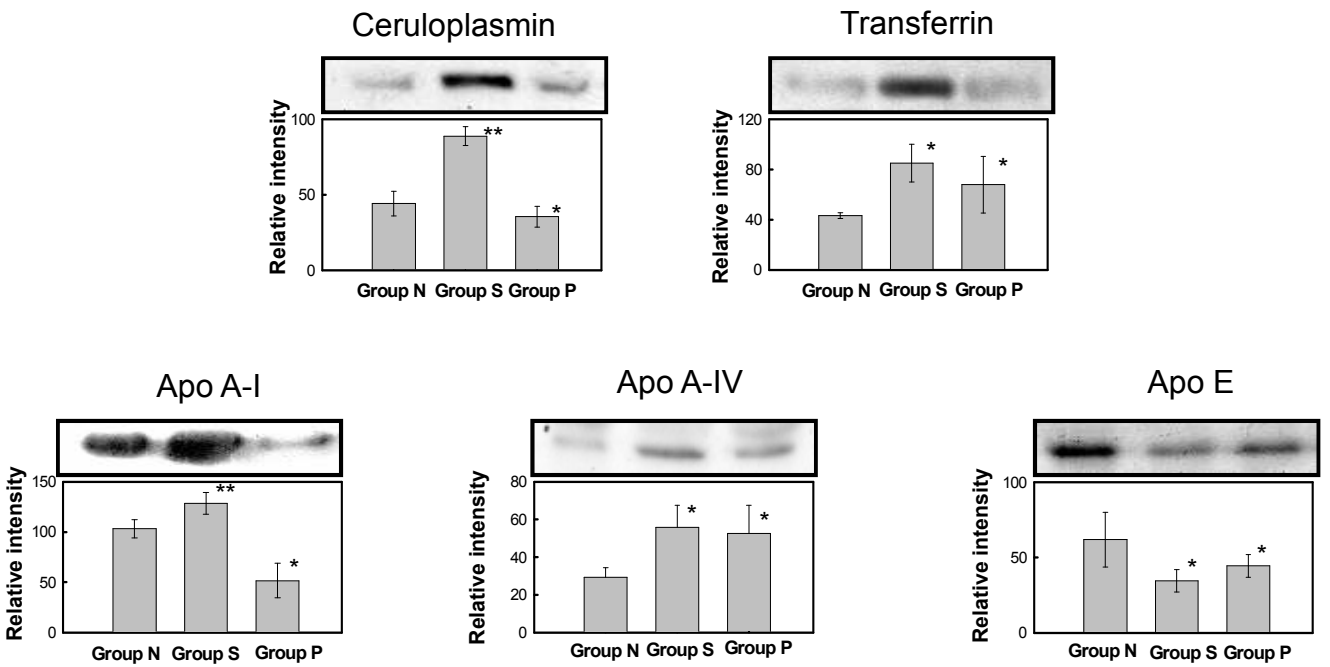


Changes in concentration of the rat plasma proteins



Kim et al. *Proteomics* 2006, 6, 5291–5302

Confirmation of differential regulation for five plasma proteins by Western blot analysis



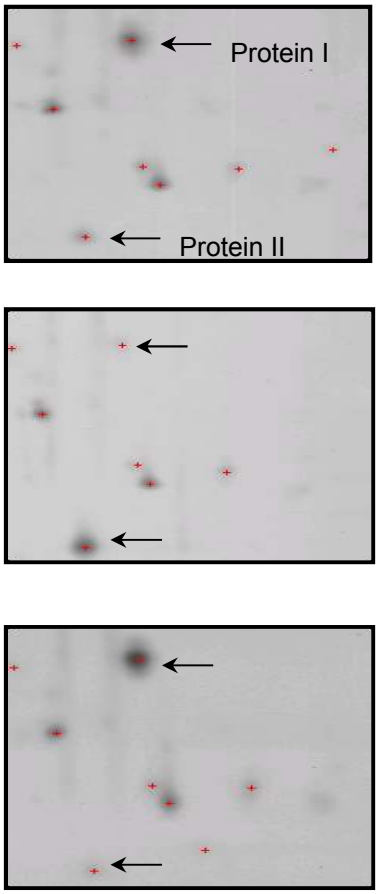
Kim et al. *Proteomics* 2006, 6, 5291–5302

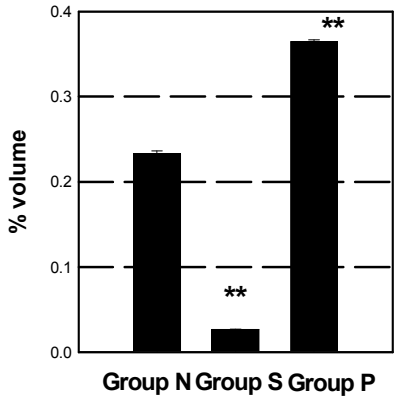
Finding novel plasma marker proteins for diabetes

Normal healthy group

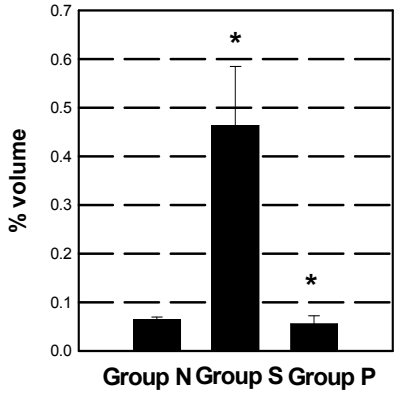
STZ-induced diabetic group

EPS-treated diabetic group





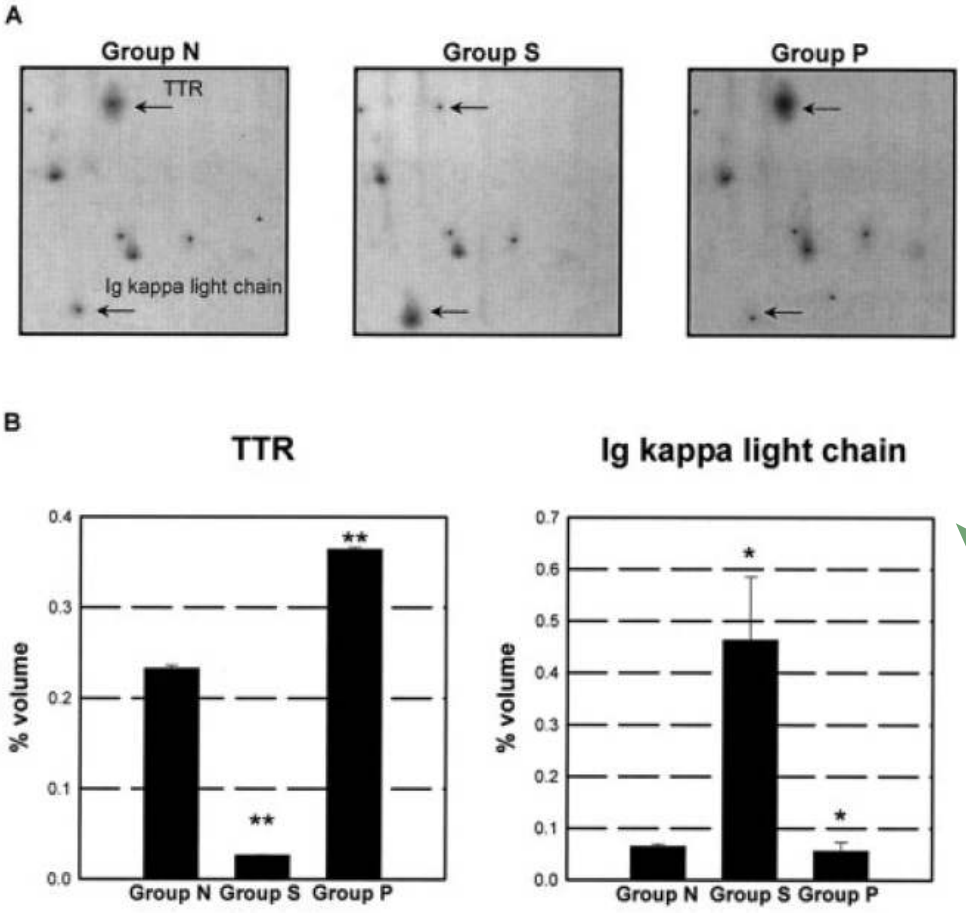
Protein I



Protein II

Kim et al. *Proteomics* 2006, 6, 5291–5302

Finding novel plasma marker proteins for type 1 diabetes



Identification by MS/MS

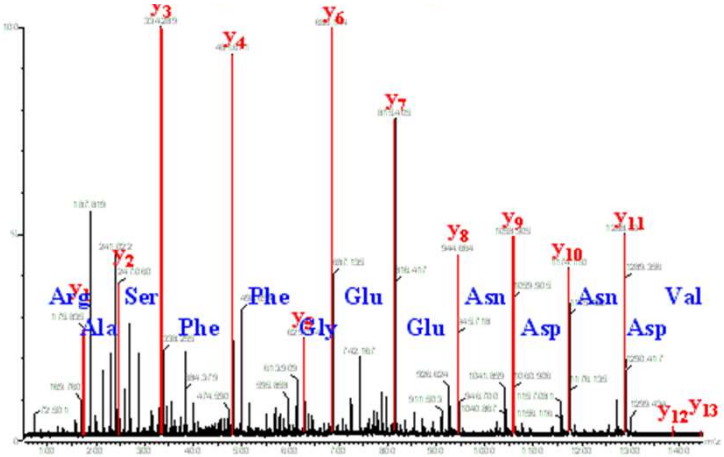
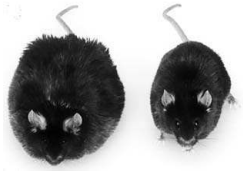
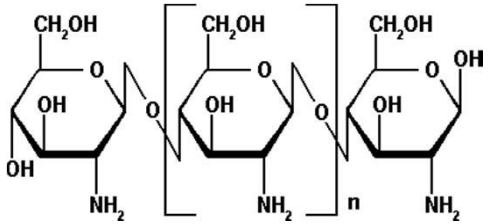


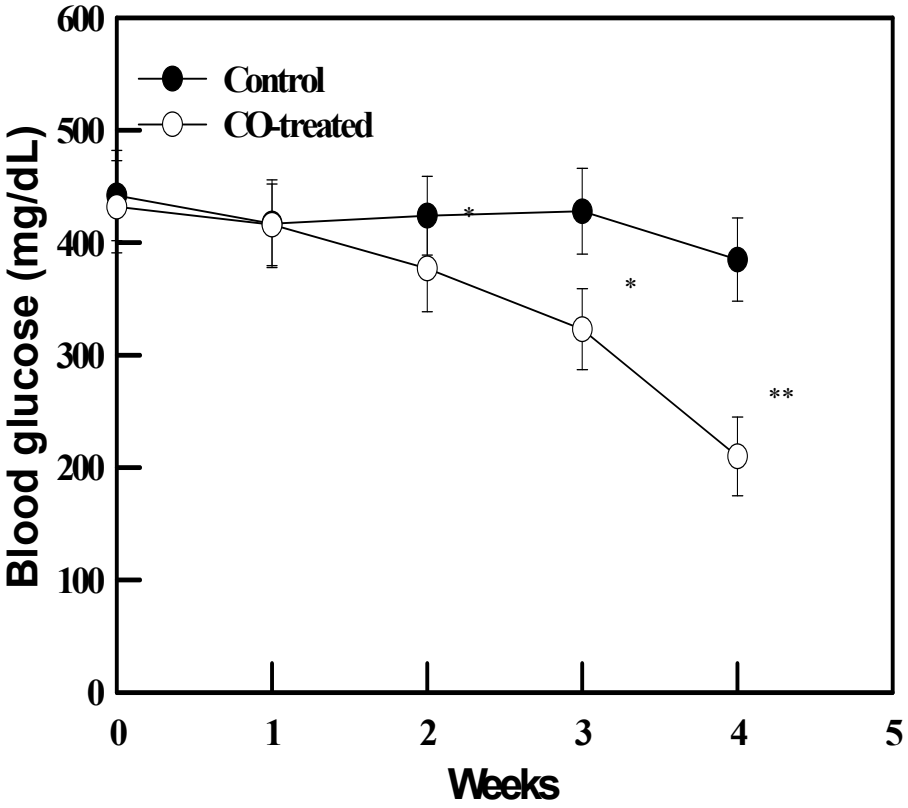
Figure 4. Protein alterations of TTR tetramer and Ig kappa light chain. (A) Patterns of TTR tetramer and Ig kappa light chain in the 2-DE map, (B) Histograms of TTR tetramer and Ig kappa light chain showing the relative spot volume by a differential protein analysis. Notations for each group are the same as in Fig. 1. For each spot, the relative intensity was averaged and expressed as a mean ± SEM of the three separate experiments. $*p < 0.05$ *vs.* Group N or S, $**p < 0.001$ *vs.* Group N or S.

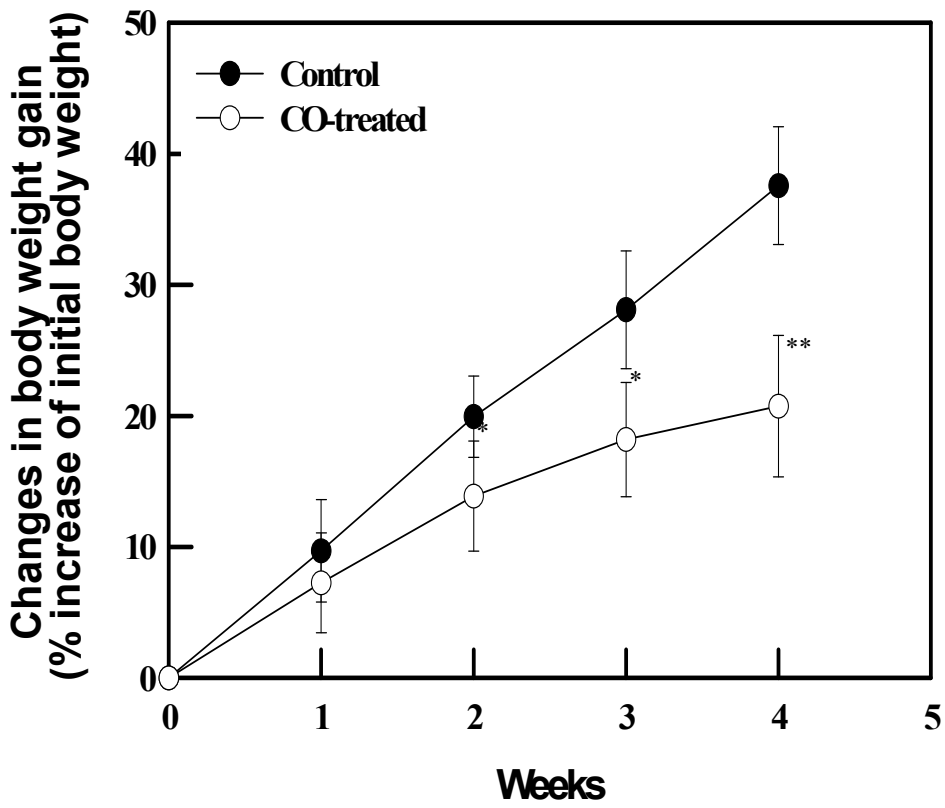
Kim et al. *Proteomics* 2006, 6, 5291–5302

Anti-diabetic and anti-obesity effects of chitosan oligosaccharides (CO)





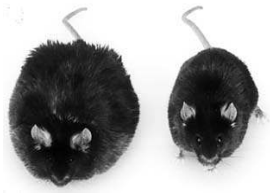


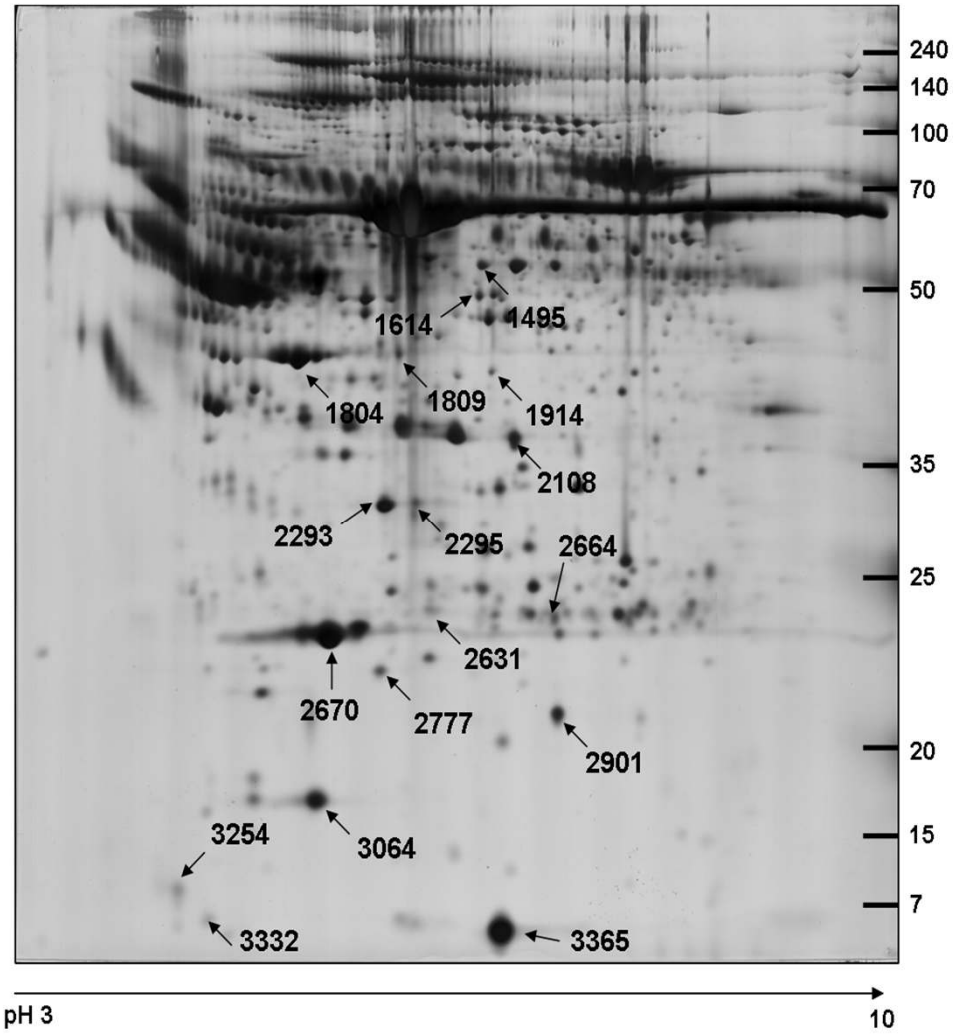


Change in body weight and blood glucose levels in control and CO-treated *ob/ob* mice

Kumar et al. Proteomics 9, 2149-2162 (2009)

Plasma proteomic analysis in *ob/ob* mice



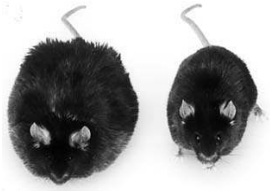


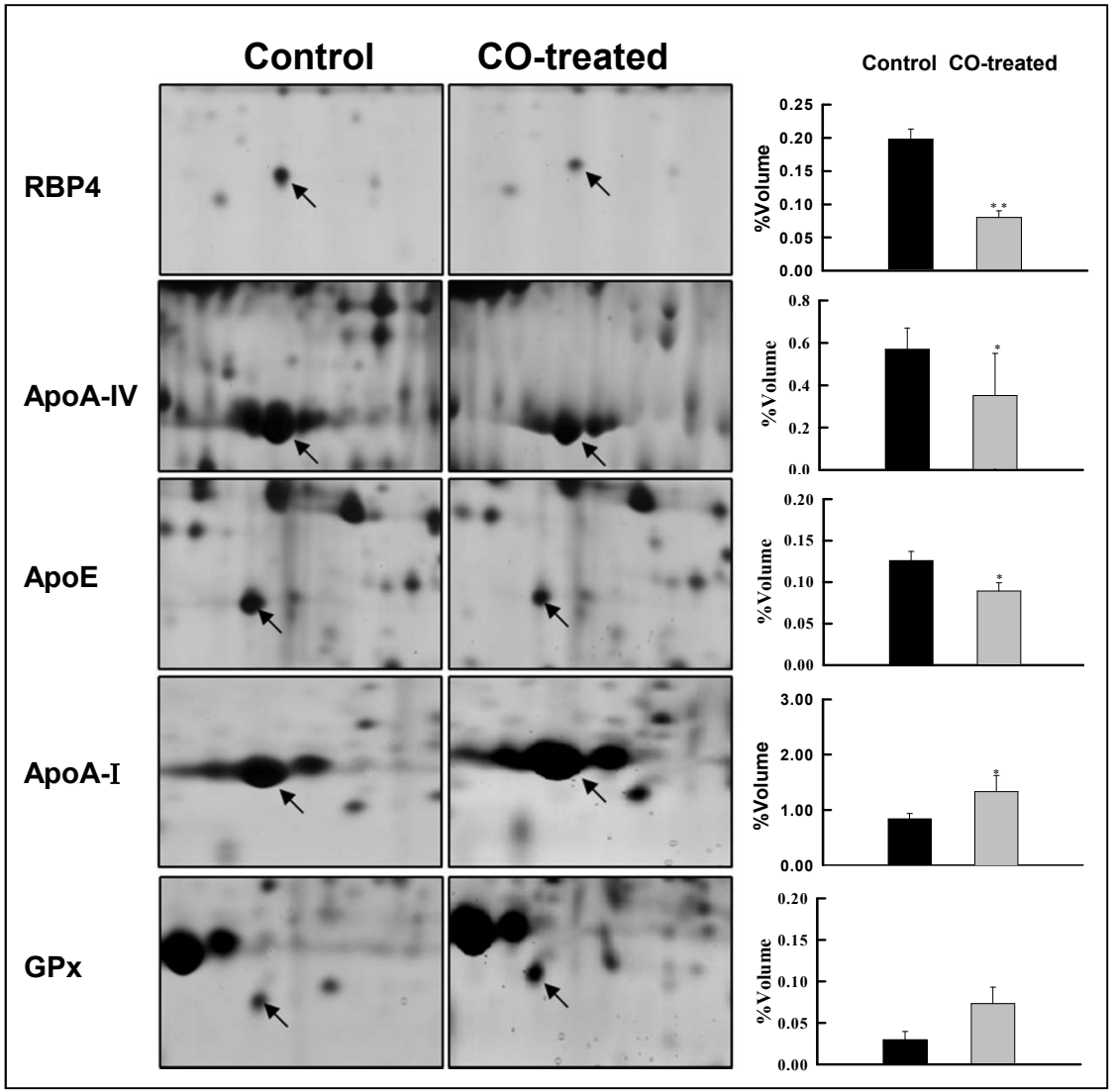
A representative 2-DE gel image of silver-stained plasma proteins in *ob/ob* mice

Kumar et al. Proteomics 9, 2149-2162 (2009)

Plasma proteomic analysis in *ob/ob* mice

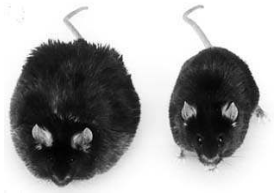
Zoom-in gel images and volume density for altered plasma proteins

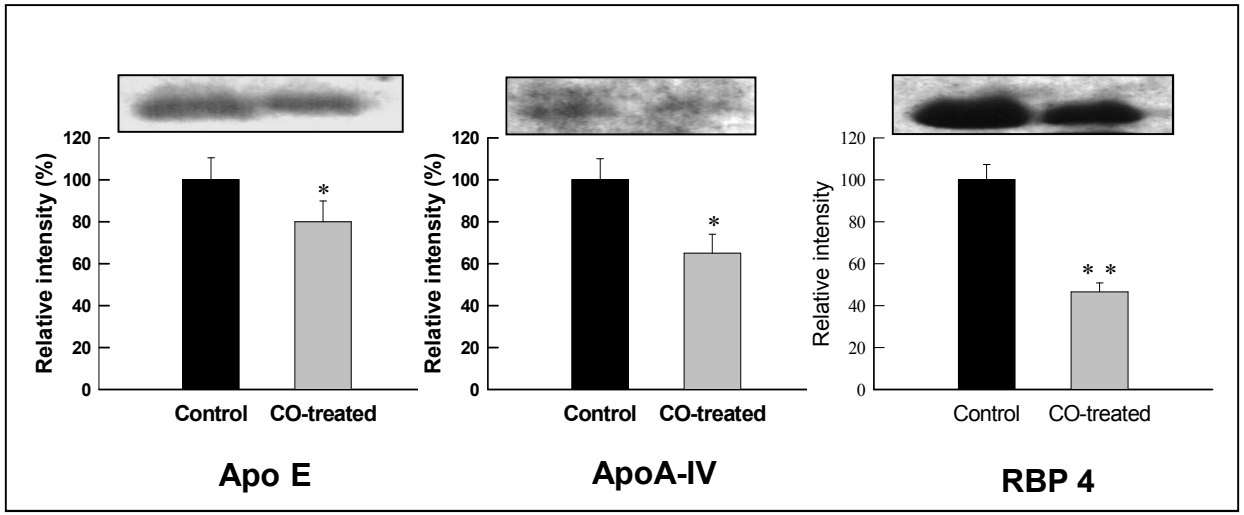


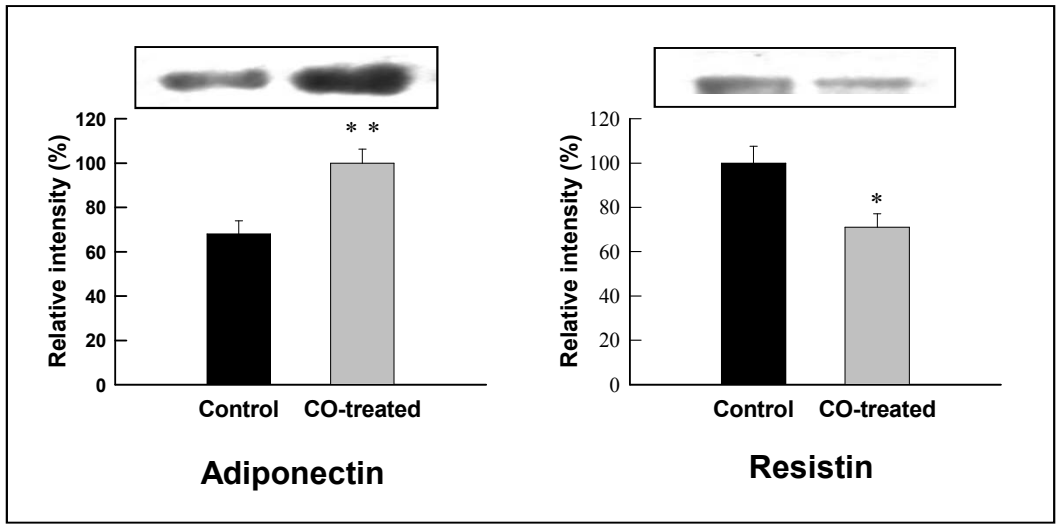


Kumar et al. Proteomics 9, 2149-2162 (2009)

Confirmation of proteomic results by Western blot analysis

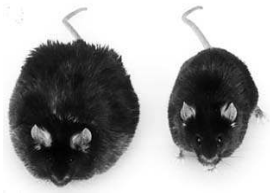


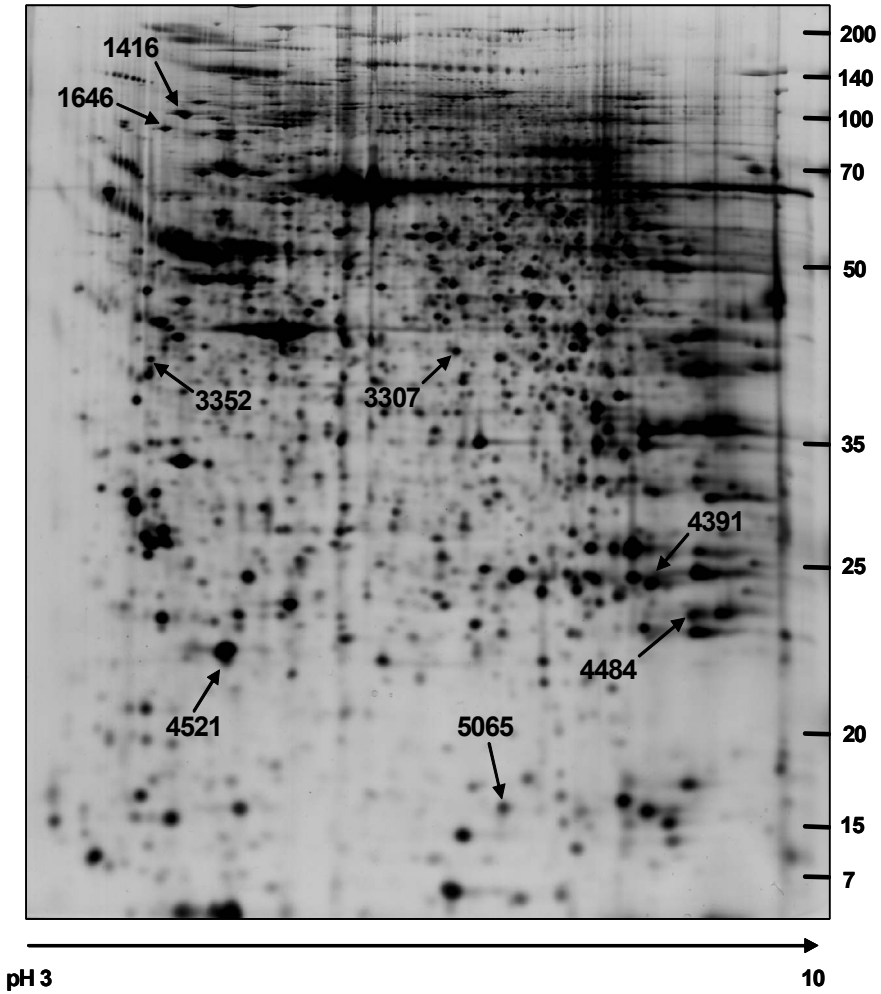




Kumar et al. Proteomics 9, 2149-2162 (2009)

Proteomic analysis WAT in *ob/ob* mice

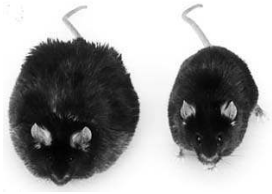


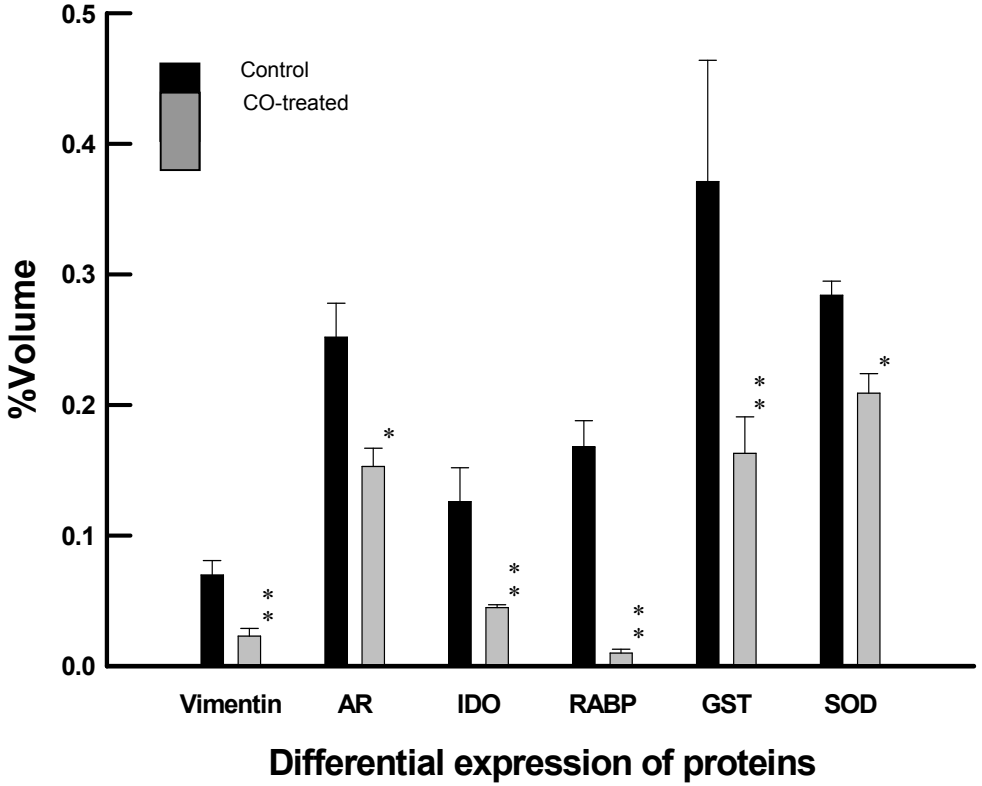


A representative 2-DE gel image of silver-stained WAT proteins in *ob/ob* mice

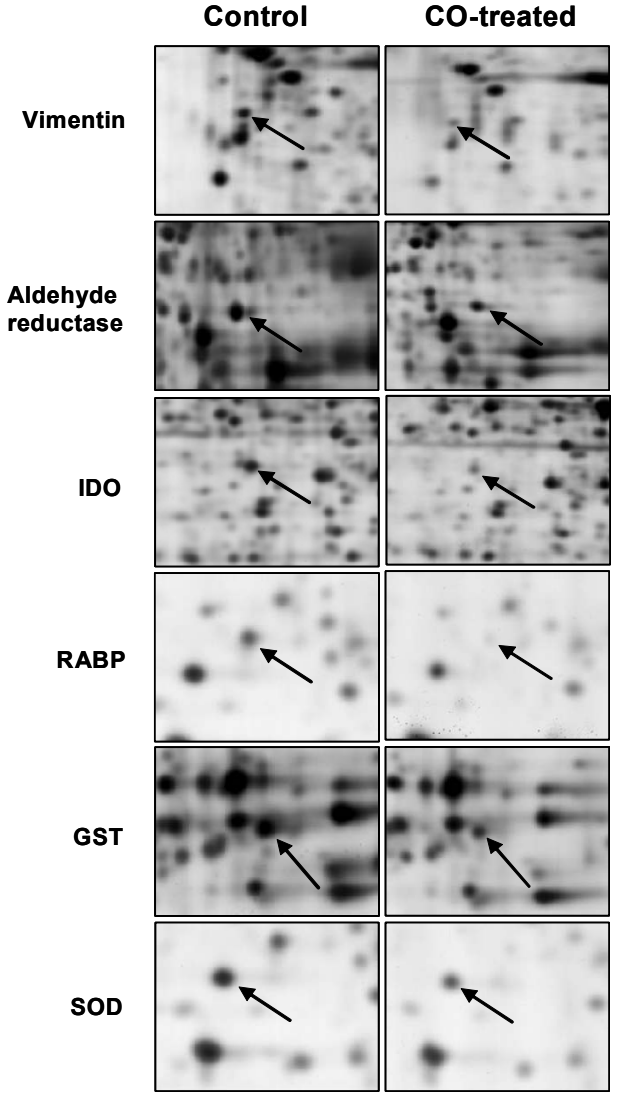
Kumar et al. Proteomics 9, 2149-2162 (2009)

Proteomic analysis WAT in *ob/ob* mice



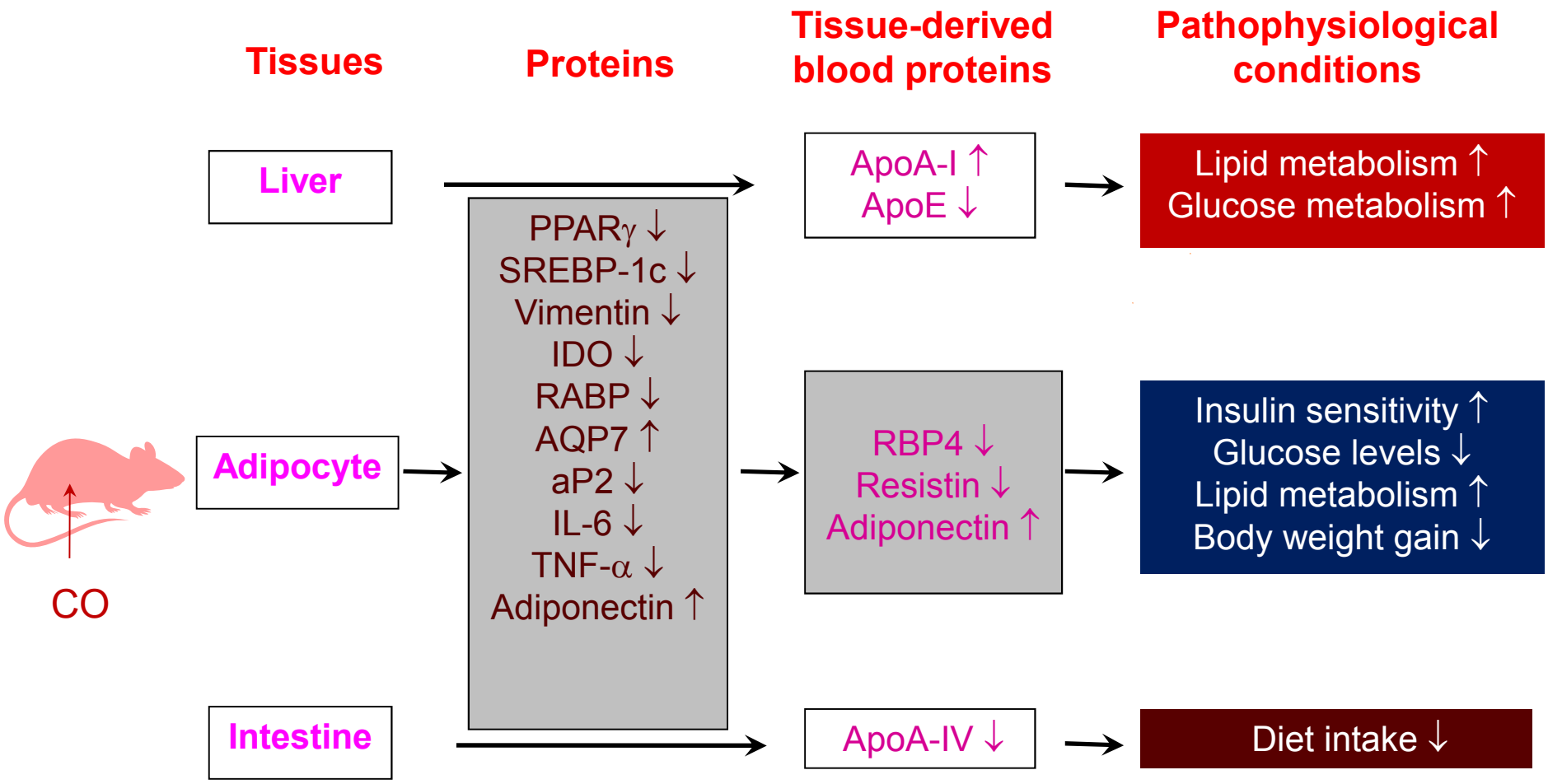


IDO, Indoleamine-pyrrole 2,3-dioxygenase
RABP, retinoic acid binding protein
GST, glutathione S-transferase
SOD, superoxide dismutase



Kumar et al. Proteomics 9, 2149-2162 (2009)

Proposed scheme for amelioration of hyperglycemia and obesity in *ob/ob* mice by CO



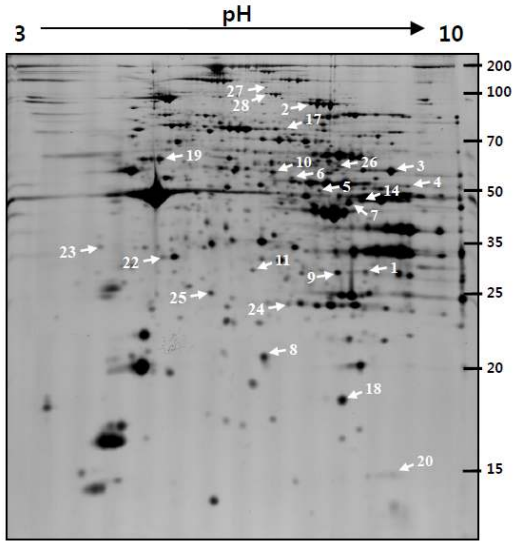
Kumar et al. Proteomics 9, 2149-2162 (2009)

Part II

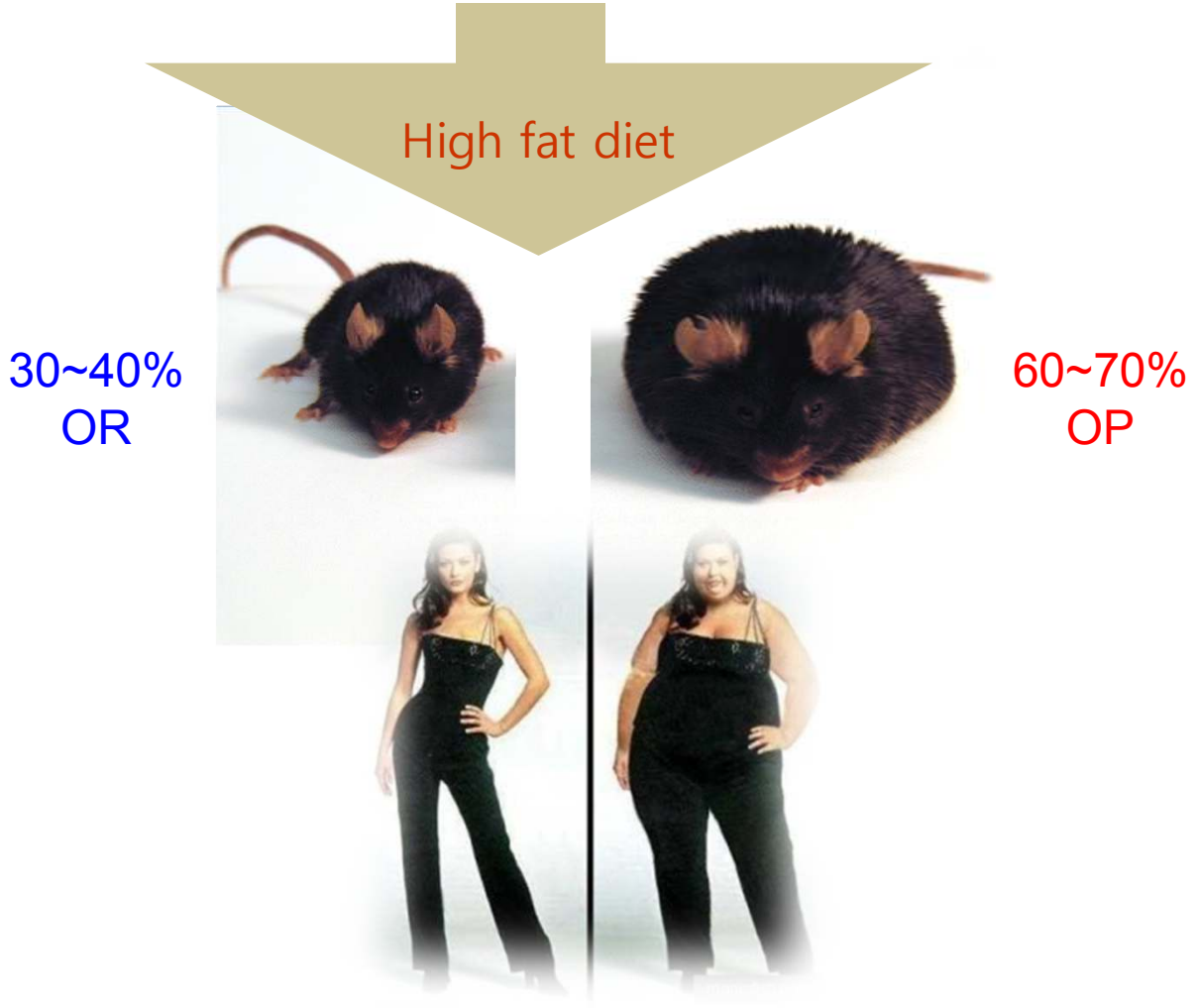
Proteomic approach for obesity

1

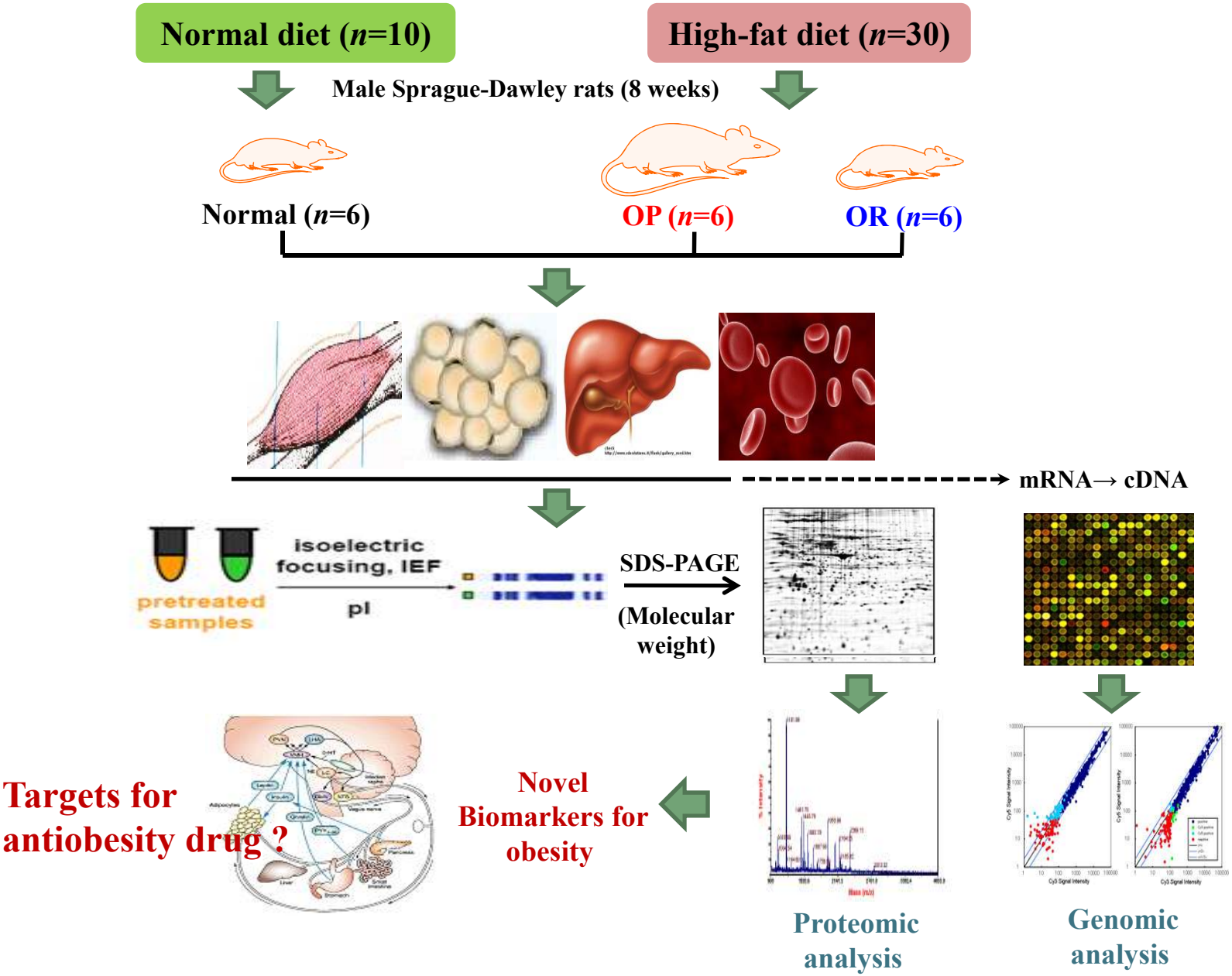
Proteomic insight into susceptibility and resistance against diet-induced obesity in rats



Why some people become obese (obesity-prone, OP) and others do not (obesity-resistant, OR) when exposed to high-calorie diets ?



Experimental design



Phenotype of diet-induced obesity-prone and obesity-resistant rats

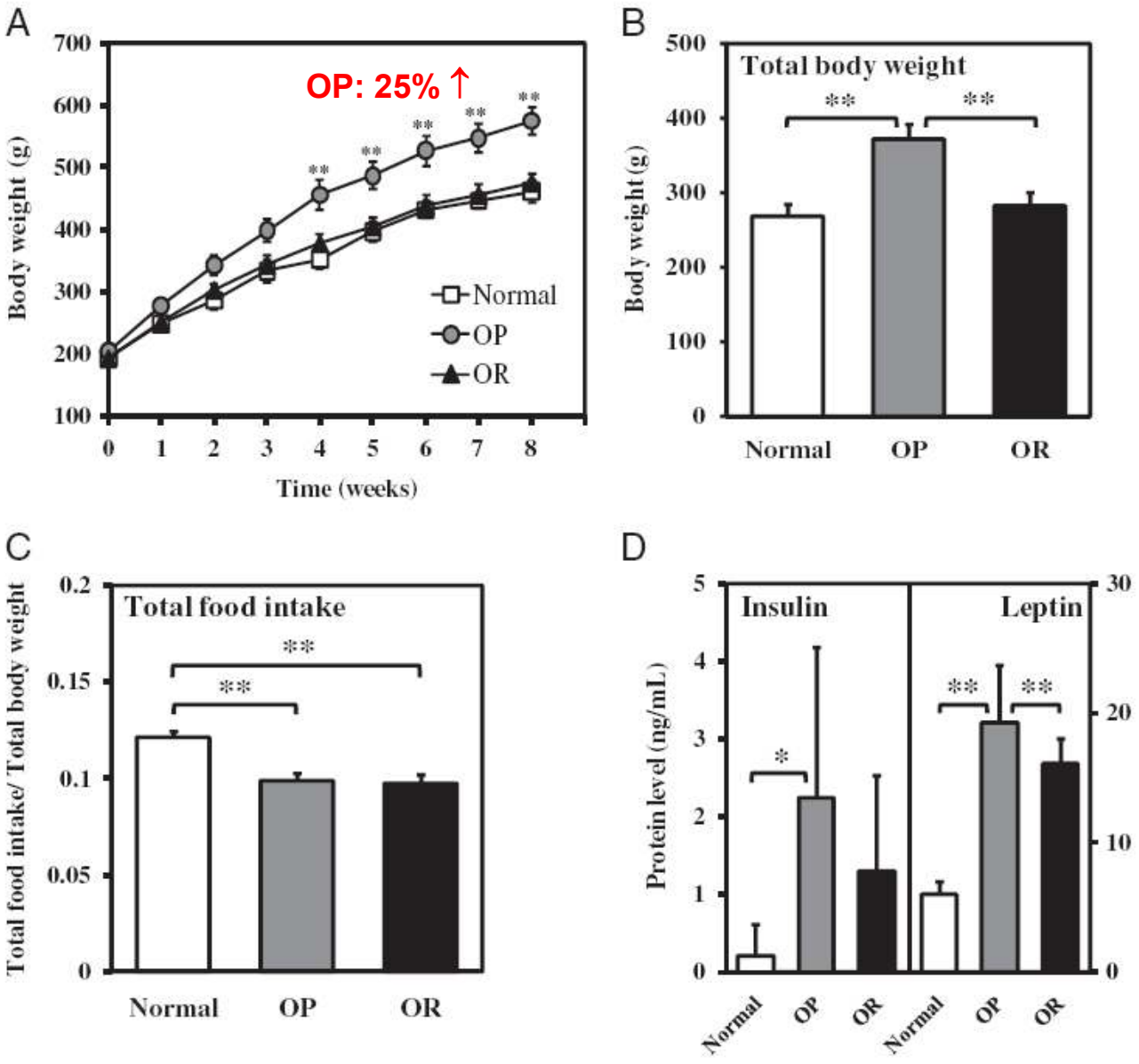
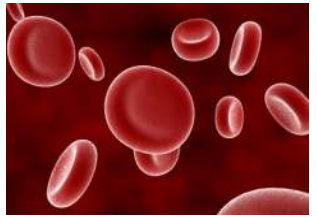


Figure 1. Phenotype of OP and OR rats fed an HFD. (A) Body weight profiles and (B) total weight gain of normal rats fed an LFD as well as OP and OR rats fed an HFD. They were weighed every alternative day for the 56-day duration of the study. (C) Total food intake *per* total body weight of rats in normal, OP, and OR rats. (D) Levels of leptin and insulin in plasma of normal, OP, and OR rats, measured using ELISA. Data was presented as mean ±SEM in six rats *per* group and were estimated using the ANOVA test. Asterisk indicates statistical significance ($^{*}p<0.05$ and $^{**}p<0.01$) between each group.

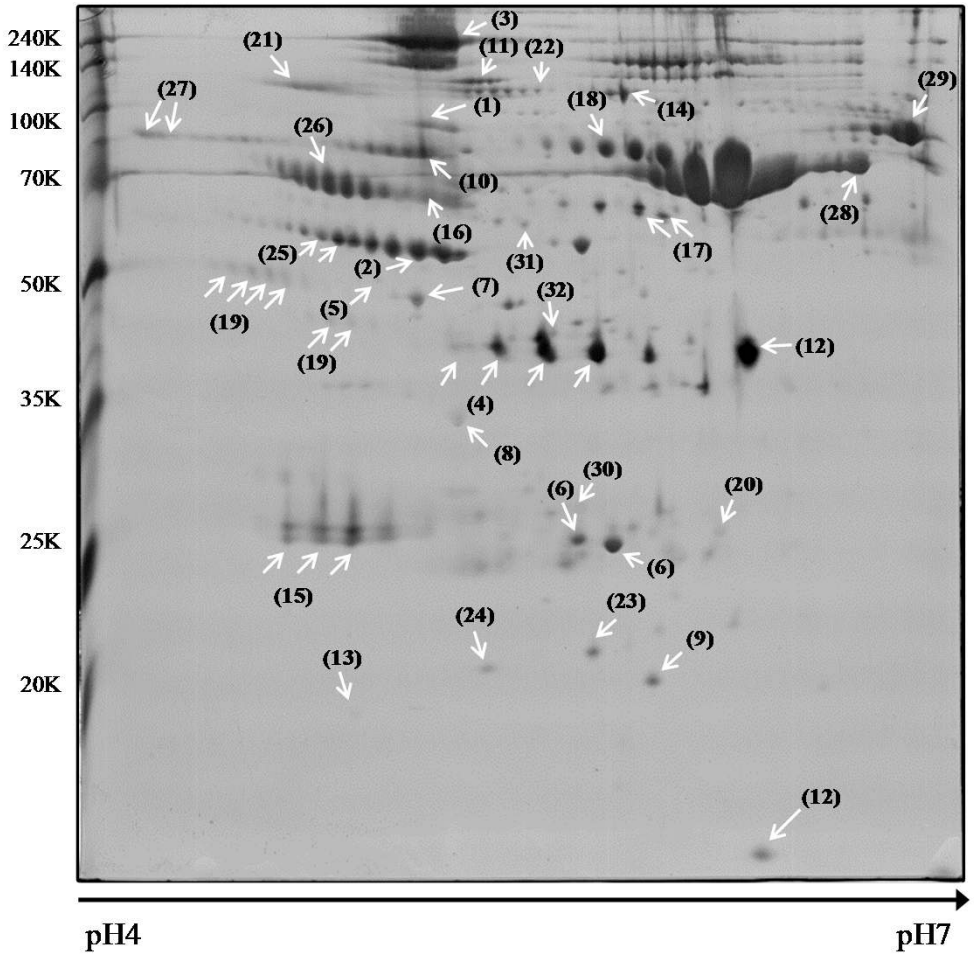
Choi et al. *Proteomics*, 10: 4386~4440 (2010)

Proteomic analysis of plasma

Changes in levels of plasma proteins are indicators for intervention of obesity risk



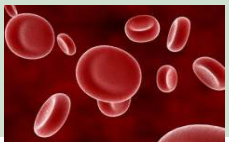
Plasma proteome analysis in OP and OR rats

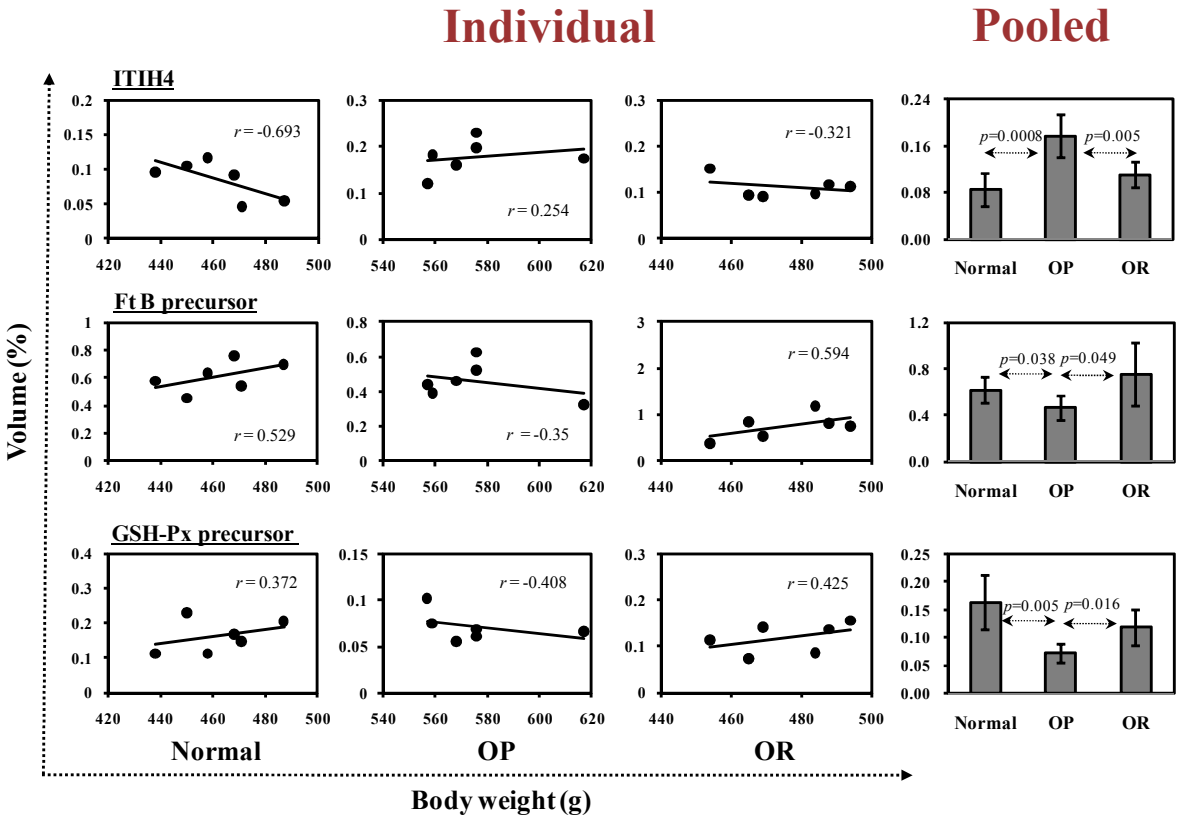


A representative silver-stained 2-DE gel image of rat plasma proteome.
Differentially regulated proteins in each group and major rat plasma proteins were marked with arrows. Proteins of numbers in gels are listed in Table 1.

Choi et al. *Proteomics*, 10: 4386~4440 (2010)

Plasma proteome analysis in OP and OR rats





No evidence linked to obesity !

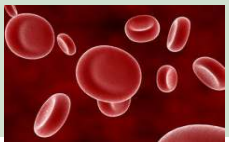
Increased insulin sensitivity ?

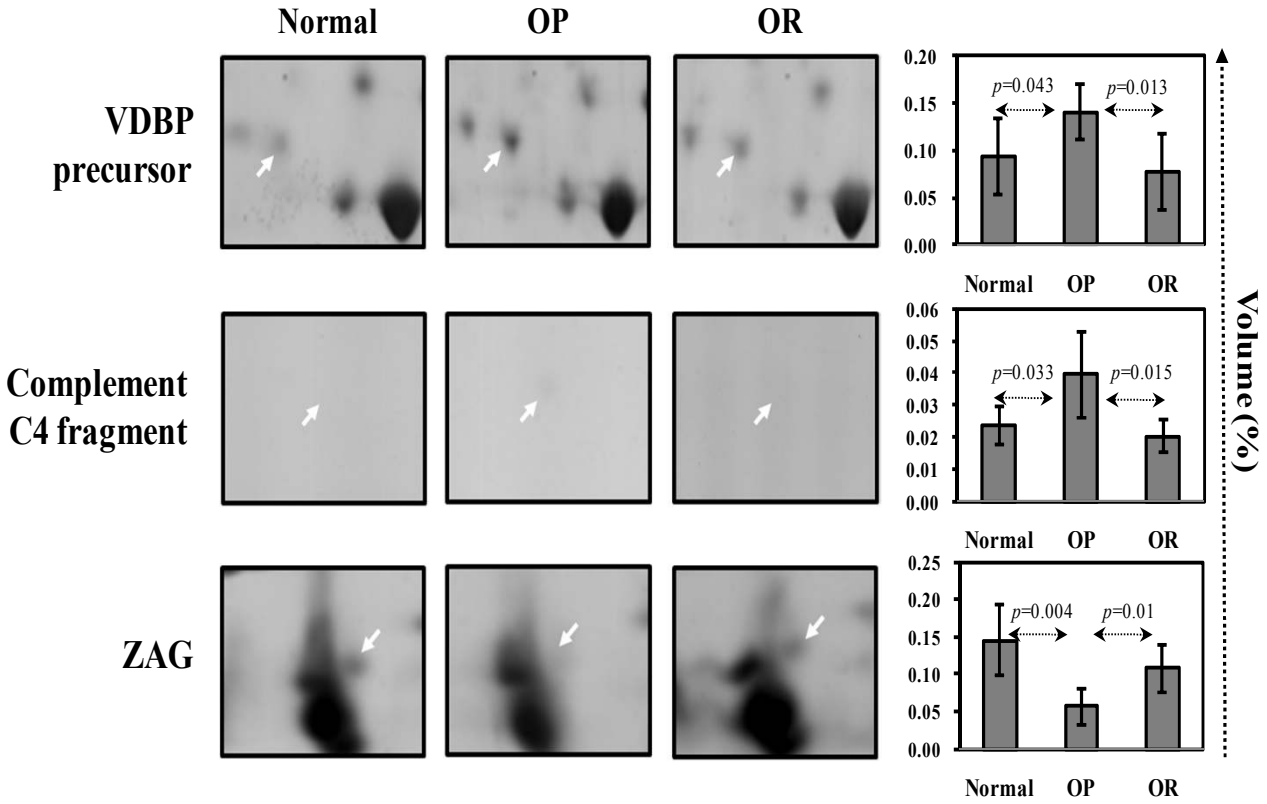
Increased resistance to oxidative stress ?

Differential protein levels of plasma proteins in OP and normal/OR rats. Line graphs refer to protein density of six individual rats as a function of body weight (left three) and bar graphs stand for average levels of six rats. These data were identified by 2-DE combined with MALDI-TOF-MS, presenting a volume density (%) of each protein spot in 2-DE gels. Data were exhibited as mean values ±SEM of volume density of target protein spot in six rats per group. They were estimated using ANOVA and Pearson's linear correlation test by SPSS program and the *p* values represent the significance of expression level between each group.

Choi et al. ***Proteomics***, 10: 4386~4440 (2010)

Plasma proteome analysis in OP and OR rats



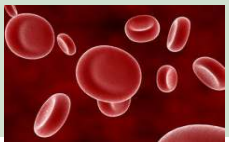


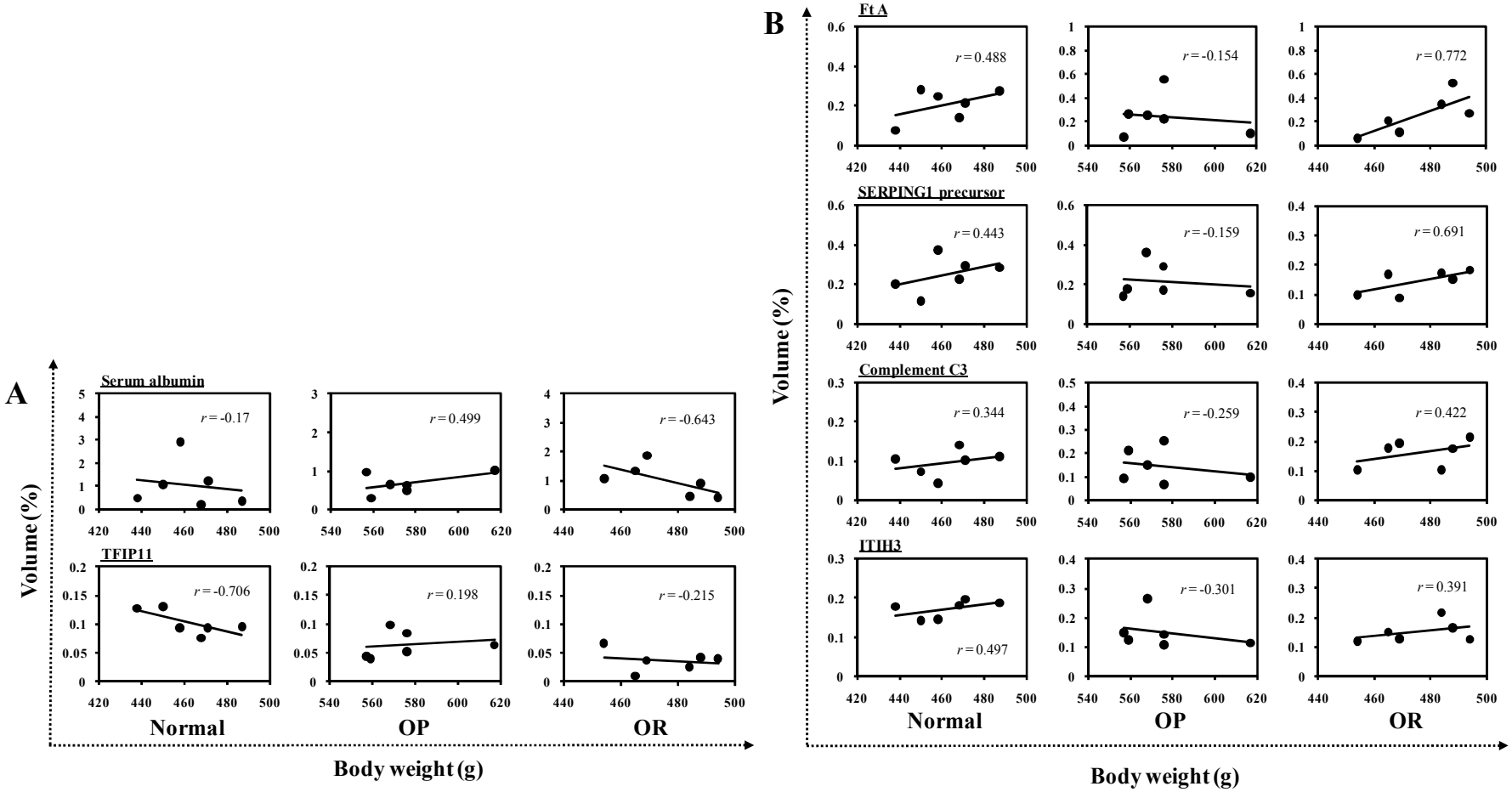
Differential protein levels of plasma proteins in OP and normal/OR rats. Left three figures are zoom-in images of representative gels and right bar graphs were produced from average protein levels of six rats.

Data were exhibited as mean values ±SEM of volume density (%) of the target spot in each group. They were estimated using the ANOVA test and the *p* values represent the significance of expression level between each group.

Choi et al. ***Proteomics***, 10: 4386~4440 (2010)

Plasma proteome analysis in OP and OR rats

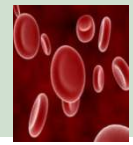


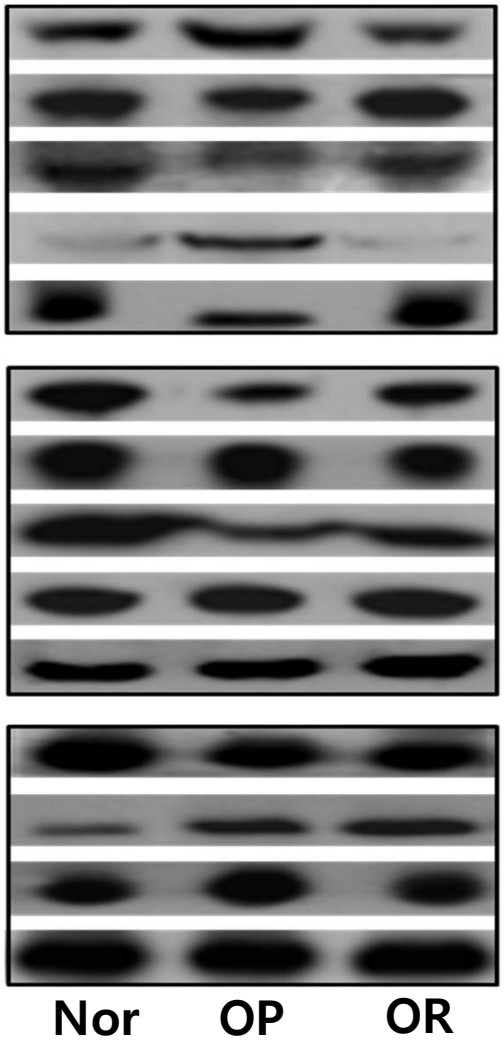


Differential protein levels of plasma proteins in OP and normal/OR rats. Line graphs were produced from protein density of six individual rats as a function of body weight. Data were exhibited as mean values ±SEM of volume density (%) of target spot in each group. They were estimated using the ANOVA test and Pearson's linear correlation test by SPSS program and the *p* values represent the significance of expression level between each group.

Choi et al. *Proteomics*, 10: 4386~4440 (2010)

Validation of plasma proteomic results by Western blot analysis

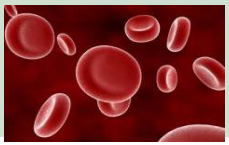




Validation of differentially regulated plasma proteins in OP and normal/OR rats by immunoblot analysis.
Levels of 5 identified proteins from 2-DE analysis (A) together with 8 important plasma proteins (B and C) were established. Data are representative of two independent experiments. Full names of proteins are presented in the abbreviations section.

Choi et al. *Proteomics*, 10: 4386~4440 (2010)

Summary from plasma proteome analysis



Proteins	OP	*Physiological functions*	OR
ITIH4	↑	Inflammation	↓
Complement C4	↑	Inflammation	↓
Ft B p	↓	Inflammation Insulin regulation	↑
RBP4	↑	Insulin resistance	↓
Resistin	-	Insulin resistance	-
ZAG	↓	Lipolysis	↑
ApoA-I, ApoE	-	Lipid metabolism	-
VDBP	↑	Glucose & vitamin D metabolism, FFA transport	↓
Adiponectin	-	Glucose metabolism, FA catabolism	-
GSH-Px	↓	Oxidative stress	↑
Cp	-	Copper transport Oxidative stress	-
Tf	-	Iron transport Oxidative stress	-

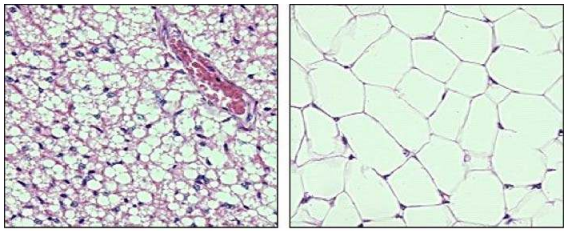
Functional study

Summary of verified plasma proteins by proteomic approach in OP and OR rats. Physiological function of each protein mentioned here indicates the most representative role of each protein in its cellular function

Choi et al. *Proteomics*, 10: 4386~4440 (2010)

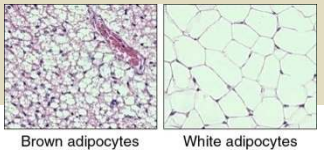
Proteome analysis in adipose tissues

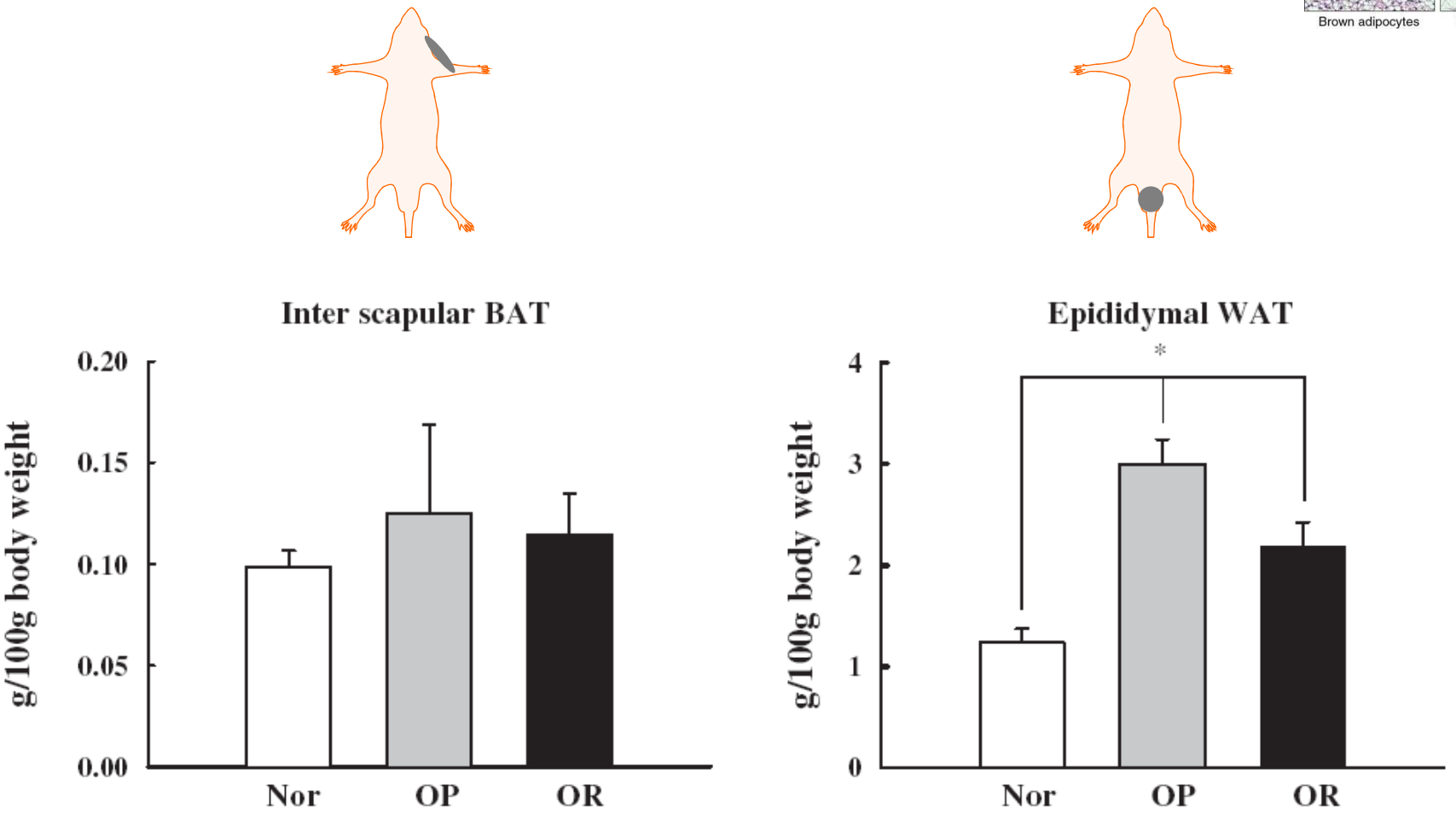
Degree of lipogenesis and fat oxidation are closely related with adiposity



Brown adipocytes White adipocytes

Adipose tissue weight in OP and OR rats

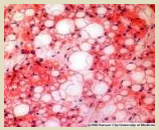


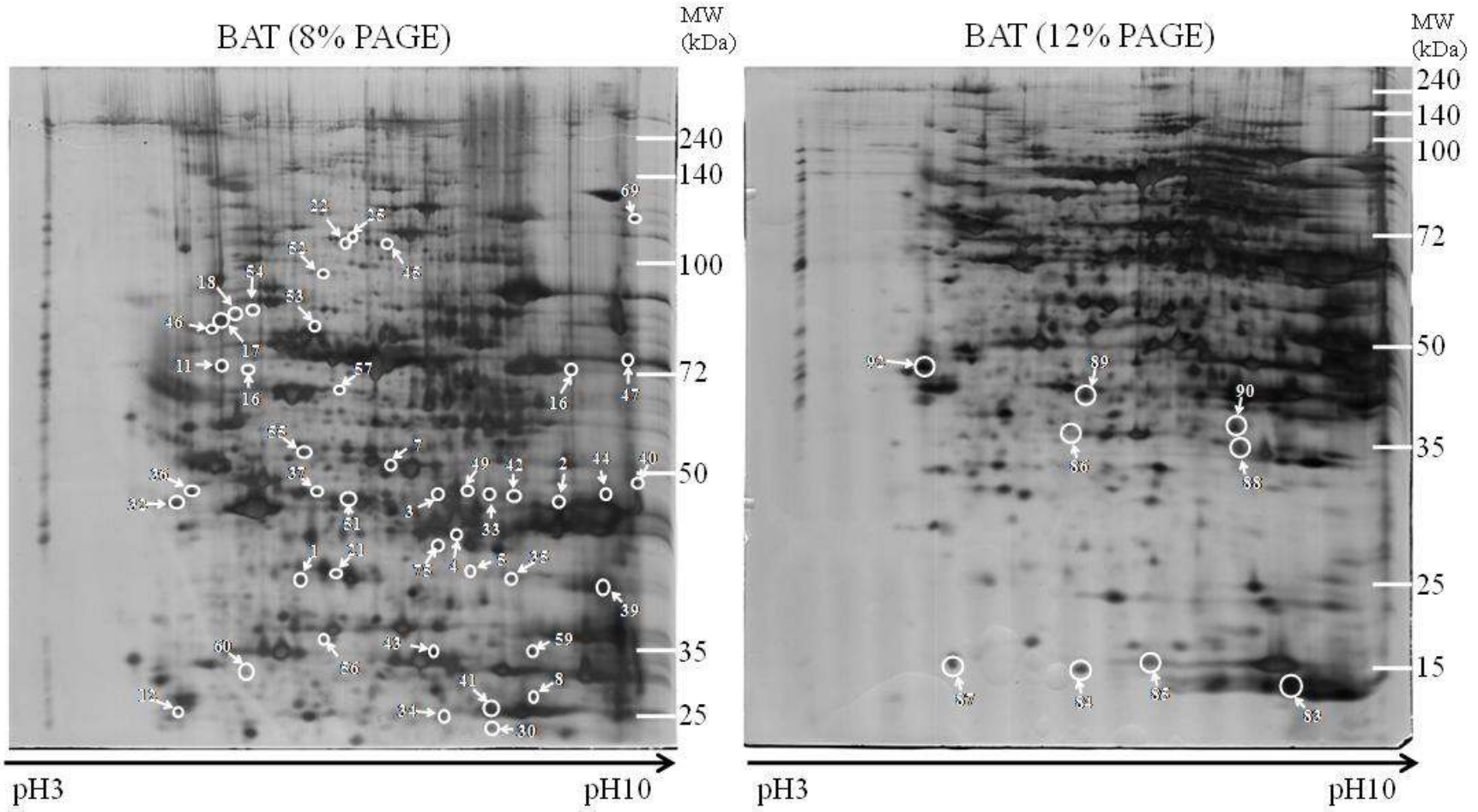


Adipose tissue weight of normal, OP, and OR rats (*n=6* in each group*)*.
**p<0.01* for significance. Error bars represent means±SEM.

Joo et al. *J. Proteome Res.* 9:2977-2987 (2010)

Proteome maps of brown adipose tissue (BAT)

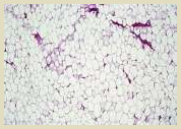


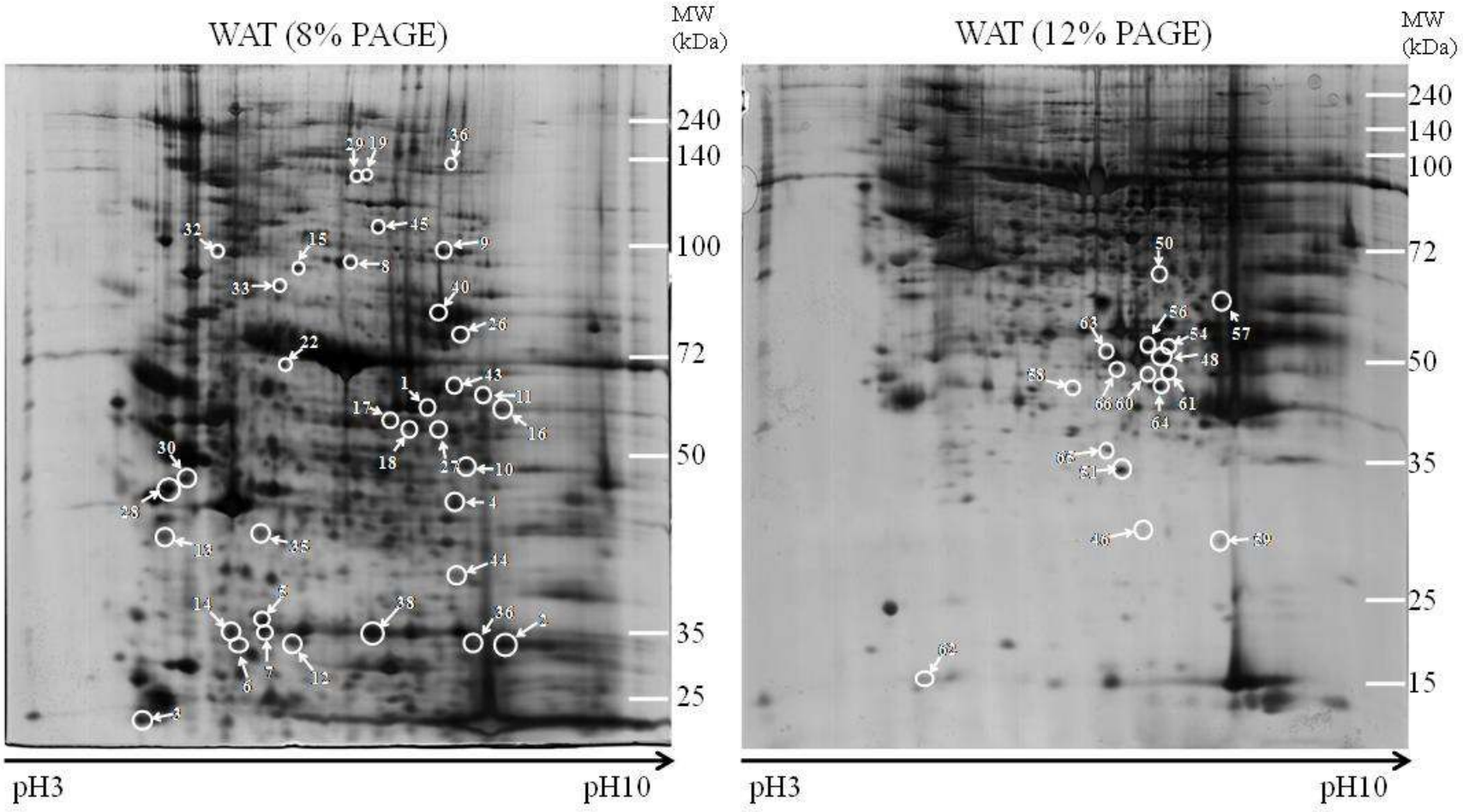


Representative 2-DE gel images of BAT protein homogenate. Proteins were extracted and separated on pH 3−10 IPG strips for the first dimension, followed by 8 or 12% (w/v) polyacrylamide gel electrophoresis (PAGE) for the second dimension. Differentially expressed proteins were marked with arrows. Protein numbers in gels are listed in Table 1.

Joo et al. *J. Proteome Res.* 9:2977-2987 (2010)

Proteome maps of white adipose tissue (WAT)

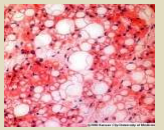


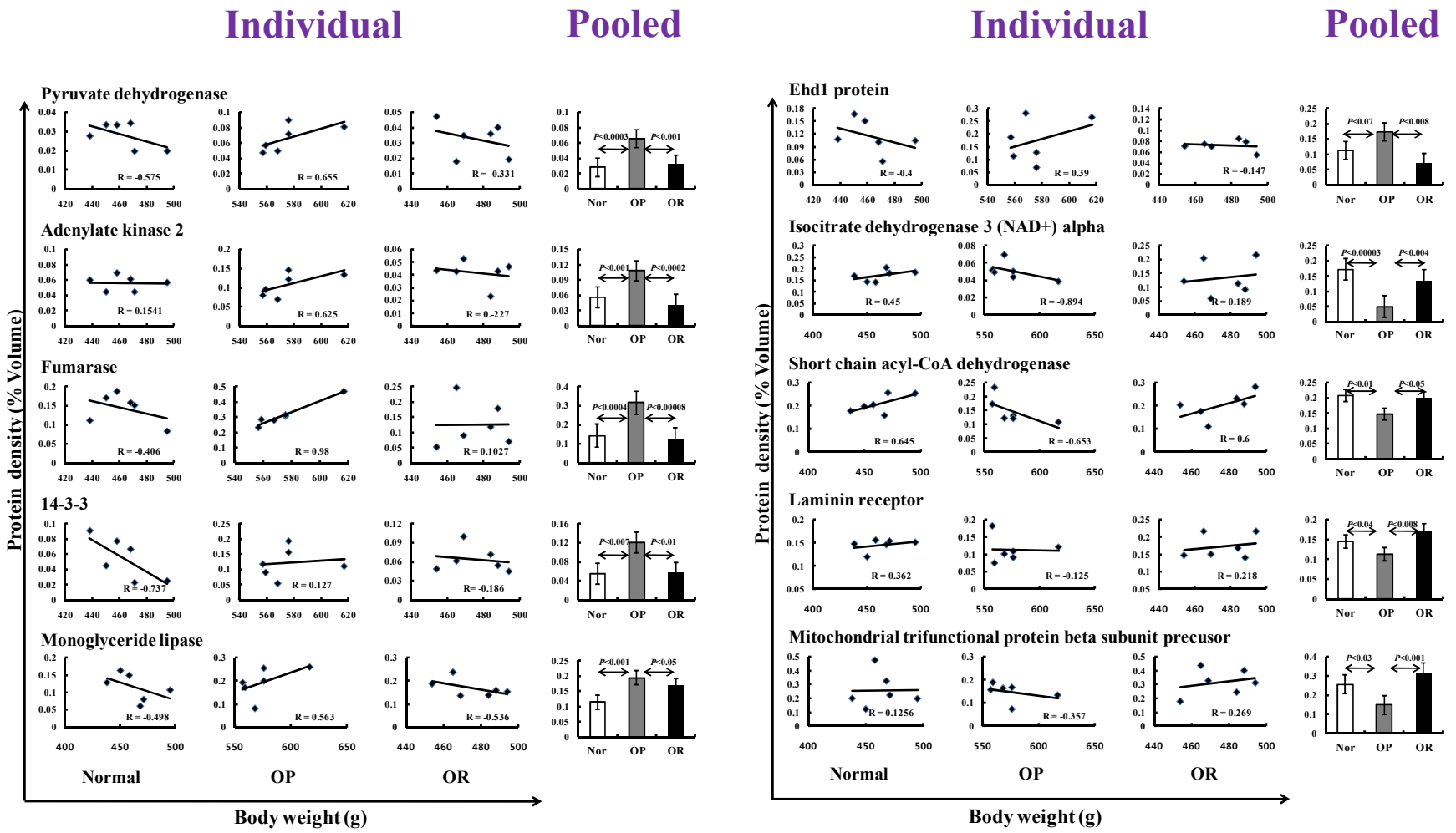


Representative 2-DE gel images of WAT protein homogenate. Proteins were extracted and separated on pH 3−10 IPG strips for the first dimension, followed by 8 or 12% (w/v) polyacrylamide gel electrophoresis (PAGE) for the second dimension. Differentially expressed proteins were marked with arrows. Protein numbers in gels are listed in Table 1.

Joo et al. *J. Proteome Res.* 9:2977-2987 (2010)

Proteome analysis in BAT of OP and OR rats

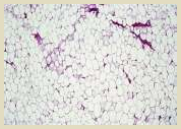


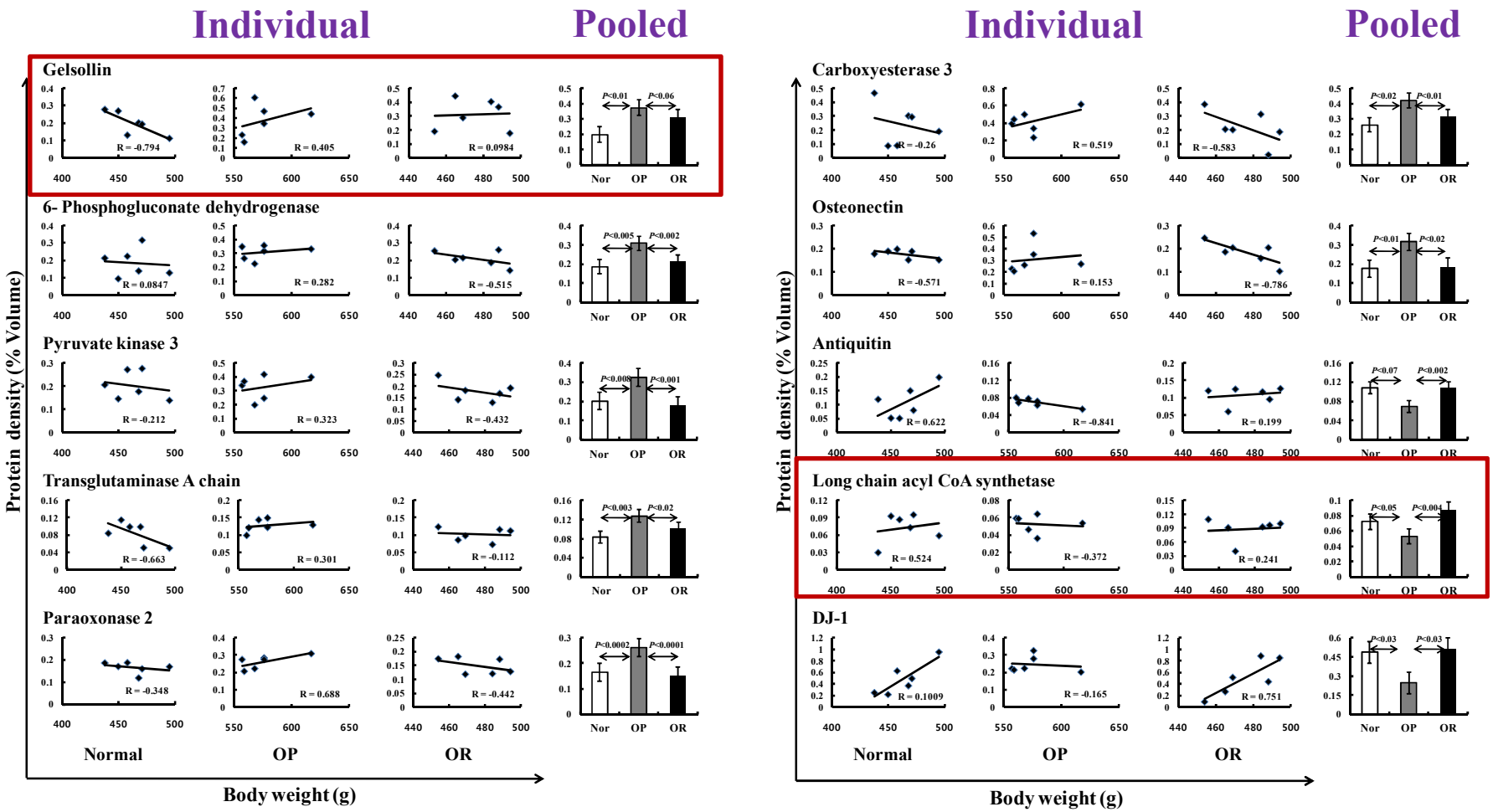


Proteins of BAT showing differential expression among three experimental groups. Each scatter-plot was made from individual protein density of six rats in each group as a function of body weight (left three columns) and bar graphs were constructed from average protein density of six rats in each group (right column). Data are presented as mean values ±S.E.M. of volume density (%) of target spot in each group. They were estimated using ANOVA test and Pearson's linear correlation test by SPSS program and the *p* values represent the significance of expression level between each group.

Joo et al. *J. Proteome Res.* 9:2977-2987 (2010)

Proteome analysis in WAT of OP and OR rats

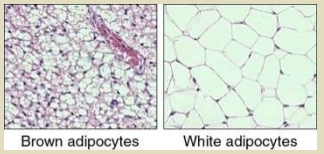


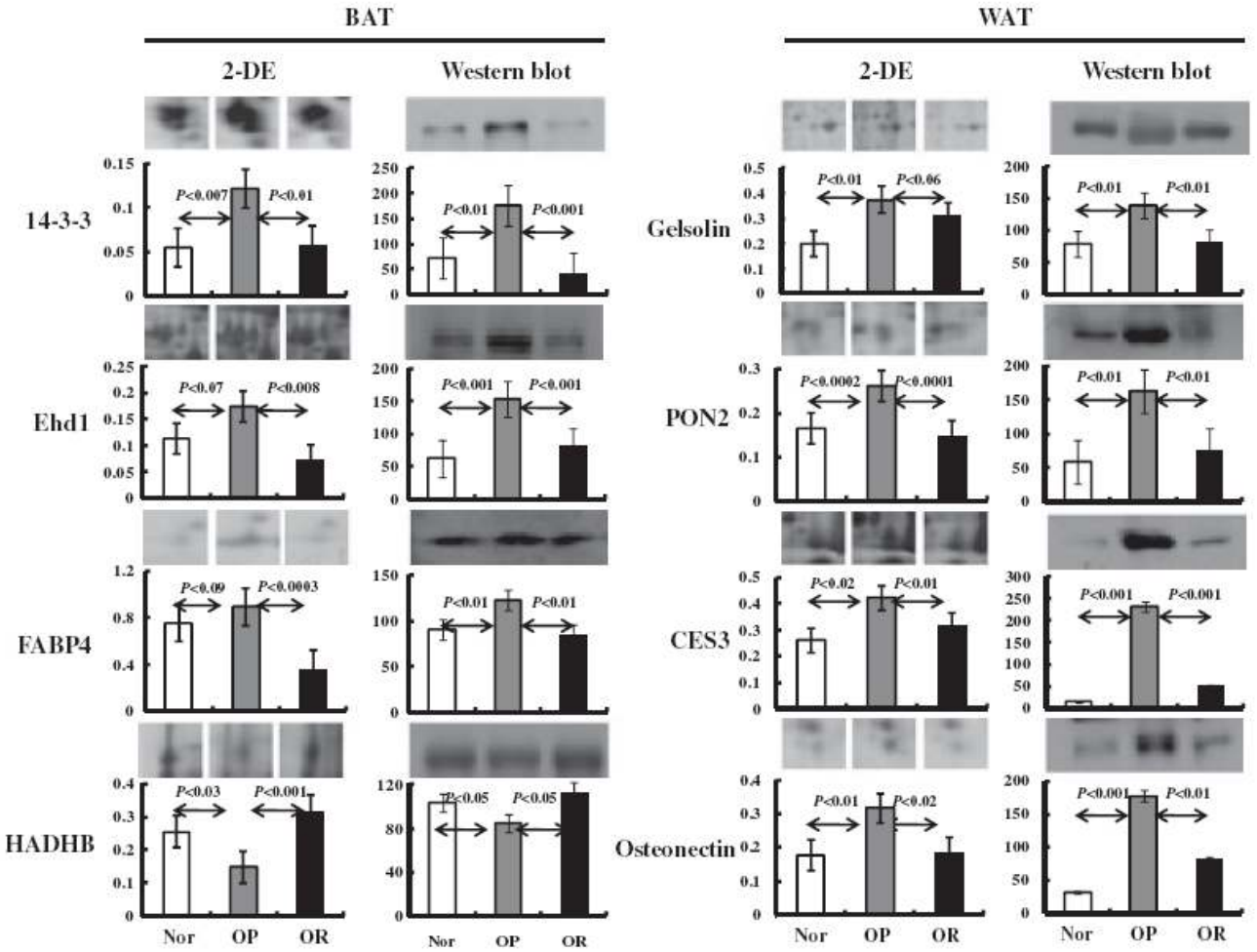


Proteins of WAT showing differential expression among three experimental groups. Each Scatter-plot was made from individual protein density of six rats in each group as a function of body weight (left three columns) and bar graphs were constructed from average protein density of six rats in each group (right column). Data are presented as mean values ±S.E.M. of volume density (%) of target spot in each group. They were estimated using the ANOVA test and Pearson's linear correlation test by the SPSS program and *p* values represent the significance of expression level between each group.

Joo et al. *J. Proteome Res.* 9:2977-2987 (2010)

Western blot analysis for validation of proteomic data

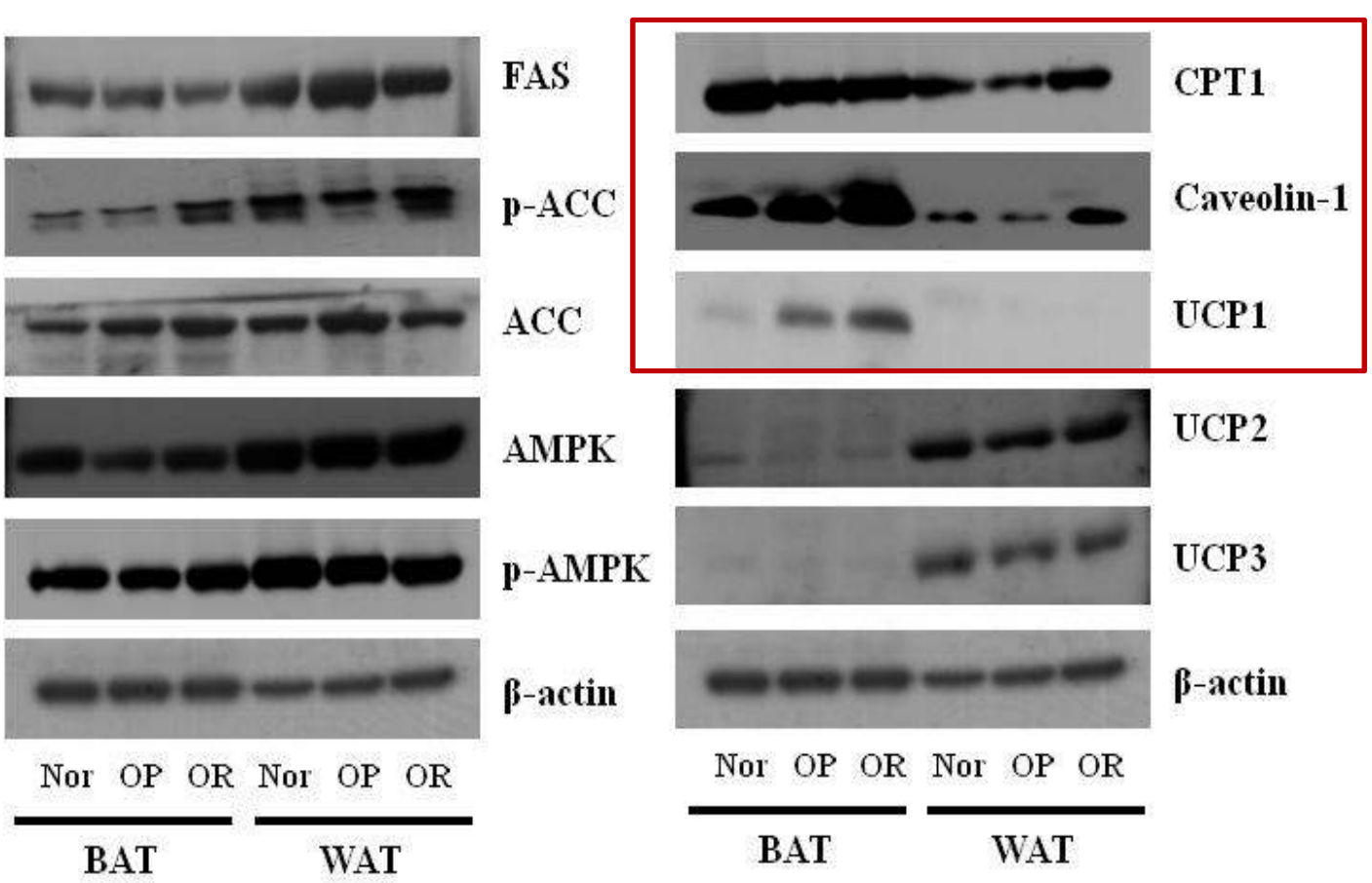


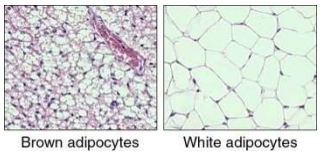


Validation of proteomic data for some proteins of interest in normal, OP, and OR rats by immunoblot analysis. 2-DE spot densities (*Y-axes in bar graphs*) are presented as mean values±SEM of volume density (%) of target proteins in each group, whereas Western blot densities (*Y-axes in* bar graphs) are presented as percentage of changes in protein concentration in each group.

Joo et al. *J. Proteome Res.* 9:2977-2987 (2010)

Differential expressions of key thermogenic and lipogenic proteins





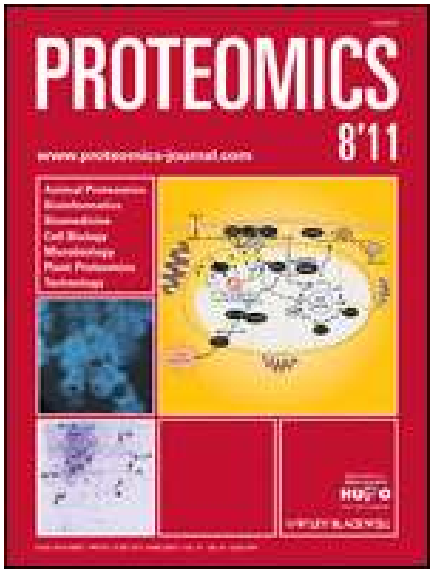
Differential expression patterns of key thermogenic and lipogenic proteins in normal, OP, and OR rats by immunoblot analysis.

Joo et al. *J. Proteome Res.* 9:2977-2987 (2010)

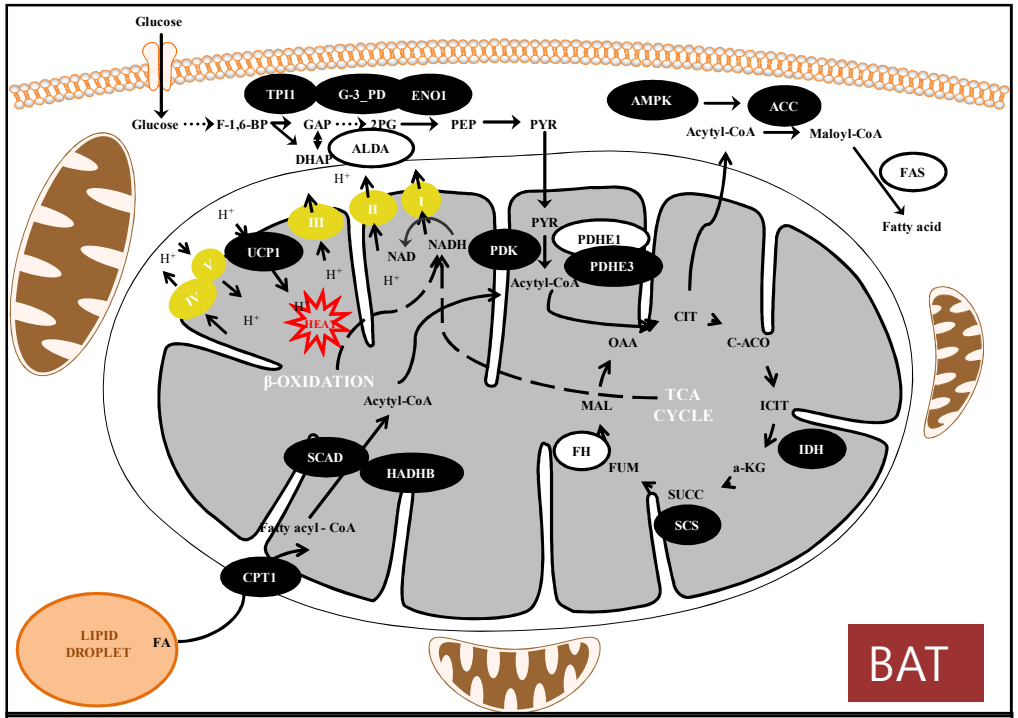
Summary on adipose proteome

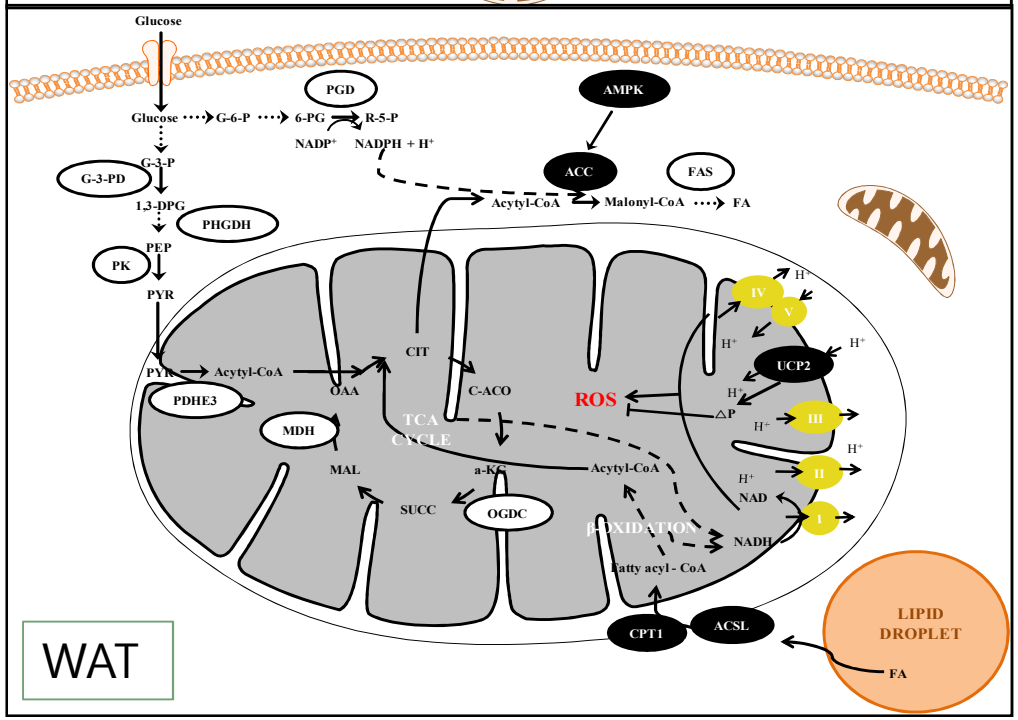
Substantial difference in protein expression between OP and OR rat adipocyte tissues

Altered expressions of many proteins were associated with obesity-resistance in BAT (upper figure) and WAT (lower figure) of OR rats fed a high fat diet. Up- and down-regulated proteins were marked in black and white circle, respectively. See abbreviation section for the full name of each protein.



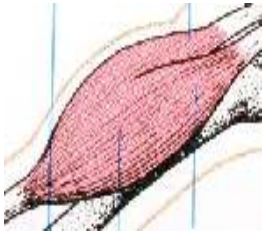
Published in *PROTEOMICS* with a cover picture (April 2011)



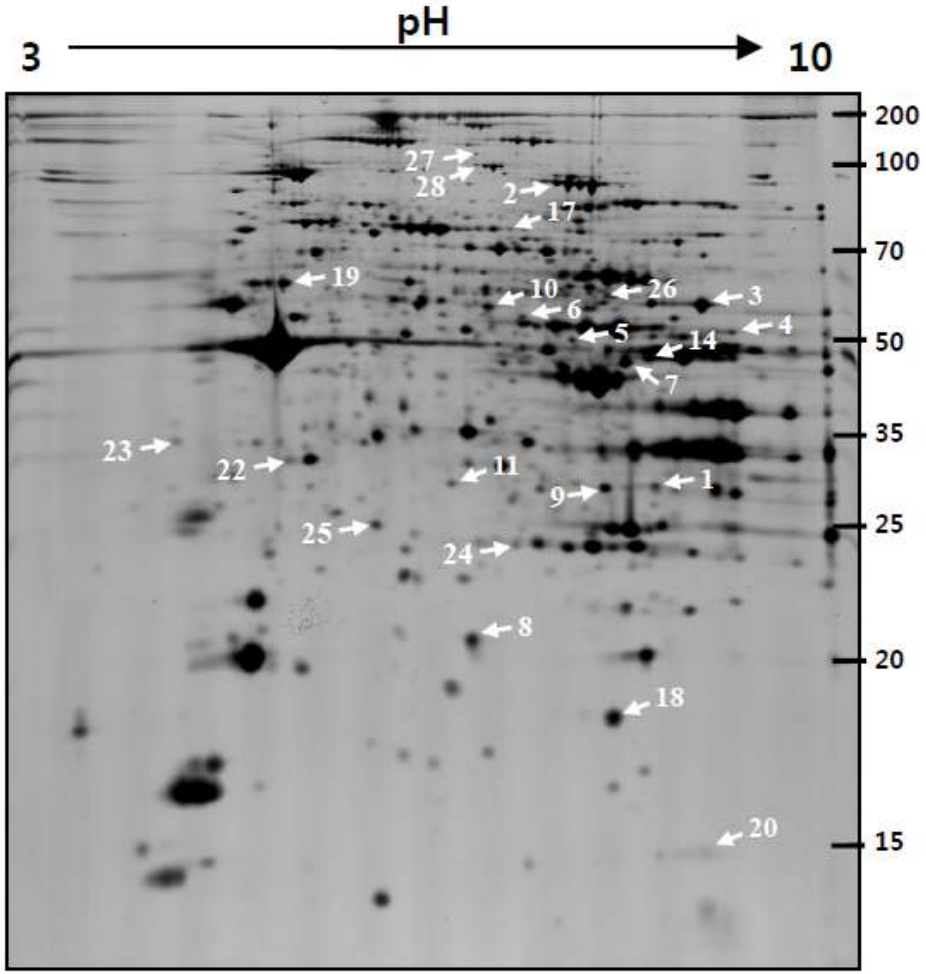


Proteomic analysis of skeletal muscle

Inefficient muscle lipid utilization may be associated with development of obesity



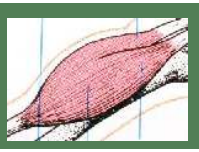
Skeletal muscle proteome map

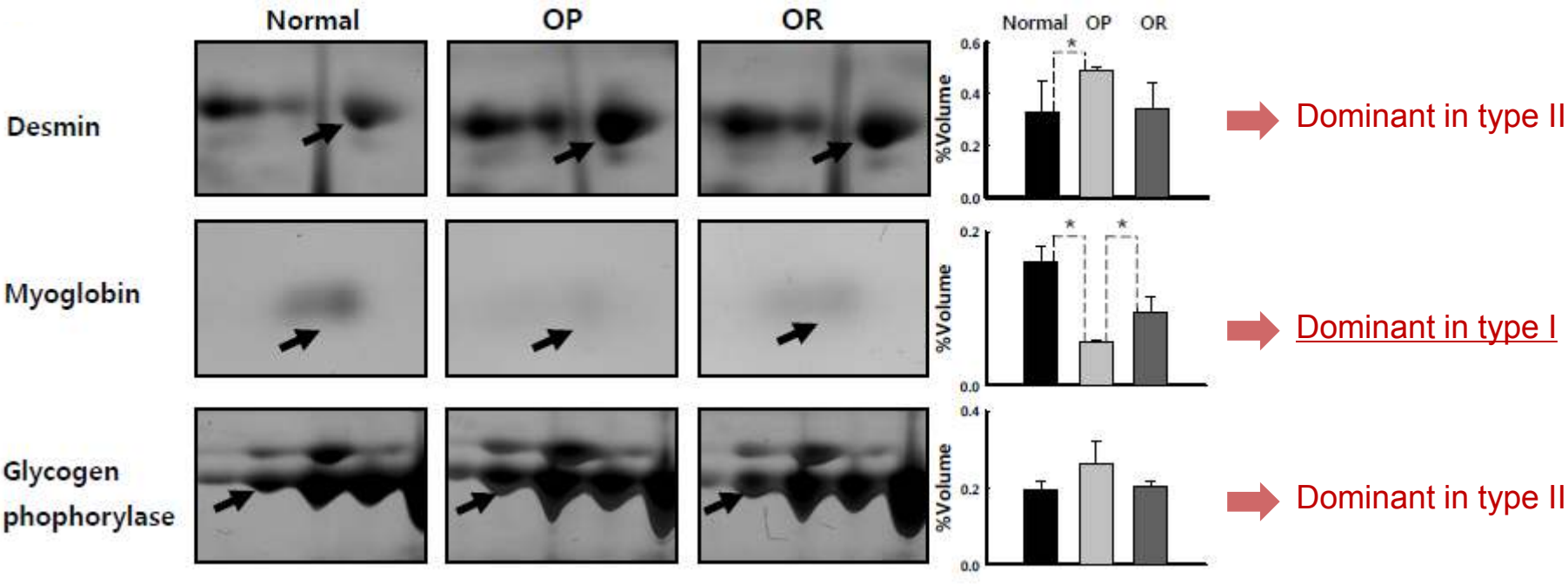


A representative silver-stained 2-DE gel image of rat skeletal muscle proteome. Differentially regulated proteins in each group and major rat plasma proteins were marked with arrows. Proteins of numbers in gels are listed in Table 1.

Kim et al. *J. Proteome Res.*10:281~1292 (2011)

Proteomic analysis of skeletal muscle



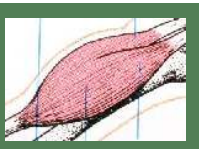


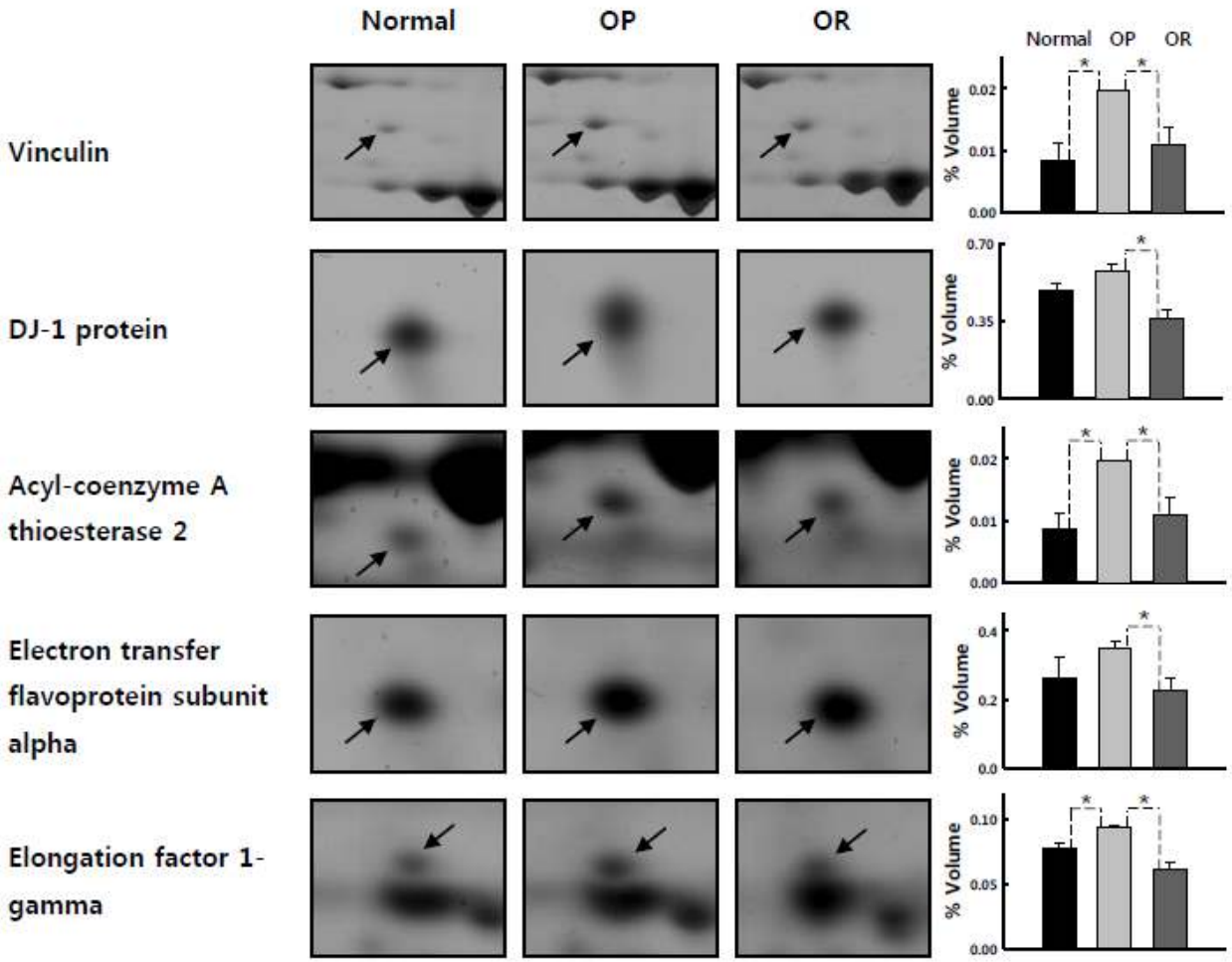
Shifting of muscle fiber distribution from type I (fat oxidation) to type II (glycolytic) due to resistance to developing obese state

Comparison of expression patterns of various proteins determining fast- and slow- muscle types in normal, OP, and OR rats. Zoom-in gel images for altered levels of muscle fiber marker proteins. Asterisk indicates statistical significance (*p<0.05, **p<0.01).

Kim et al. *J. Proteome Res.*10:281~1292 (2011)

Proteomic analysis of skeletal muscle



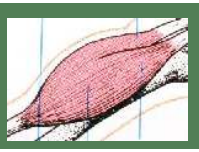


Newly found muscle marker proteins that were not linked to obesity

Comparison of expression patterns of various proteins determining fast- and slow-muscle types in normal, OP, and OR rats. Zoom-in gel images for altered levels of muscle fiber marker proteins. Asterisk indicates statistical significance (*p<0.05, **p<0.01).

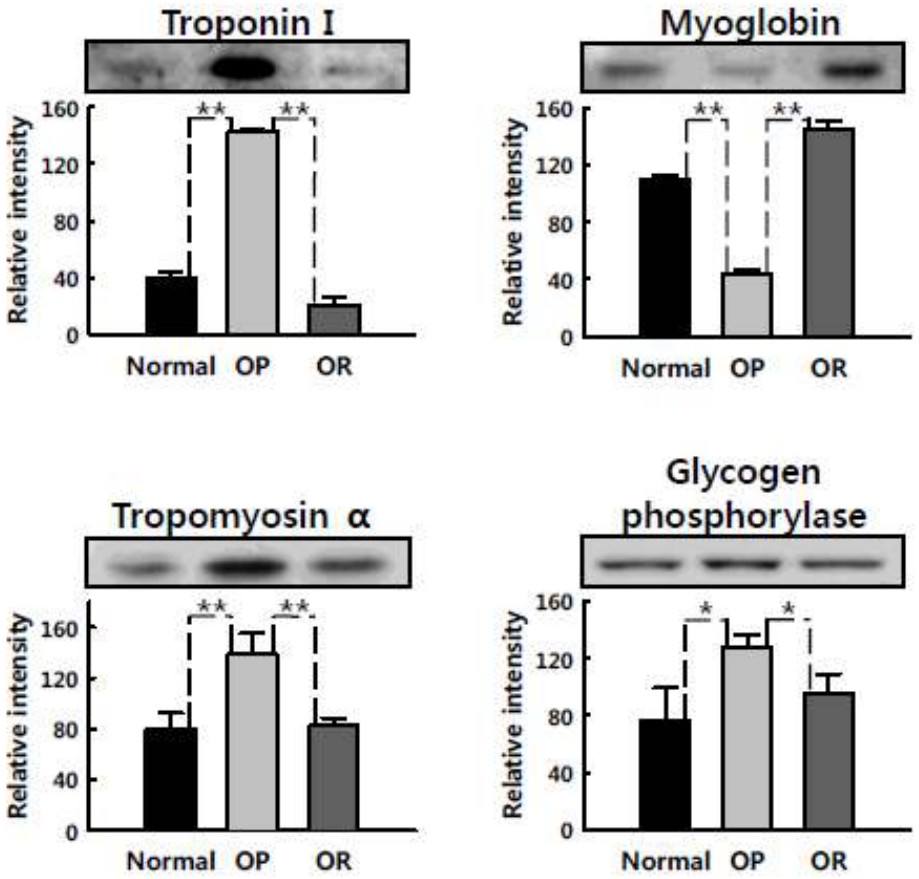
Kim et al. *J. Proteome Res.*10:281~1292 (2011)

Expression patterns of important muscle proteins



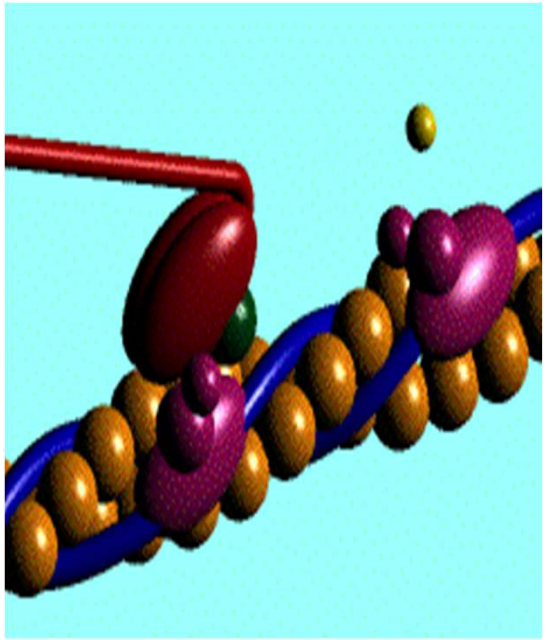
Dominant in type II

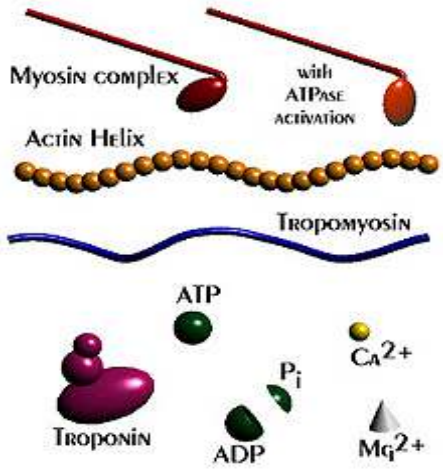
Dominant in type I



Dominant in type II

Dominant in type II

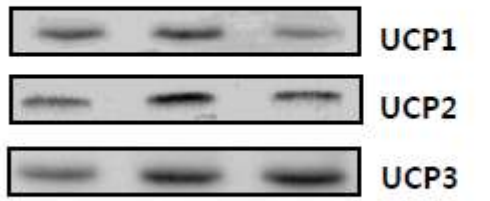




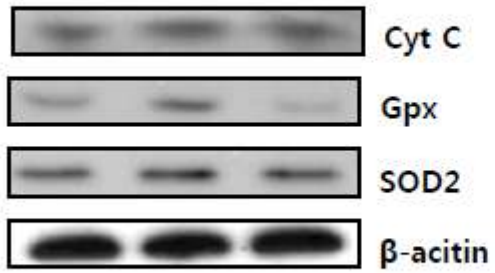
Comparison of expression patterns of various proteins determining fast- and slow- muscle types in normal, OP, and OR rats. Western blot analysis of muscle fiber markers. Asterisk indicates statistical significance (*p<0.05, **p<0.01).

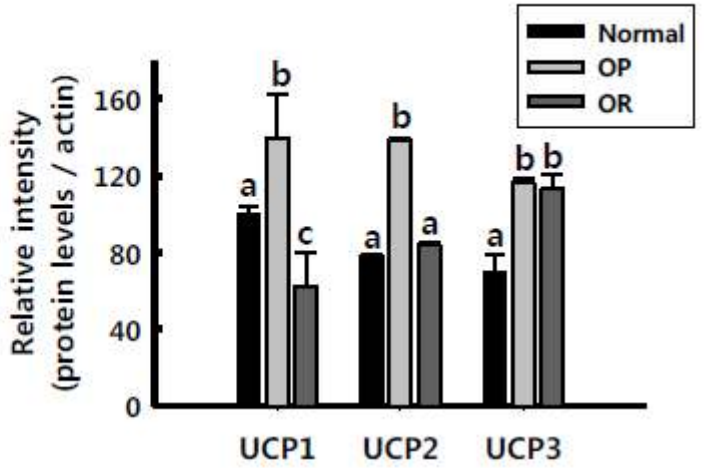
Kim et al. *J. Proteome Res.*10:281~1292 (2011)

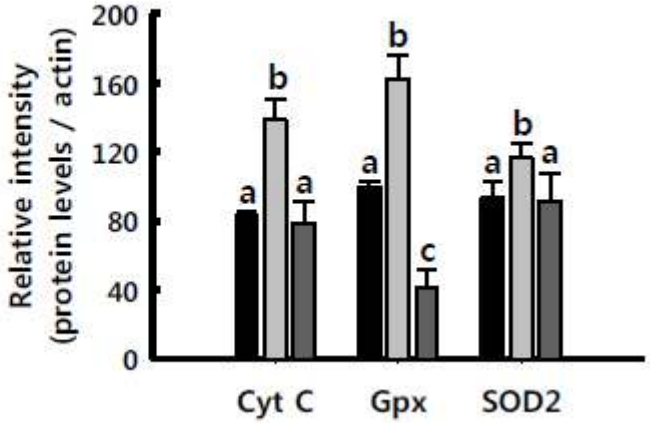
Expression patterns of UCPs and antioxidative proteins



Nor OP OR





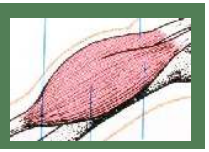


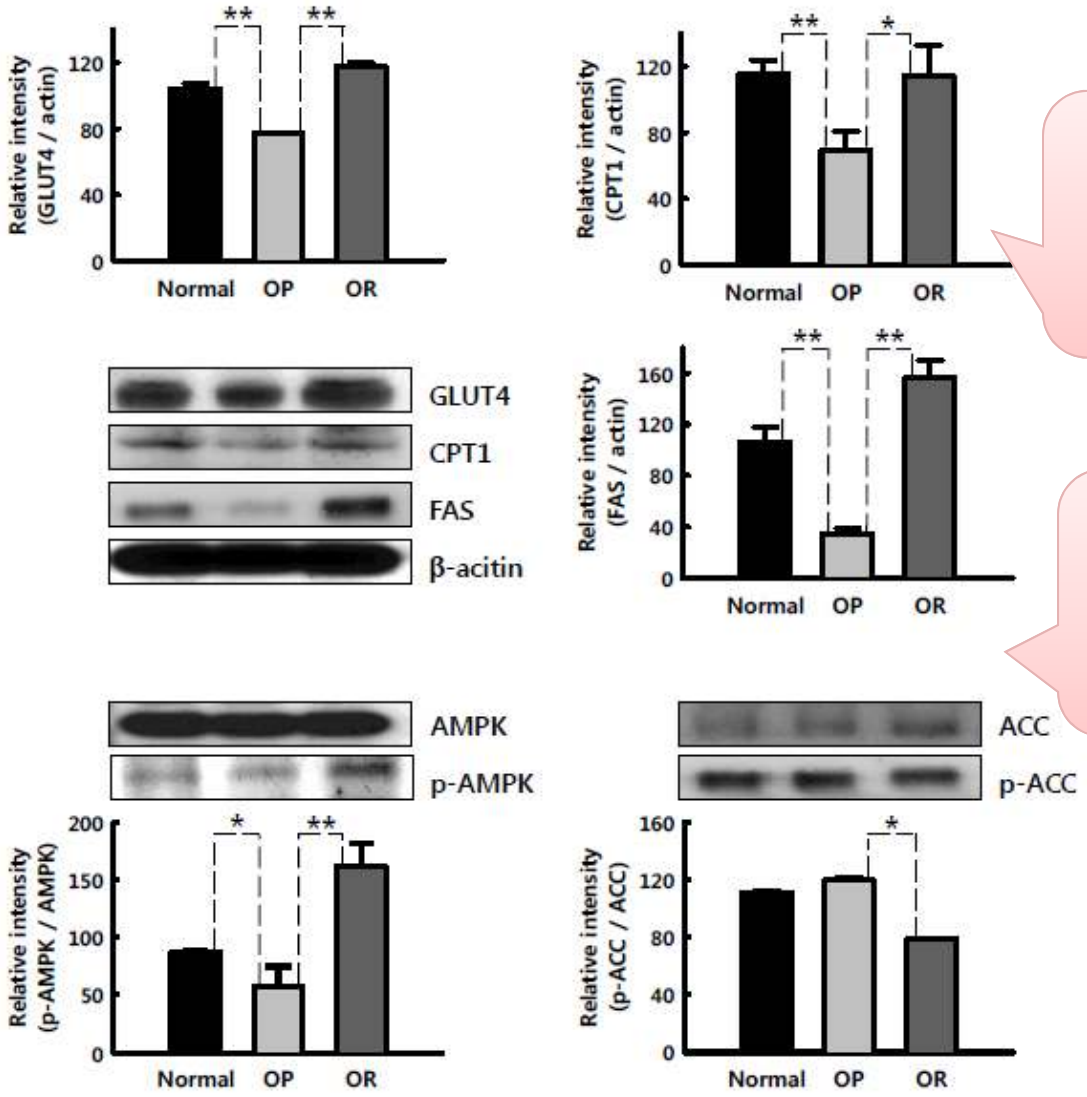
UCPs and anti-oxidative proteins were also differentially expressed !

Effects of HFD on expression of UCPs and some antioxidant proteins in normal, OP, and OR rats. Asterisk indicates statistical significance (*p<0.05, **p<0.01).

Kim et al. *J. Proteome Res.*10:281~1292 (2011)

Expression patterns of important metabolic proteins





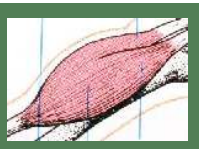
Increased fat oxidation in OR rat muscle !

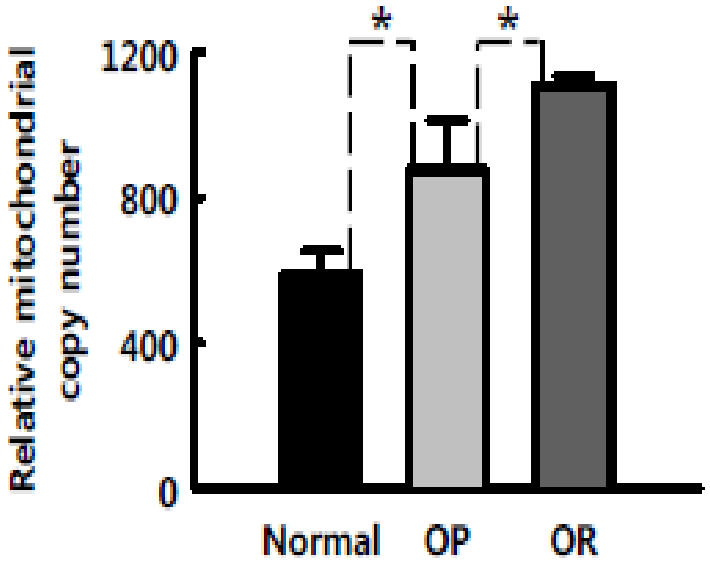
Increased lipogenesis in OR rat muscle ??

Changes in expression of proteins associated with energy expenditure and lipid metabolism in rat skeletal muscle. Asterisk indicates statistical significance (*p<0.05, **p<0.01).

Kim et al. *J. Proteome Res.*10:281~1292 (2011)

Mitochondrial contents

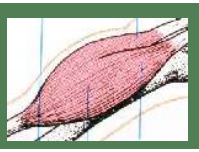




Effects of HFD on the mitochondrial contents in normal, OP, and OR rats. Asterisk indicates statistical significance (*p<0.05).

Kim et al. *J. Proteome Res.*10:281~1292 (2011)

Summary from muscle proteome analysis



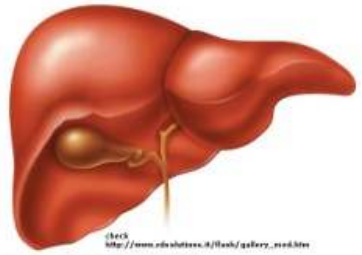
- Enhanced regulation of proteins involved in lipid metabolism and muscle contraction resulted in OR of HFD-fed rats.

- Increased expression of marker proteins for oxidative muscle type (type I) contributed to OR phenotype.

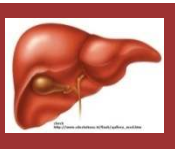
Kim et al. *J. Proteome Res.* 10:281~1292 (2011)

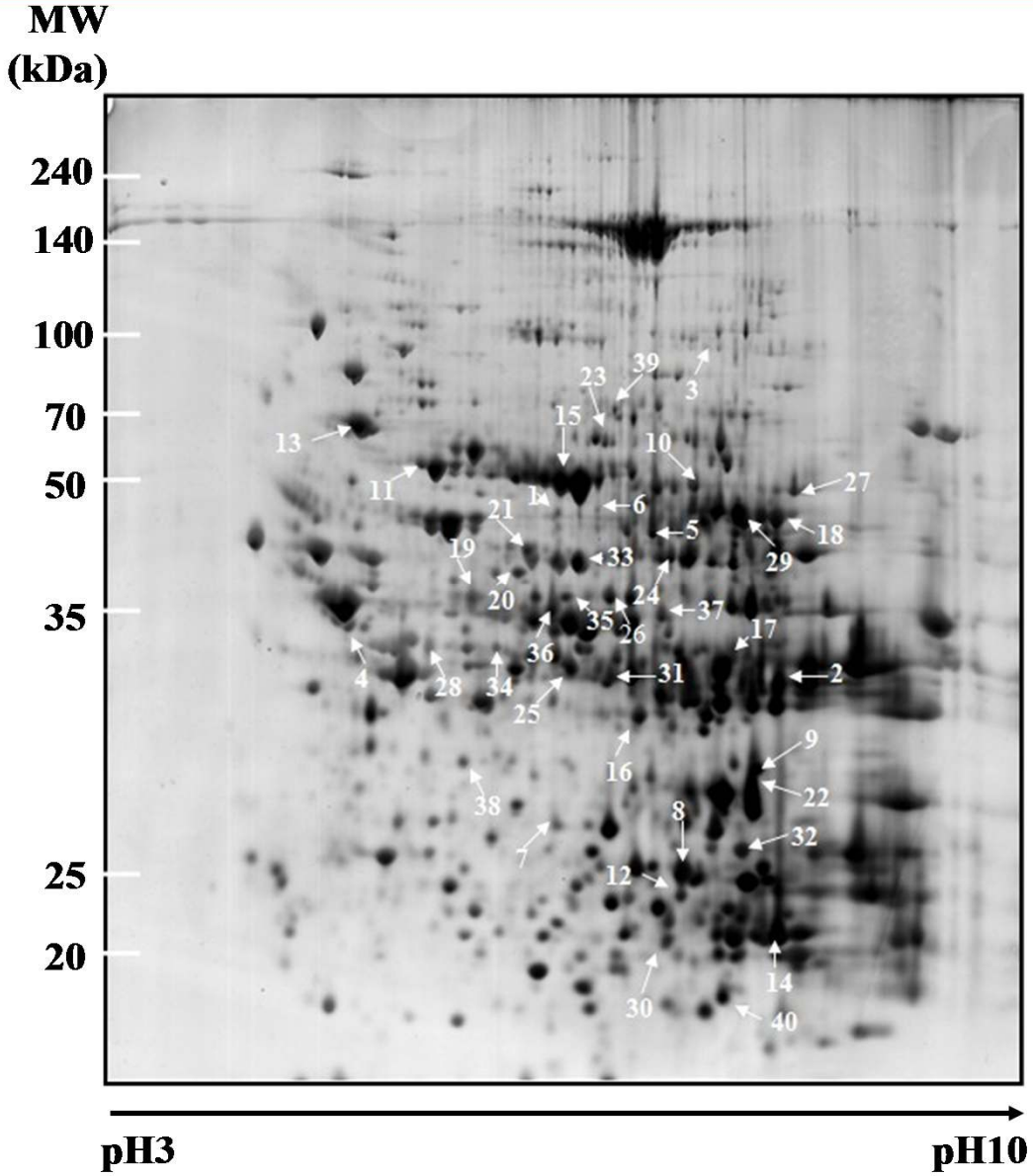
Proteomic analysis of liver

Liver plays a pivotal role in lipid homeostatic response to feeding conditions



Proteome map of liver

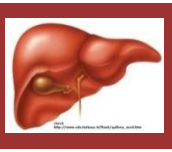


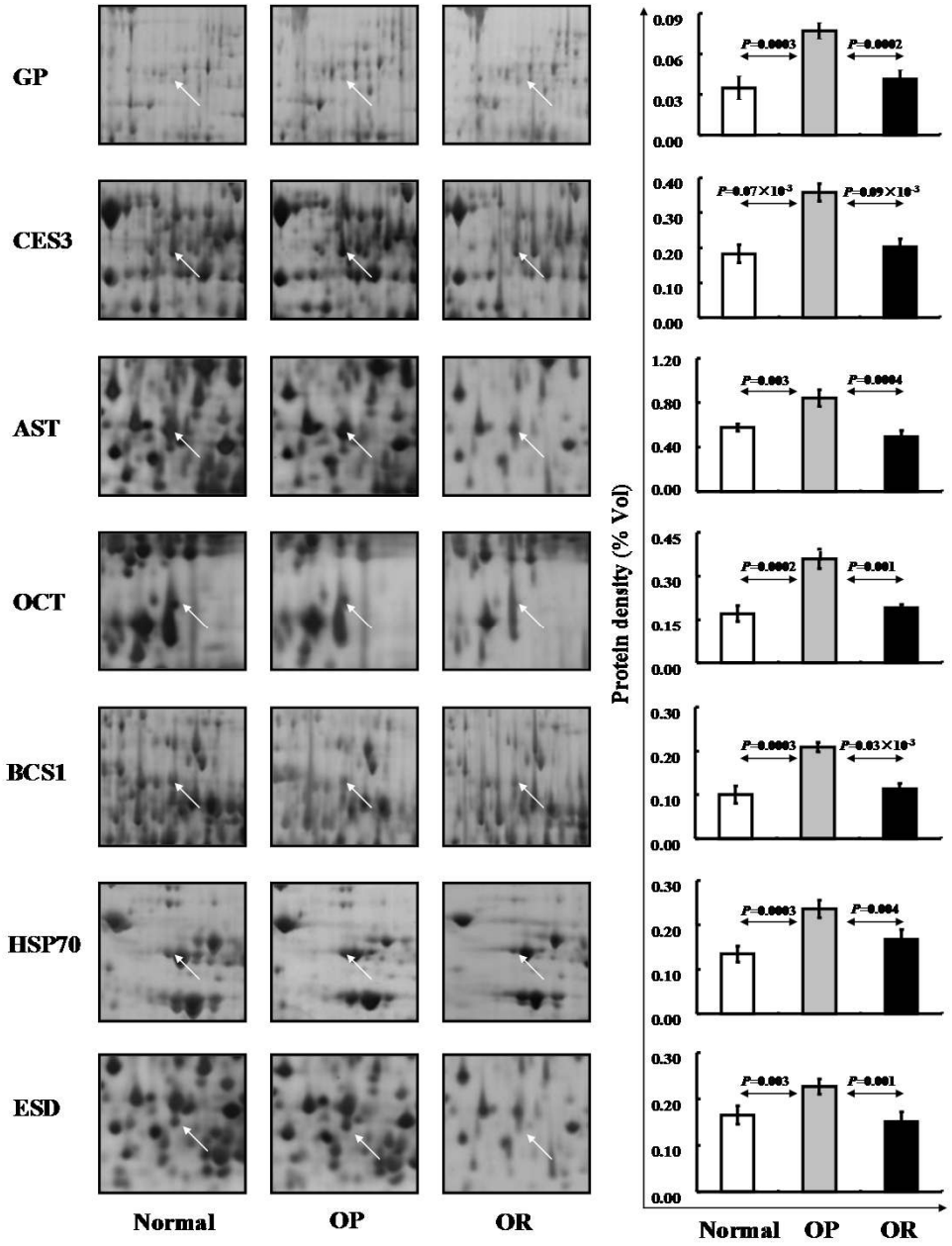


Representative 2-DE gel images of liver protein homogenate. Proteins were extracted and separated on pH 3–10 IPG strips for the first dimension, followed by 8% (w/v) polyacrylamide gel electrophoresis (PAGE) for the second dimension. Differentially expressed proteins were marked with arrows.

Wang et al. *Br. J. Nutr.* 106:612~626 (2011)

Proteomic analysis of liver





➡ Reduced insulin resistance

➡ Reduced hepatic fat

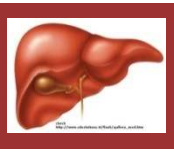
➡ No evidence linked to obesity !

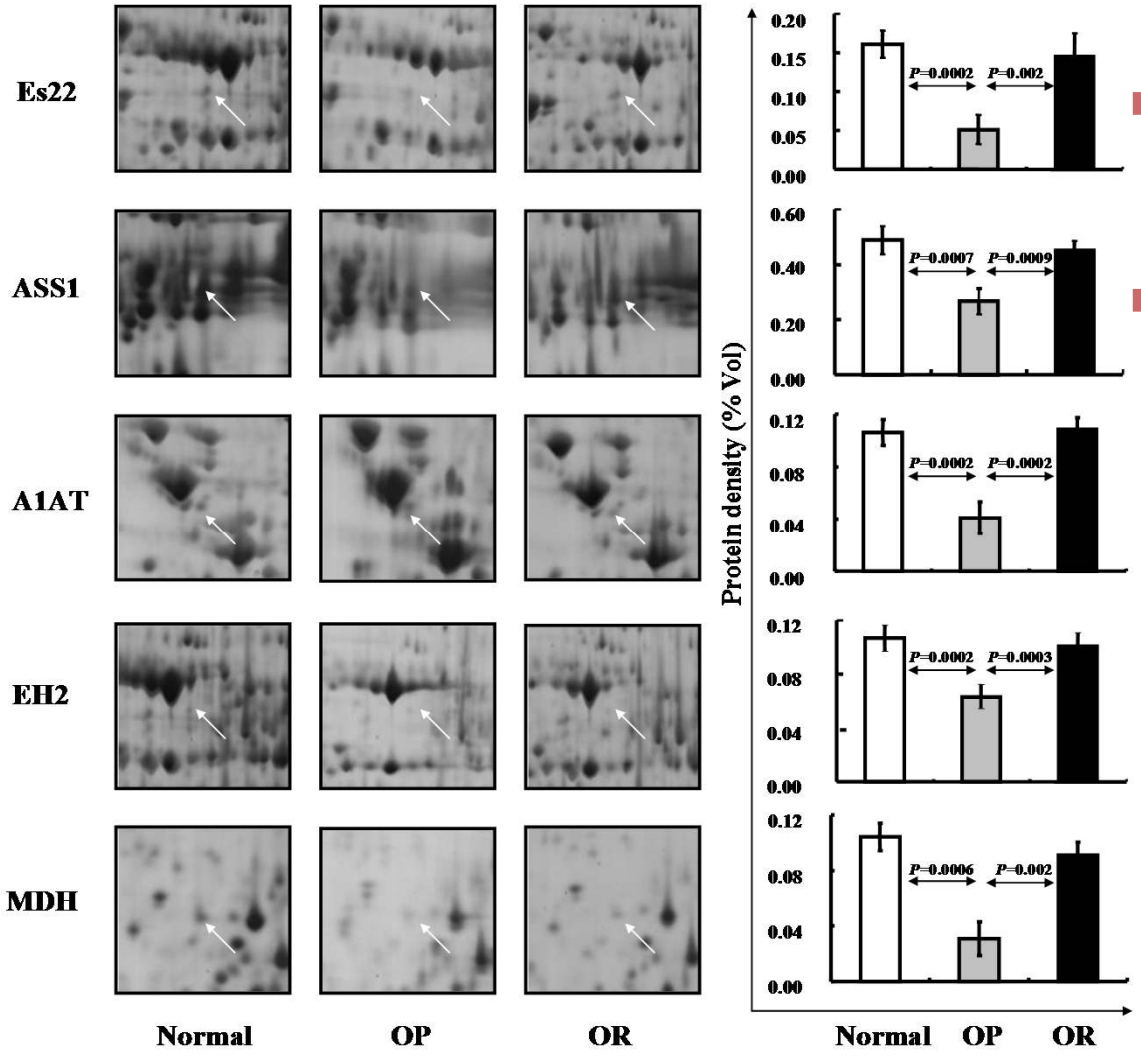
➡ No evidence linked to obesity !

Significantly increased liver proteins in OP rats compared to control and OR rats. Zoom-in-gel images of each protein are shown with their average expression levels in three rats in each group. Data are presented as mean values ±S.E.M. of volume density (%) of target spot in each group. They were estimated using the ANOVA test and the *p* values represent the significance of expression level between each group.

Wang et al. *Br. J. Nutr.* 106:612~626 (2011)

Proteomic analysis of liver





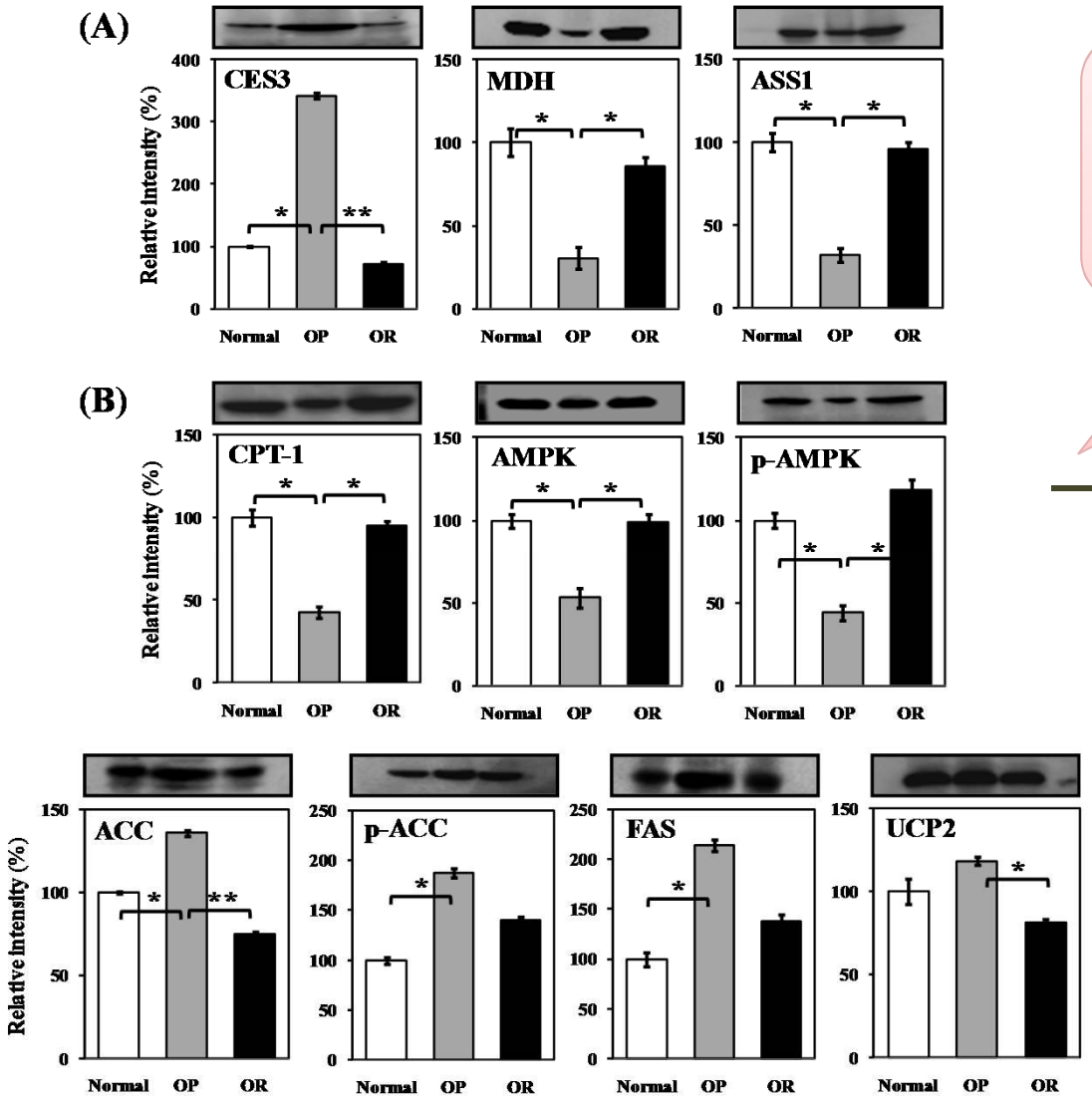
➡ Reduced insulin resistance

➡ No evidence linked to obesity !

Significantly decreased liver proteins in OP rats compared to control and OR rats. Zoom-in-gel images of each protein were shown with their average expression levels in three rats in each group. Data are presented as mean values ±S.E.M. of volume density (%) of target spot in each group. They were estimated using the ANOVA test and the *p* values represent the significance of expression level between each group.

Wang et al. *Br. J. Nutr.* 106:612~626 (2011)

Western blot analysis for metabolically important proteins

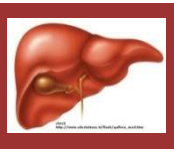


Increased fat oxidation and reduced lipogenesis in OR rat liver

Validation of proteomic data for some proteins of interest (A) and differential expression patterns of seven metabolic liver proteins (B) in normal, OP, and OR rats by immunoblot analysis. Band density was calculated by ImageMaster 2D software V4.95 and relative intensity (%) demonstrated that values of target proteins were normalized to those of β-actin. Asterisk indicates statistically significant (*p<0.05 and **p<0.01). Full names of proteins are presented in the abbreviations section.

Wang et al. *Br. J. Nutr.* 106:612~626 (2011)

Summary from liver proteome analysis



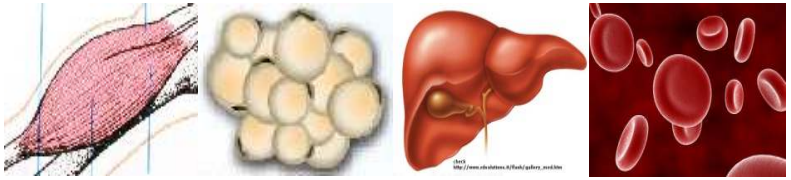
- Enhanced regulation of proteins involved in lipid metabolism resulted in OR phenotype.

- Increased expression of liver proteins for β-oxidation contributed to OR phenotype.

Wang et al. *Br. J. Nutr.* 106:612~626 (2011)

Validation of marker proteins through gene knock-down technique using siRNA

Strategy for functional study



Identified biomarkers by proteomic analysis

Cell line-based functional study
(Transiently transfected or established transgenic cell line)

siRNA design
- siRNA design tool
- Effective BLAST searches

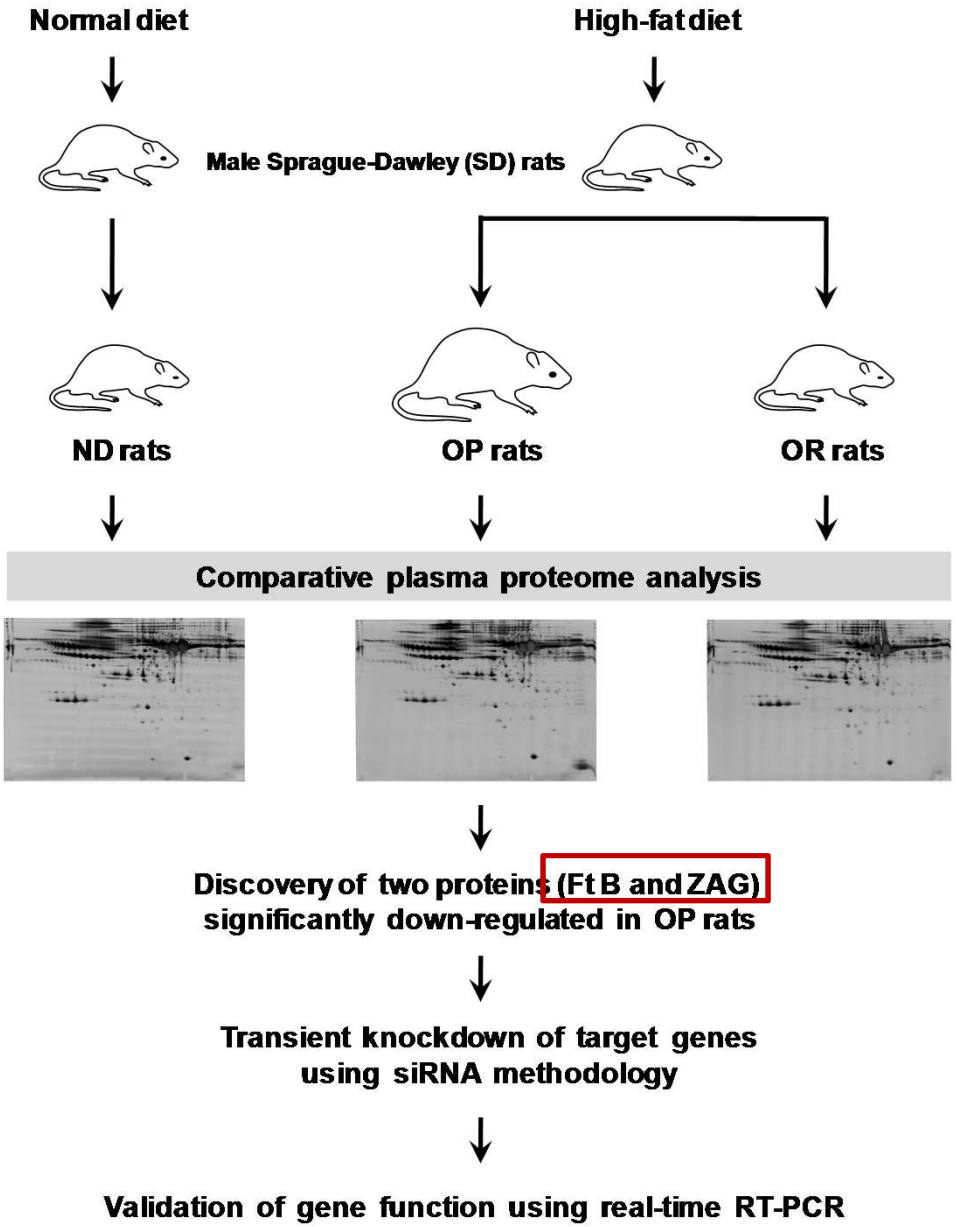
Produce the siRNA
- Chemical synthesis
- *In vitro* transcription
- Vector-based expression

Effective introducing siRNA into cells
- Parameter optimization
  ex) cell densities, transfection reagent, RNA ratio, quality of the siRNA

Screening for siRNA effects
- Western blotting
- RT-PCR
- Real time PCR

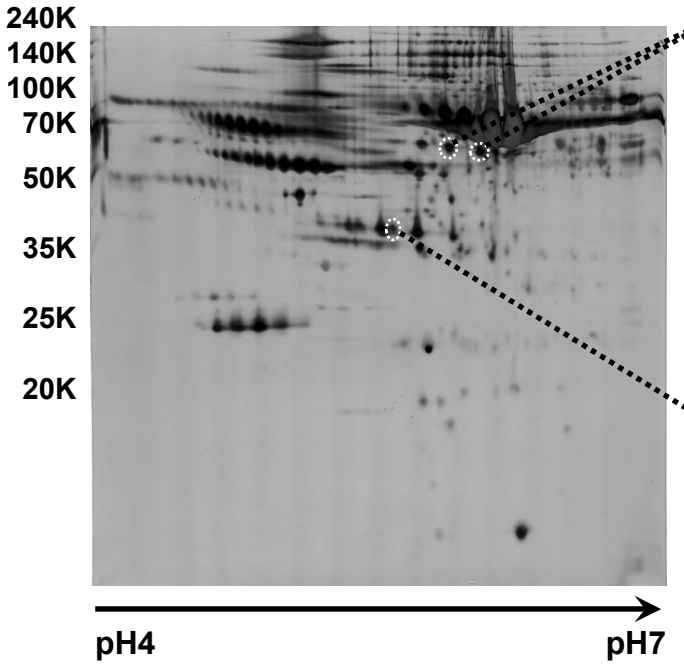
Animal model-based functional study
(Knock-out or transgenic animal model)

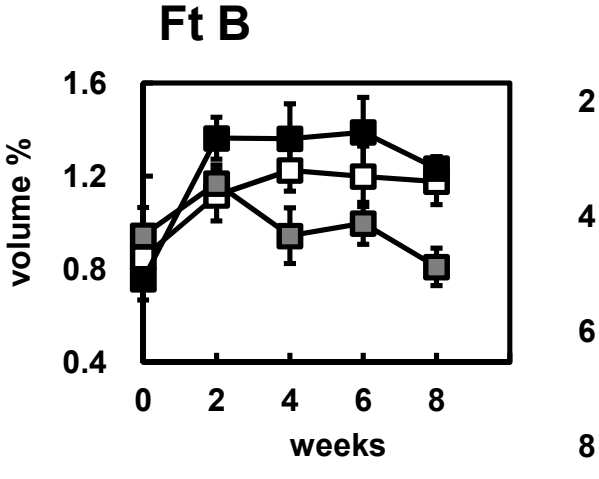
Experimental design

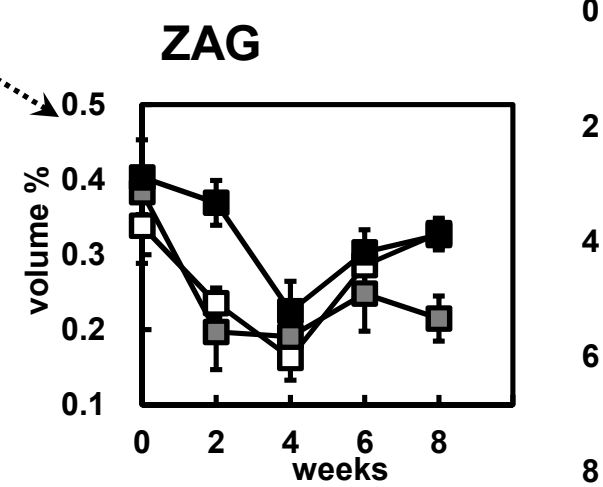


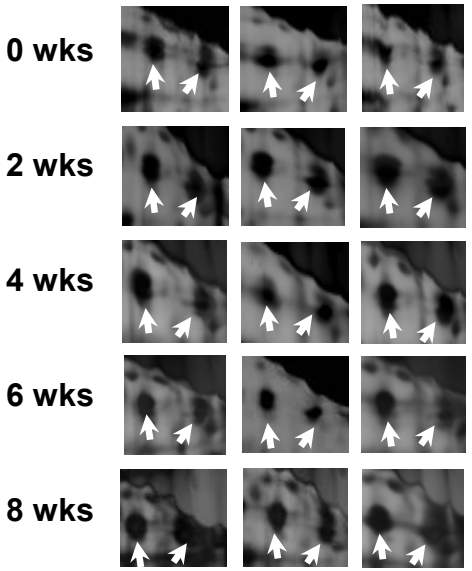
Proteomic results of feutin B and zinc-alpha-2-glycoprotein

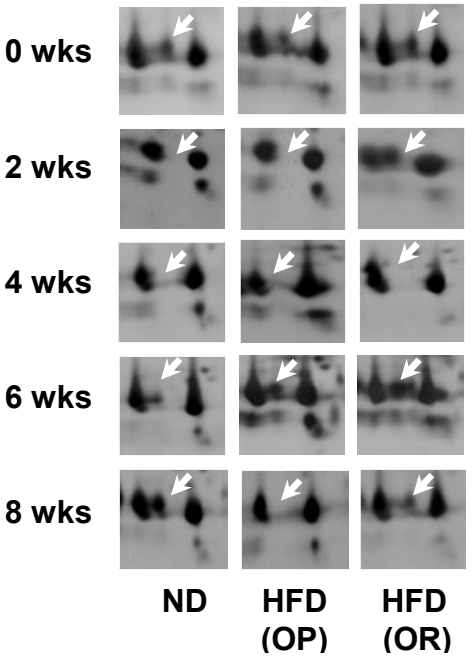
Biomarkers: must be consistently regulated !









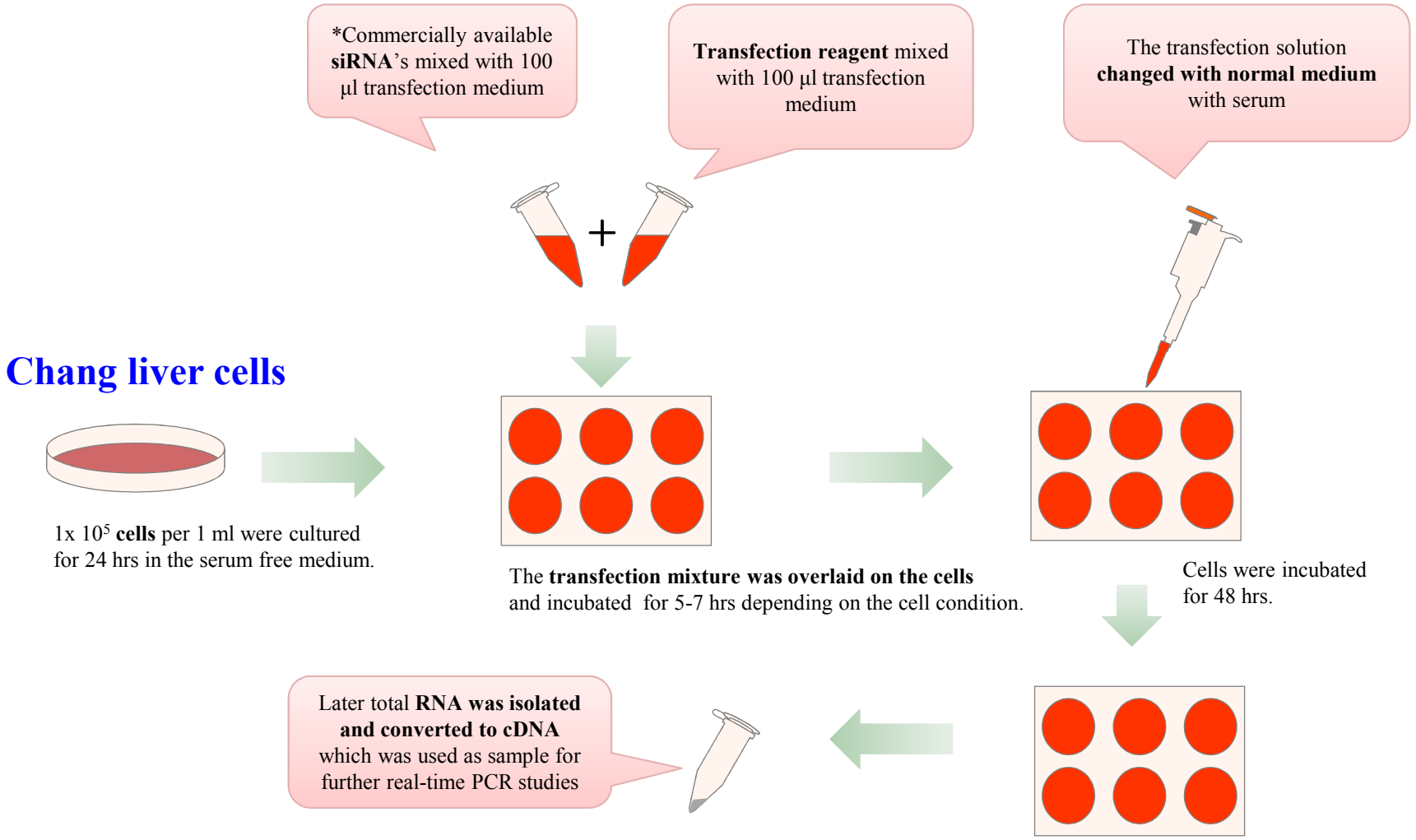


Secretary proteins from liver

Manuscript in preparation (2012)

How to knock down the target gene ?

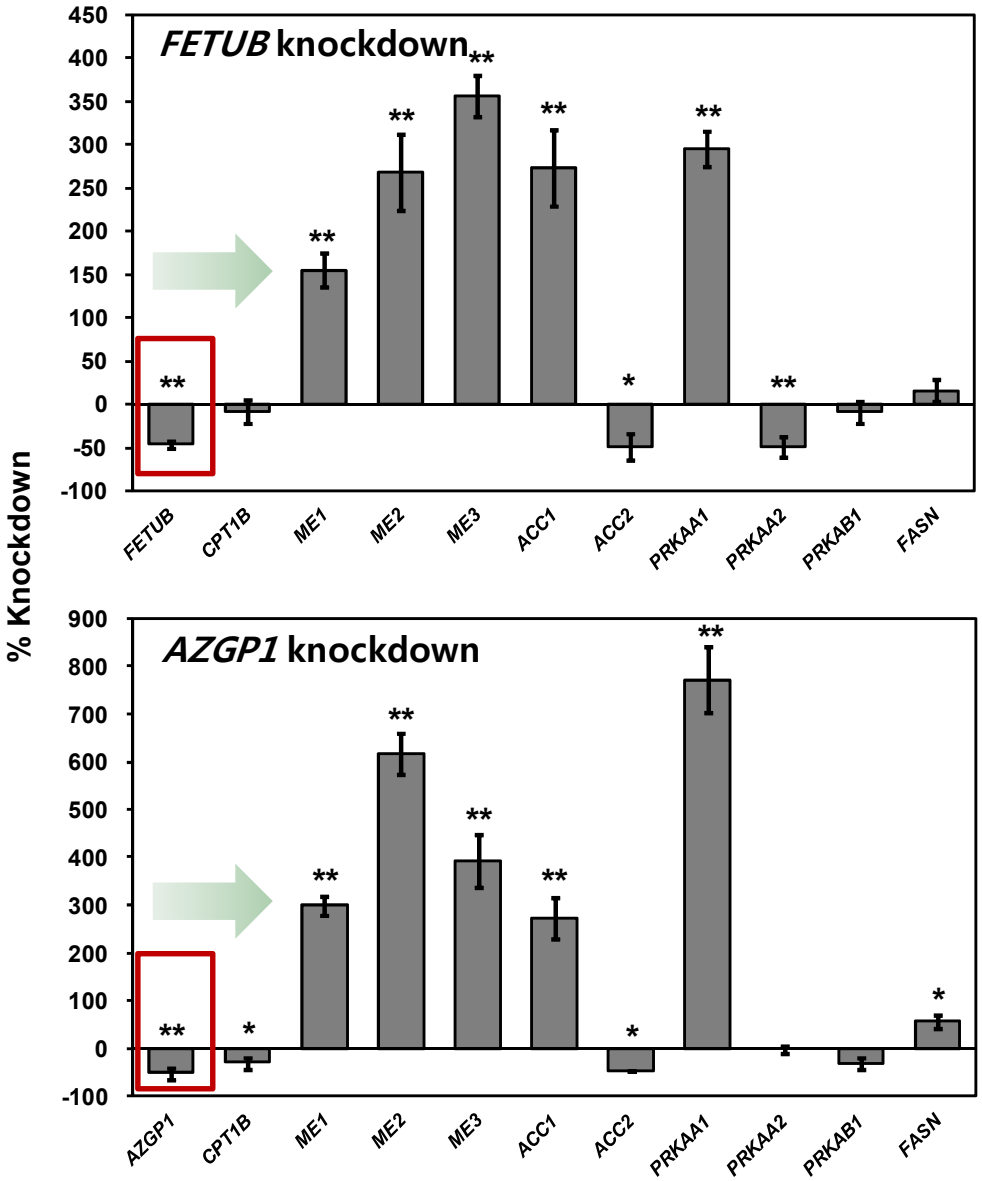
Procedure of Knock-down experiment using siRNA technique



* Commercially available siRNA is a pool of 3 target-specific 20-25 nt siRNAs designed to knock down gene expression.

Effects of *FETUB* or *AZGP1* Knockdown

Major lipogenic genes were highly expressed in knowndown liver cells



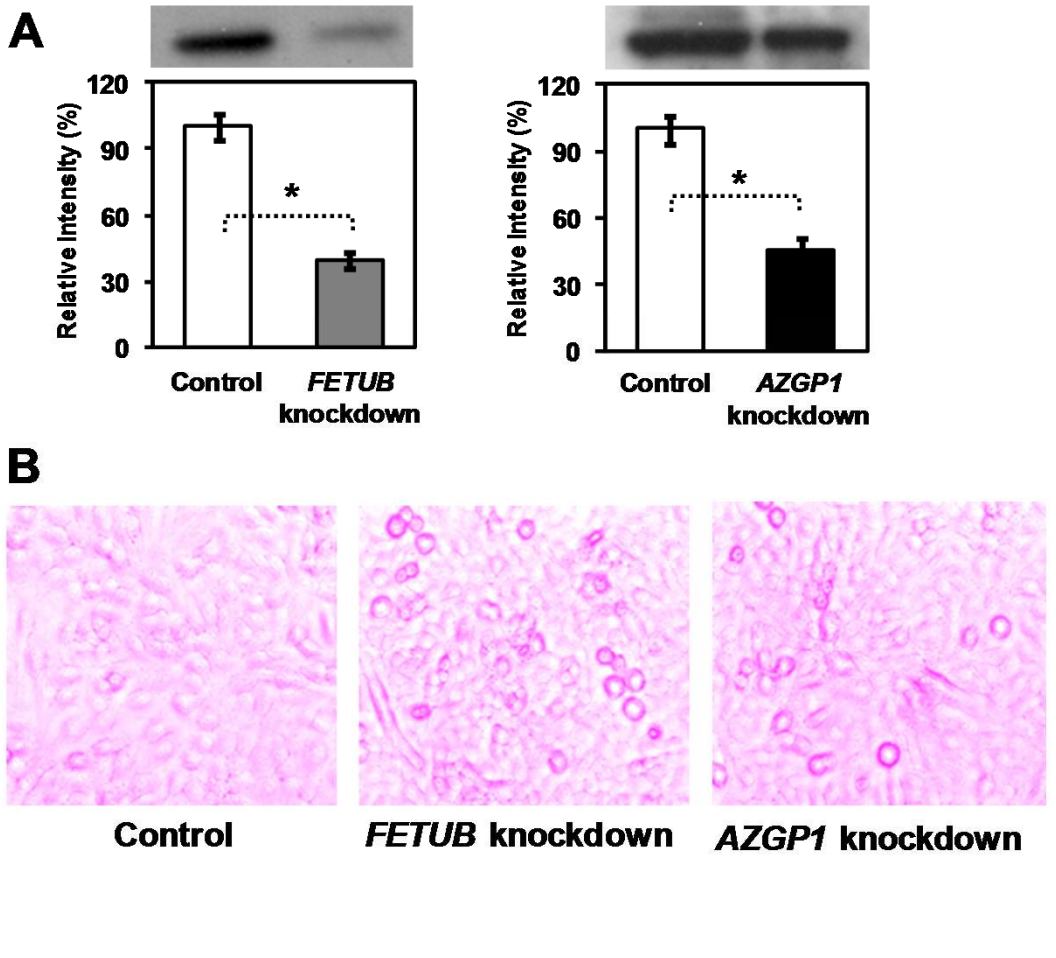
Effects of *FETUB* or *AZGP1* knockdown on transcription of the selected genes in Chang liver cells. Transcript levels of each gene were normalized to *GAPDH* transcript levels. Data are exhibited as mean values ±SD of % Knockdown. Data are representative of three independent assays. Statistical significance between each group was determined by ANOVA test, where *p* value is $^{*}p<0.05$ and $^{**}p<0.01$

FETUB, fetuin B; ***CPT1B***, carnitine palmitoyltransferase 1B; ***ME***, malic enzyme, NADP(+)-dependent, cytosolic; ***ACC***, acetyl-CoA carboxylase; ***PRKAA***, protein kinase, AMP-activated, alpha catalytic subunit ; ***PRKAB***, protein kinase, AMP-activated, beta catalytic subunit; ***FASN***, fatty acid synthase; ***AZGP1***, alpha-2-glycoprotein 1, zinc-binding

% Knockdown = (a-b)/b x 100
a: Normalized expression levels of each gene in siRNA-transfected cells
b: Normalized expression levels of each gene in BLOCK-iT-transfected cells

Manuscript in preparation (2012)

Effects of *FETUB* or *AZGP1* Knockdown

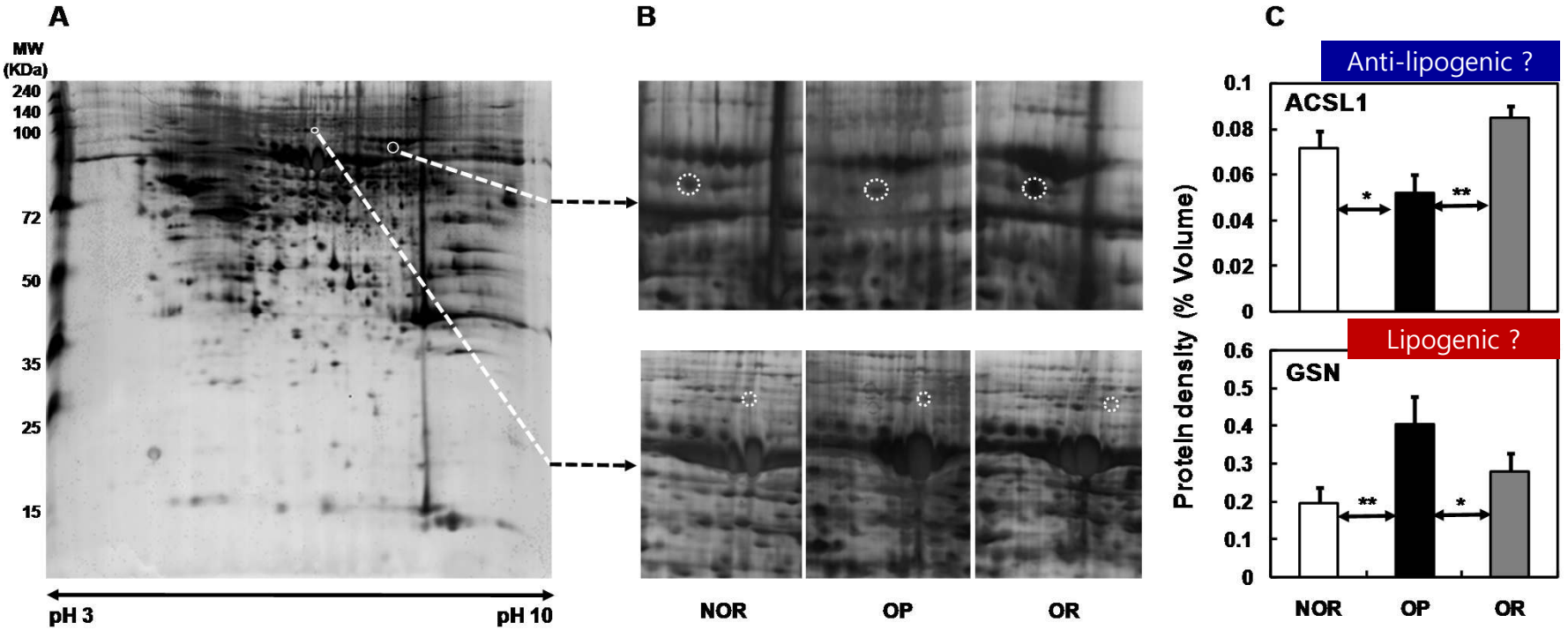


Effects of *FETUB* or *AZGP1* knockdown on fat synthesis of Chang liver cells. (A) protein levels, (B) Lipid droplets by Oil Red-O staining , (C) Triglyceride content. Data are shown representative of three experiments. $*p < 0.05$, $**p < 0.01$.

Manuscript in preparation (2012)

Functional study 2

From proteomic study in white adipose tissue, we found that..

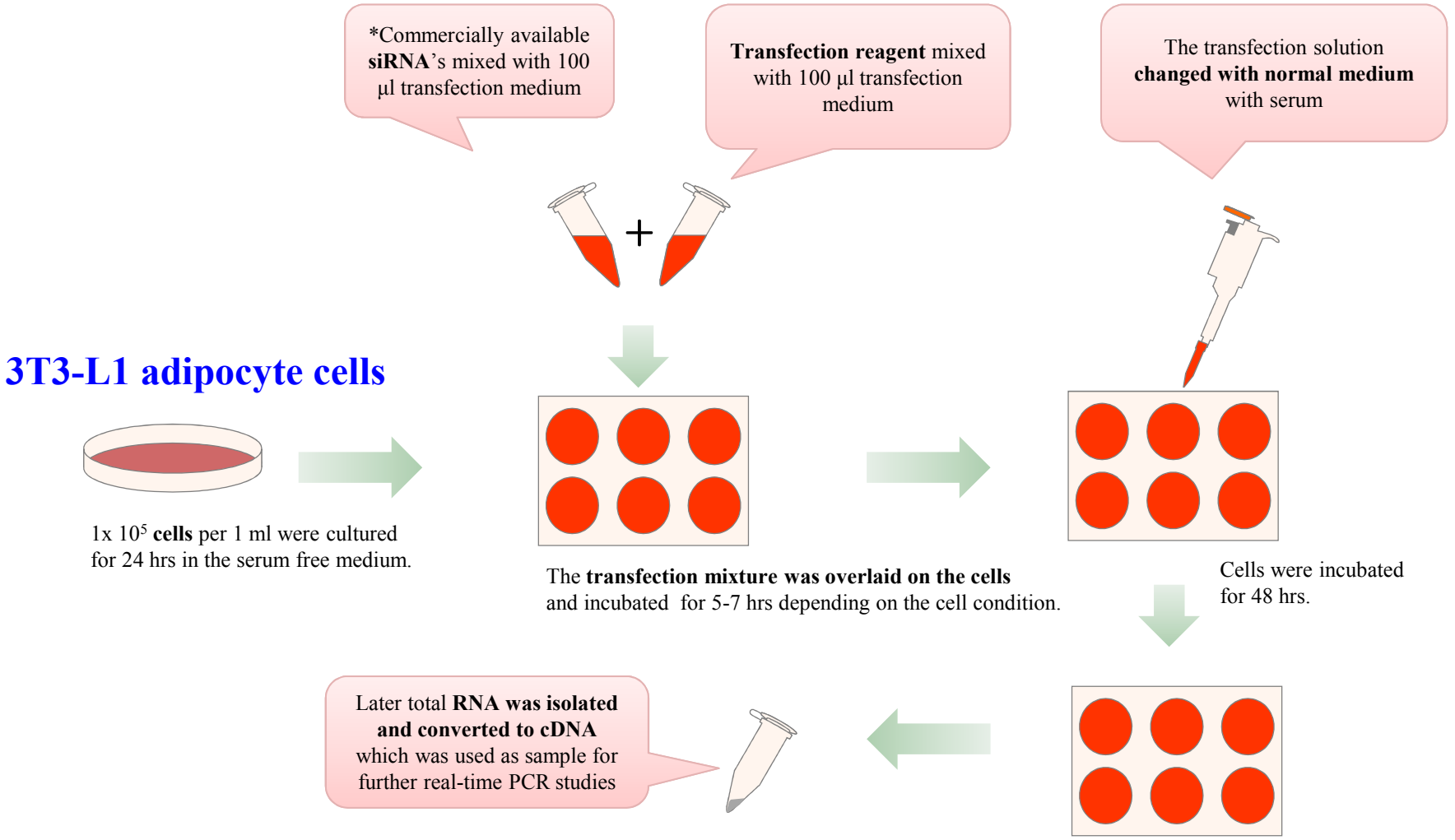


Differentially expressed ACSL1 and GSN between obesity-prone (OP) and obesity-resistant (OR) rats fed a high fat diet compared with normal rats fed a standard diet (NOR). (A) A representative whole two-dimensional electrophoresis gel image of WAT of rat, (B) zoom-in-gel images of ACSL1 and GSN, (C) comparative protein density of ACSL1 and GSN. Data are exhibited as mean values ±SD of volume density (%) of the changed spot in three individual gels using WAT tissue. Statistical significance between each group was determined by ANOVA test, where *p* value is $^{*}p<0.05$ and $^{**}p<0.01$.

Effects of gene knock-down ?

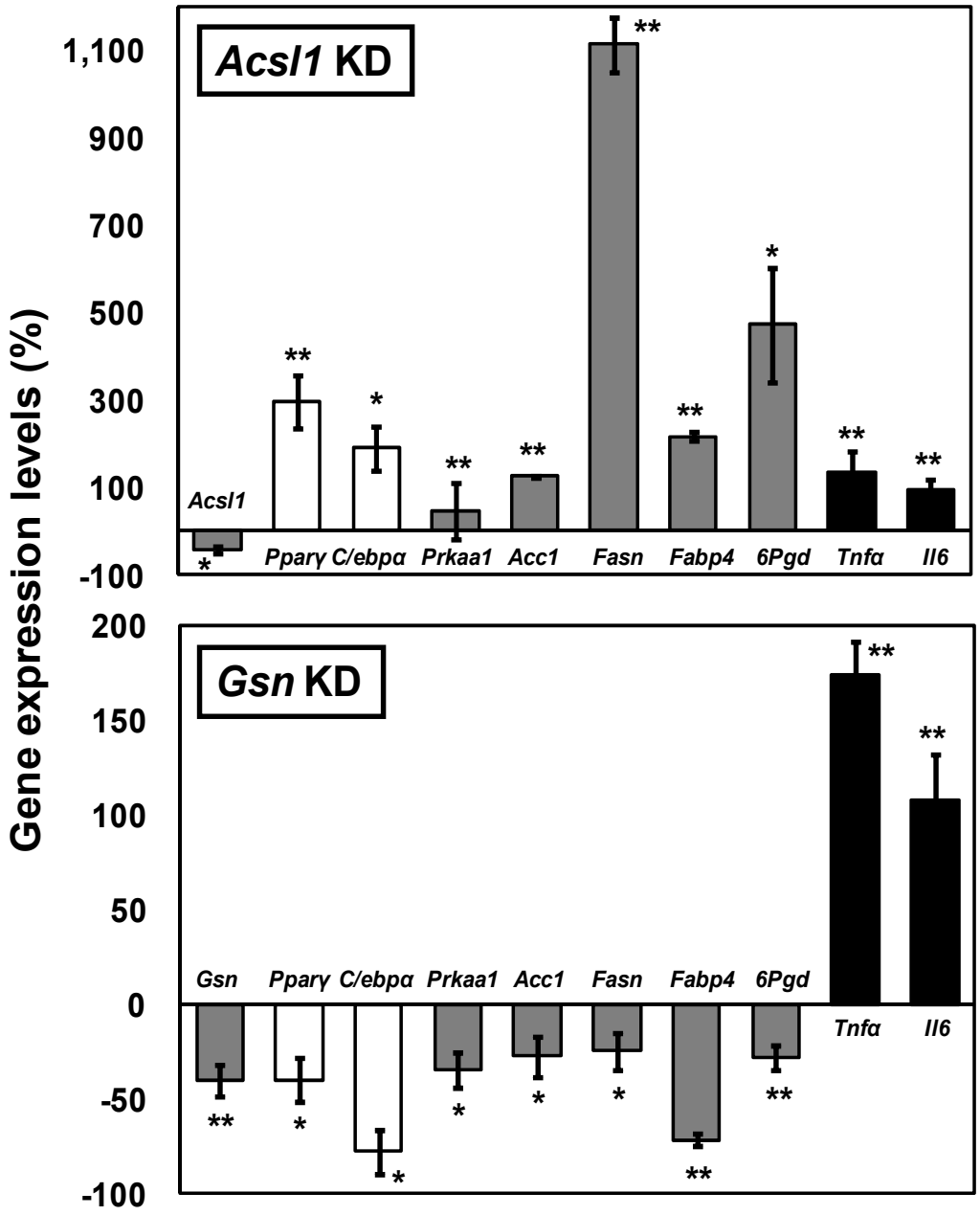
How to knock down the target gene ?

Procedure of Knock-down experiment using siRNA technique



* Commercially available siRNA is a pool of 3 target-specific 20-25 nt siRNAs designed to knock down gene expression.

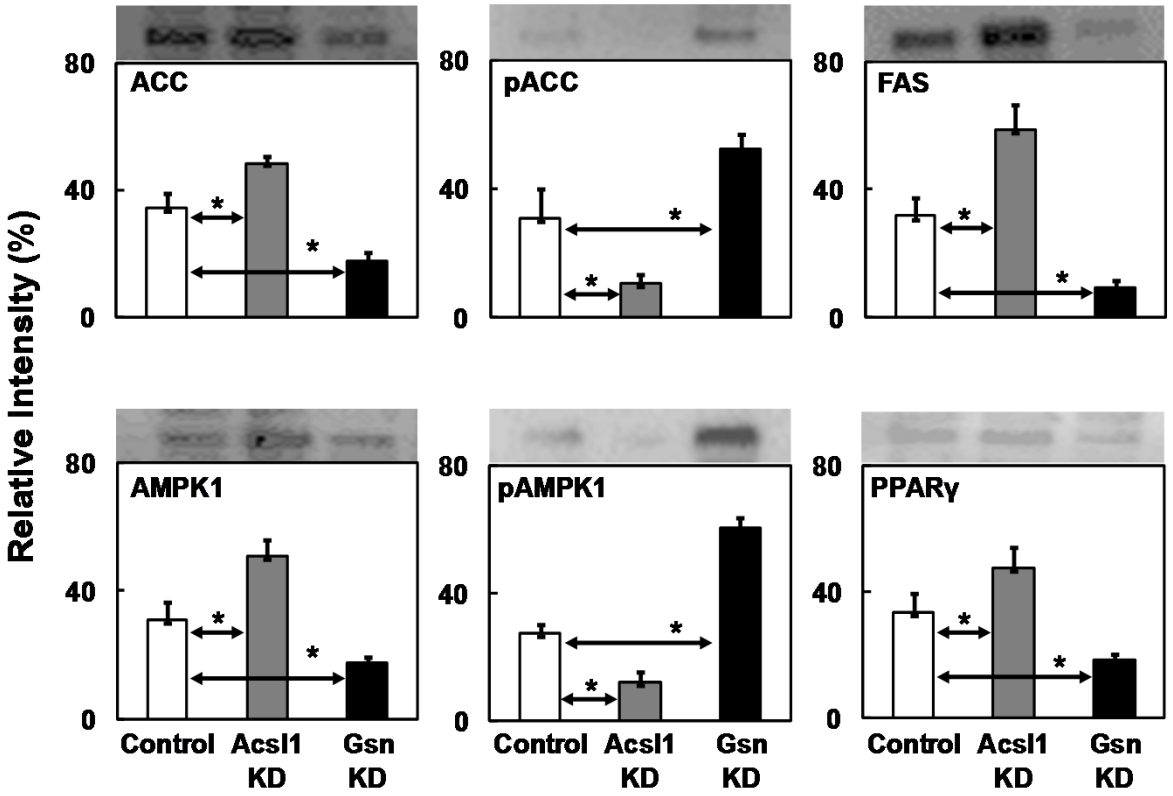
Effects of knockdown on lipogenic gene expression



Effects of *Acsl1* or *Gsn* knockdown on expression levels of adipogenic (white bars), lipogenic (gray bars), and pro-inflammatory (black) genes in 3T3-L1 cells. Transcript levels of each gene were normalized to *β-actin* transcript levels. Data are exhibited as mean values ±SD of % of differential gene expression level. Data are representative of three independent assays. Statistical significance between each group was determined by ANOVA test, where *p* value is $^{*}p<0.05$ and $^{**}p<0.01$.

Manuscript in preparation (2012)

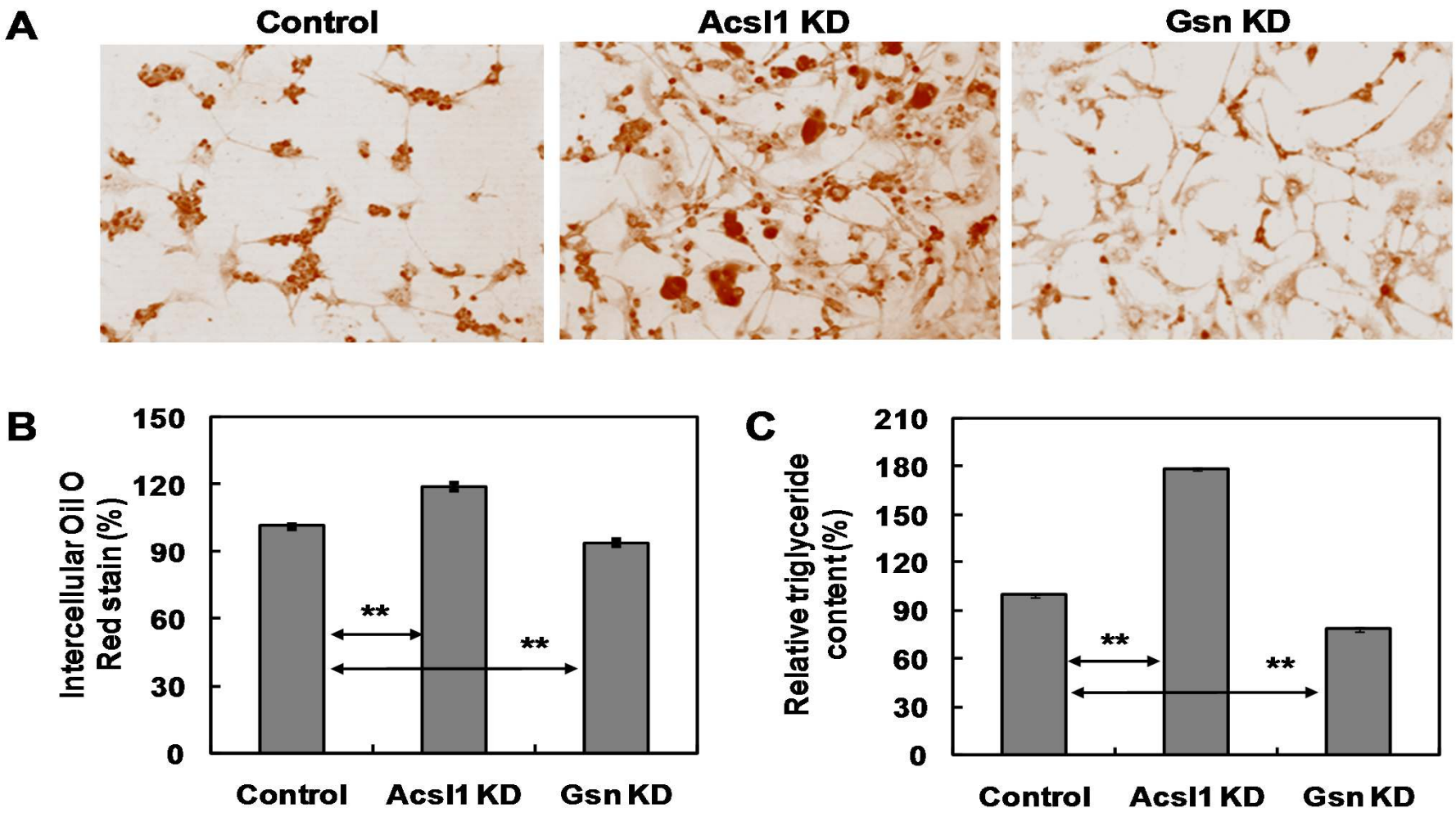
Effects of knockdown of protein expression



Effects of *Acsl1* or *Gsn* knock down on protein expression levels of some selected lipogenic proteins in 3T3-L1 cells by immunoblot analysis. Band density was calculated by Image-Master 2D software version 4.95, and relative intensity (%) demonstrated that values of protein were normalized to those of β-actin. Data are shown representative of three independent experiments. $*p<0.05$ and $**p<0.01$.

Manuscript in preparation (2012)

Effects of knockdown on fat formation

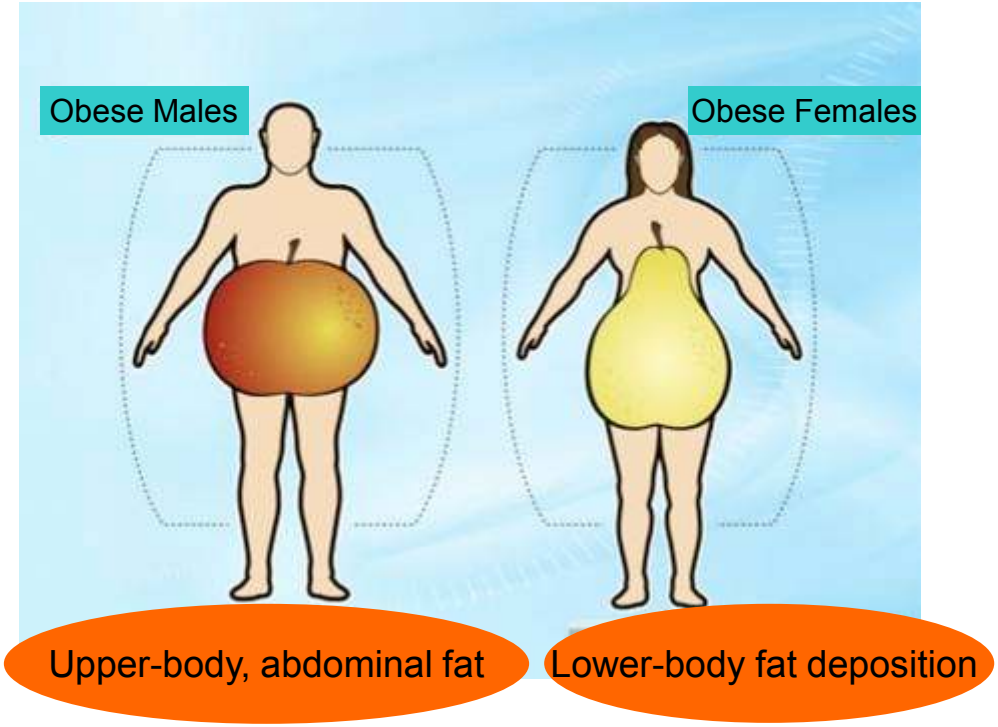


Effects of *Acsl1 & Gsn* knockdown on TG accumulation in 3T3-L1cells. (A) Oil red O staining (B) Intercellular ORO content (C) Triglyceride content assay. Data are shown representative of three experiments. *$p < 0.05$, **$p < 0.01$.

Manuscript in preparation (2012)

2

Proteome profiling for mining marker proteins for gender difference in obesity



Gender difference in obesity:
Earlier findings from biochemical studies

✓ Gender difference in obesity stems from metabolic and hormonal differences between the sexes, and contributes to differences between women and men in health risks attributable to obesity.

✓ Numerous studies; differences in body fat distribution, physical activity, metabolic rate, oxidative capacity, sex hormones, energy expenditure, adipokine signaling, appetite regulation.

✓ A line of evidence has demonstrated that males have a higher susceptibility to becoming obese, compared with females. However, some contradictory results have been found in the literature that may be explained by differences between rat strains and gender, as well as differences in the nutritional state of the animals.

➡ Need more accurate mechanisms underlying gender difference in obesity !

Hormonal effects on gender different obesity

Testosterone : stimulates fat mobilization in **males (lipolytic)**

: promotes fat storage in **females (lipogenic)**
: protects preadipocyte differentiation **(anti-adipogenic)**
: reduces insulin sensitivity by reduced GLUT levels and type I fiber in muscle
: reduces LPL activity→reduces FFA delivery→ prevents adipogenesis ??
: inhibits leptin expression
: stimulates muscle mass
: decreases UCP1 levels in BAT
: stimulates lipolysis in WAT
: increases calorie intake
: androgen receptor KO→lean
: decreases the levels of GLUT, type I fiber in **female** muscle
: decreases visceral fat in **males** but increases it in **females**

Estrogen : protects central fat accumulation in **females (anti-lipogenic)**

: stimulates preadipocyte differentiation **(pro-adipogenic)**
: reduces adipose mass and adipocyte size
: stimulates insulin sensitivity
: reduces LPL levels (becomes lean)
: stimulates thermogesis in **females**
: down-regulates lipogenic enzymes/genes
: partition of FFA toward oxidation away from TG storage
: estrogen receptor (α-ER) KO → obese
: decreases hypothalamic NPY, AgRP and increases POMC
: increases plasma leptin levels (higher leptin resistance←subcutaneous fat)

Difference in metabolism between the genders

Males

: lower basal rate of lipolysis

: lower rate of non-oxidative FFA clearance

: higher protective capacity against obesity gene variants (e.g. FTO)

: greater enzymatic potential for glycolysis → utilize higher carbohydrates

: higher resting metabolic rate → larger quantity of fat-free mass

: greater gene expression of FA transporters (FA uptake) in abdominal fat

: in aging and obese men, increased aromatase activity (androgen→estrogen)

Females

: higher β-adrenergic lipolysis (more sensitive to β-AR) → lower abdominal fat

: lower cognitive inhibition → higher desire for food stimulation

: more responsive to leptin administration

: higher type I muscle fiber → greater capacity to oxidize fat

: less abundant glycolytic proteins → greater reliance on lipid oxidation

: subcutaneous fat is the preferred energy source for gestation and lactation

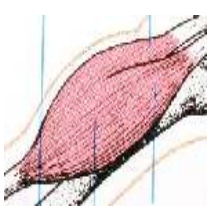
Experimental design

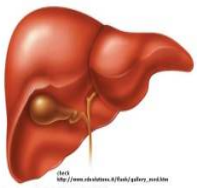
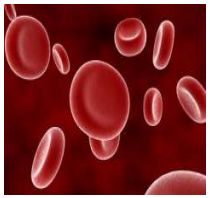
Normal diet (*n*=20) **High-fat diet (*n*=40)**

Sprague-Dawley rats (8 weeks)




Males (*n*=7) **Females (*n*=7)** **Males (*n*=7)** **Females (*n*=7)**



mRNA→ cDNA

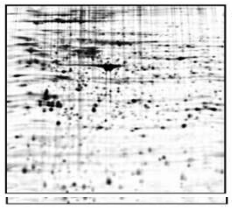
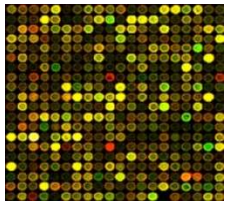
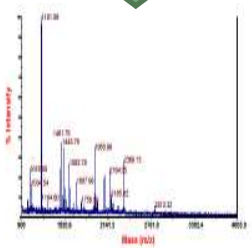
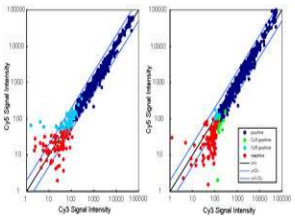
pretreated samples

isoelectric focusing, IEF

pI

SDS-PAGE

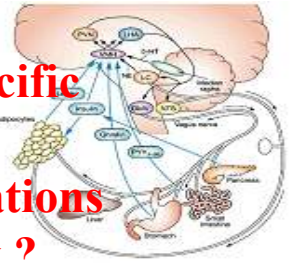
(Molecular weight)

Proteomic analysis

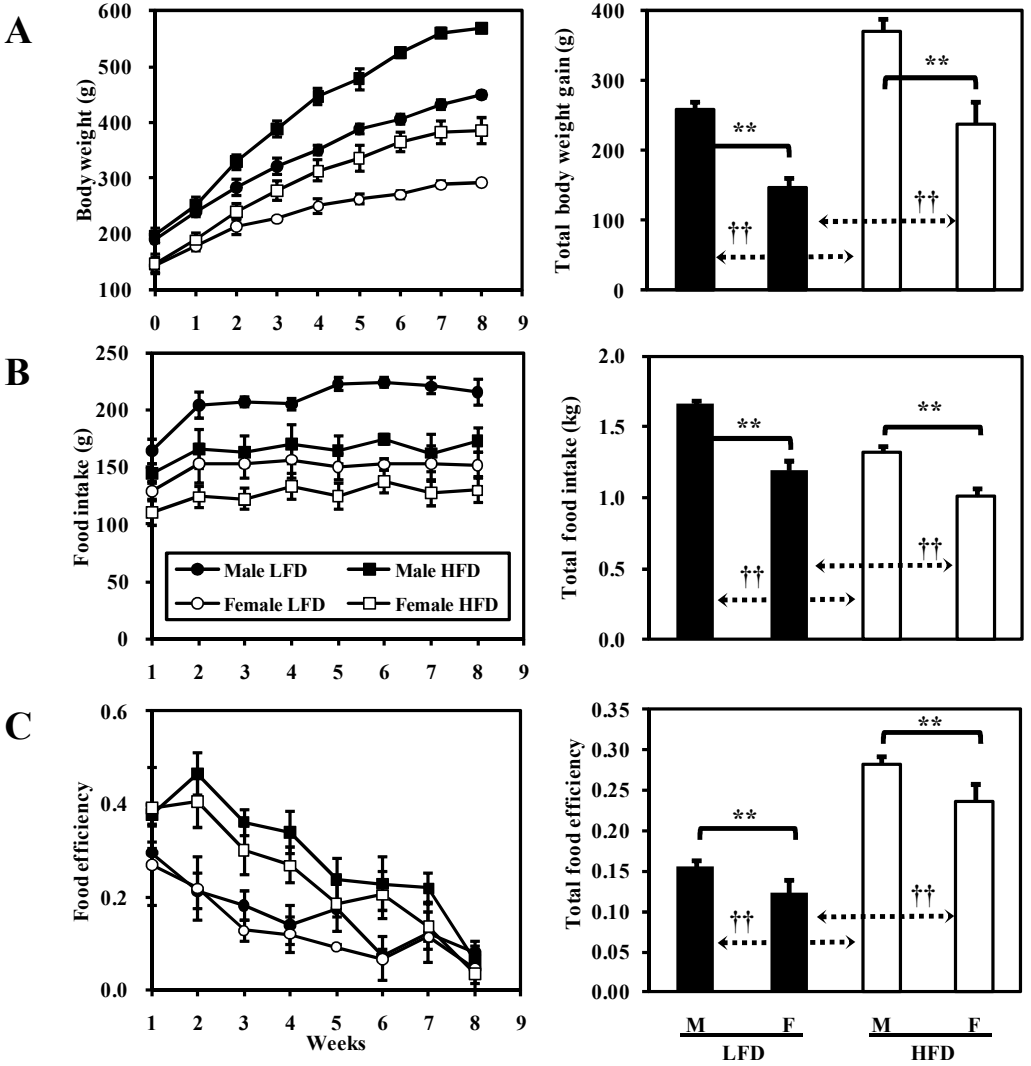
Genomic analysis

Novel biomarkers showing gender differences to obesity



Gender specific clinical recommendations to obesity ?

Male rats become more obese than females when exposed to HFD



Body weight (A), food intake (B), and food efficiency (C) in male and female rats fed LFD and HFD. Left figures represent changes of these parameters with time and the right figures display total changes. Data are presented as mean ±SEM in seven rats per group and were estimated using the ANOVA test. Statistical significance between male and female rats was determined by a t-test, where *p* value is $^{*}p<0.05$ and $^{**}p<0.01$ and significance between LFD and HFD rats was represented by $^{\dagger}p<0.05$ and $^{\dagger\dagger}p<0.01$.

Liu et al. *Proteomics , 12(2)* (2012)

Plasma biochemical parameters

Parameter	ND		HFD	
	Male	Female	Male	Female
Glucose (mg/ml)	2.08±0.18**†	1.64±0.1†	2.39±0.24	2.21±0.58
TG (mg/ml)	0.49±0.09†	0.47±0.07†	0.71±0.23	0.67±0.19
Total cholesterol (mg/ml)	0.36±0.16†	0.32±0.2†	0.6±0.21	0.54±0.16
HDL cholesterol (mg/ml)	0.52±0.11††	0.51±0.09†	0.32±0.11	0.35±0.12
Free fatty acid (μmol/ml)	1.78±0.2†	1.79±0.14	2.05±0.26	1.97±0.25
Leptin (ng/ml)	6.62±2.62*††	2.77±2.15†	21±4.28**	11.2±6.51
Insulin (ng/ml)	1.67±0.48**†	0.57±0.3	2.43±0.65	1.5±1.27
Estrogen (ng/ml)	5.56±0.55**††	8.3±0.78††	6.39±0.47**	10.83±1.44
Testosterone (ng/ml)	1.95±1.85	0.31±0.07	0.92±0.56**	0.35±0.1

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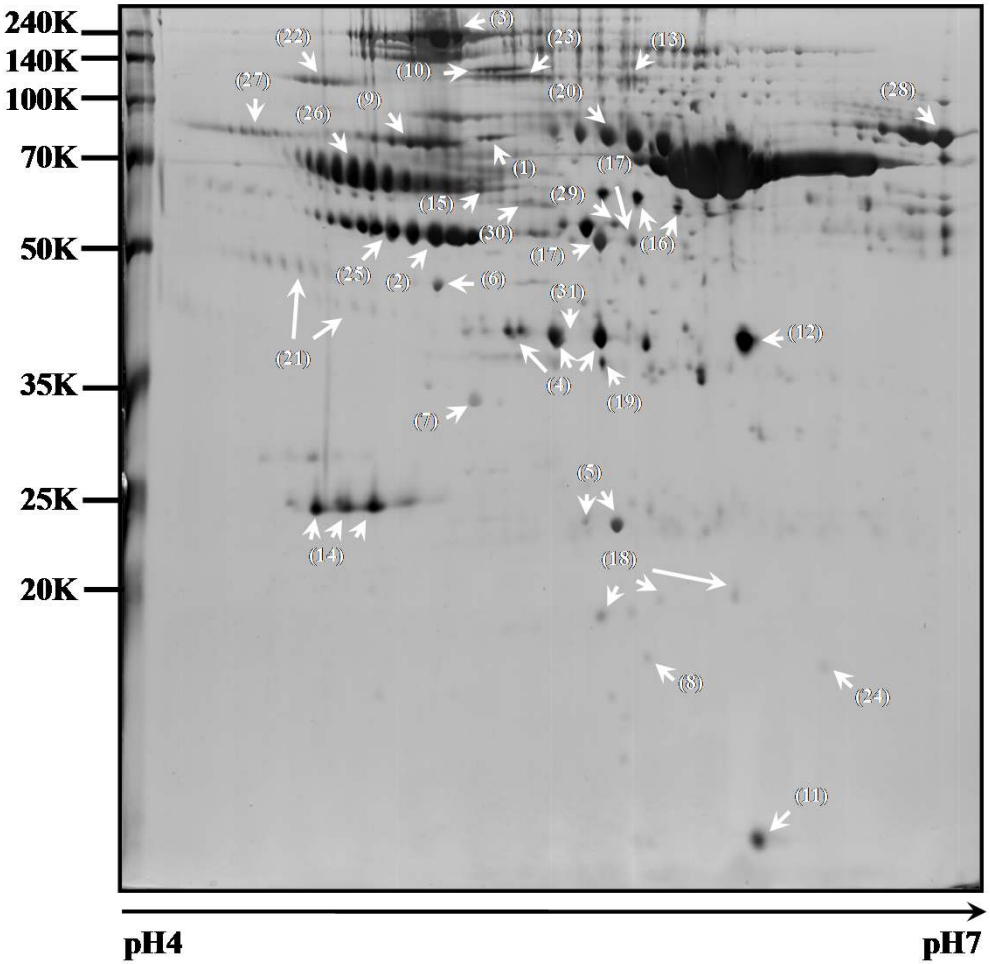
1 Plasma proteome of male and female rats



A representative silver-stained 2-DE gel image of rat plasma proteome.
Differentially regulated proteins in each group are marked with arrows and proteins of numbers in gel are listed in Table 2.

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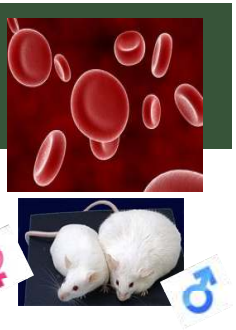
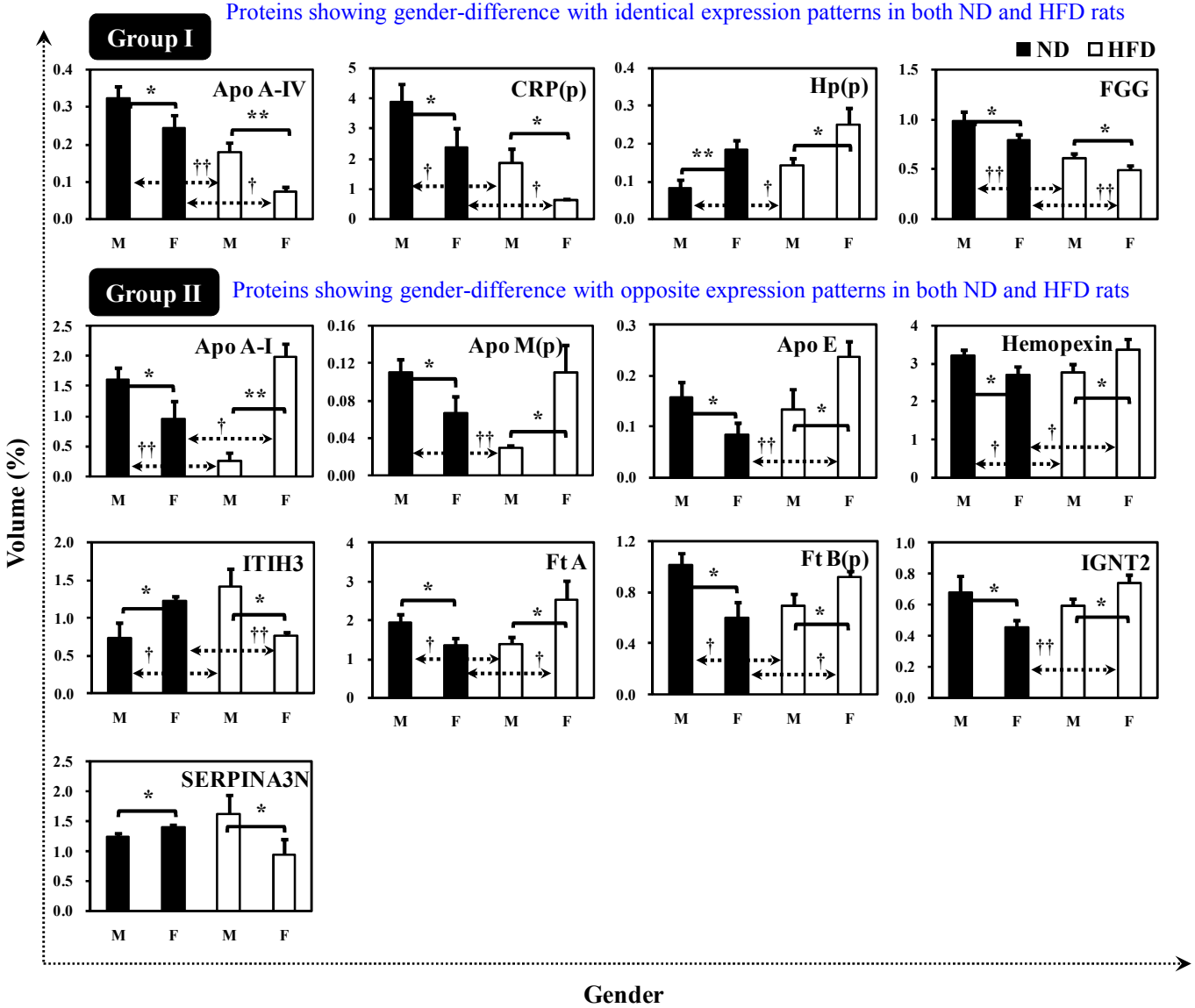
Plasma proteome analysis





Differentially expressed plasma proteins showing gender difference. Data are exhibited as mean values ±SEM of volume density (%) of the changed spot in three individual gels using pooled plasma of seven rats per group. These 13 proteins have *p* values below 0.05 between males and females fed ND and/or HFD. Statistical significance between male (M) and female (F) rats was determined by a *t*-test, where *p* values were $^{*}p<0.05$ and $^{**}p<0.01$and significance between ND and HFD rats was represented by $^{\dagger}p<0.05$ and $^{\dagger\dagger}p<0.01$. (p); precursor

Apo, apolipoprotein; **CRP**, C-reactive protein; **Hp**, haptoglobin; **FGG**, fibrinogen gamma chain; **ITIH**, inter-α-inhibitor H heavy chain; **Ft**, fetuin; **IGNT2**, I-branching beta-1,6-acetylglucosaminyltransferase family polypeptide 2; **SERPIN**, serine (or cysteine) peptidase inhibitor

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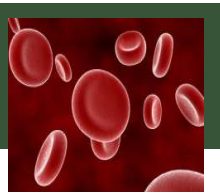
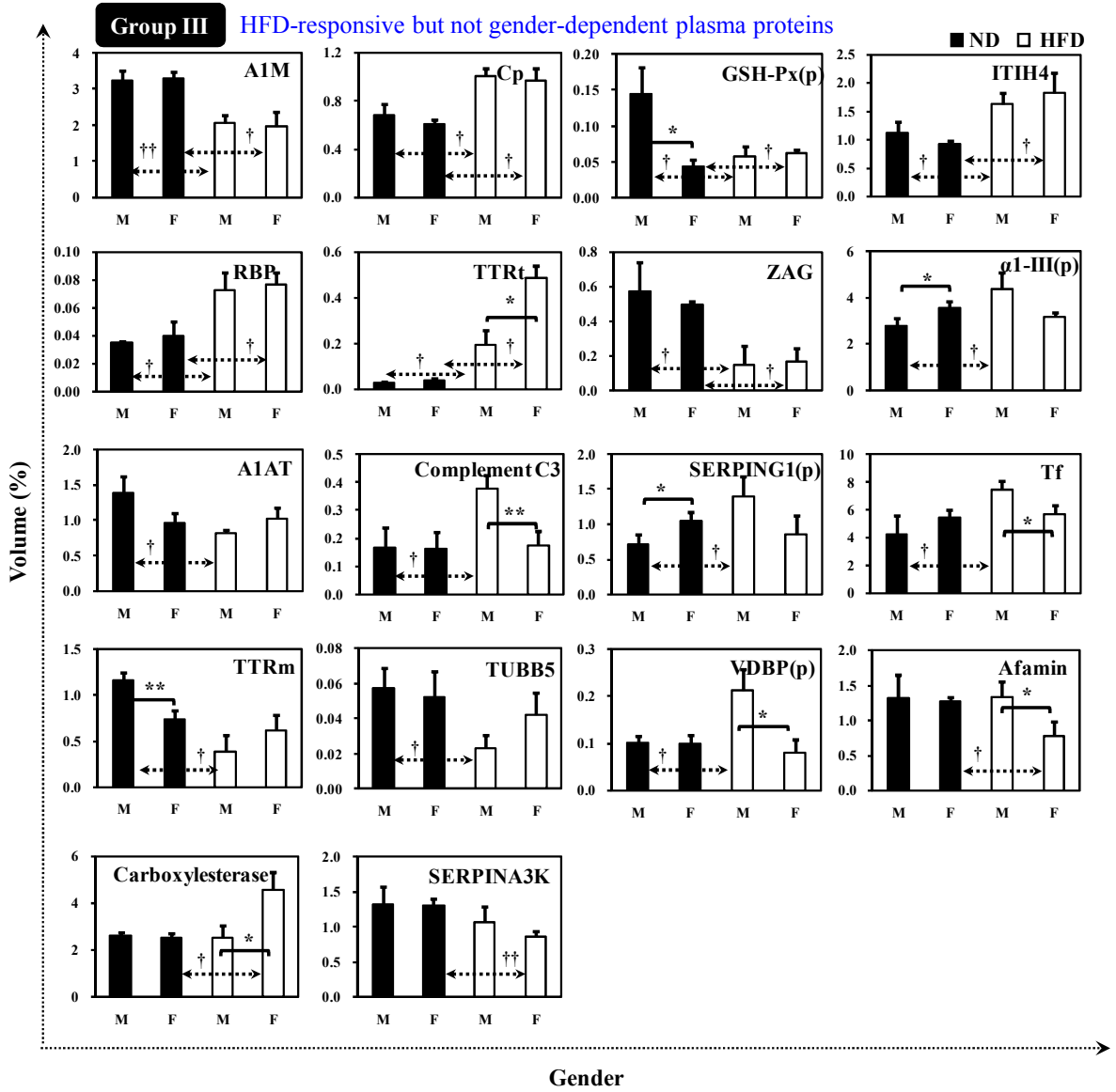
Plasma proteome analysis





Differentially expressed plasma proteins showing no gender difference but HFD-responsive. Data are exhibited as mean values ±SEM of volume density (%) of the changed spot in three individual gels using pooled plasma of seven rats per group. These 19 proteins have *p* values below 0.05 between males and females fed ND and/or HFD. Statistical significance between male (M) and female (F) rats was determined by a *t*-test, where *p* values were $^{*}p<0.05$ and $^{**}p<0.01$ and significance between ND and HFD rats was represented by $^{\dagger}p<0.05$ and $^{\dagger\dagger}p<0.01$. (p); precursor.

A1M, alpha-1-macroglobulin;
Cp, ceruloplasmin; **GSH-Px**, glutathione peroxidase; **RBP**, retinol binding protein; **TTR**, transthyretin; **ZAG**, zinc-alpha-2-glycoprotein; **α1-III,** alpha-1-inhibitor III; **A1AT**, alpha-1-antitrypsin; **Tf**, transferrin; **TUBB5**, tubulin beta-5 chain; **VDBP**, vitamin D-binding protein

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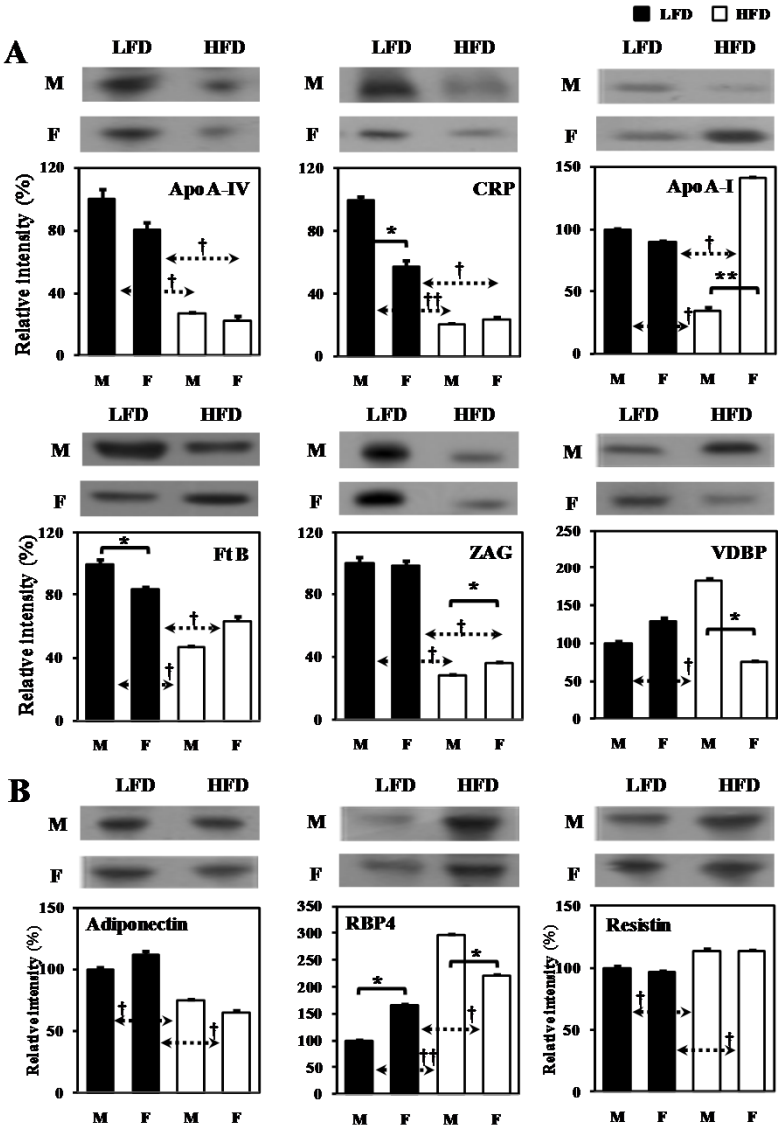
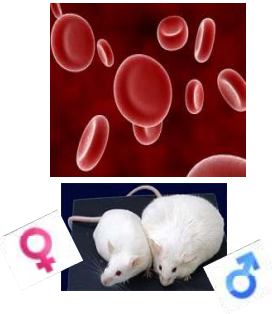
Validation of proteomic data by Western blot analysis





Validation of differentially regulated plasma proteins in male (M) and female (F) rats in response to HFD by immunoblot analysis. Levels of six identified proteins from 2-DE analysis (A) together with three important plasma proteins (B) were established using pooled plasma of seven rats per group. Data are representative of three independent experiments. For abbreviation of each protein name, see abbreviations.

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Proteins showing gender-difference in plasma


A	***Proteins showing gender difference in both LFD and HFD rats (Group I & II)***		
	ApoA-IV, CRP, FGG	↑↑	↓↓
	HP(p)	↓↓	↑↑
	Apo A-I, Apo M(p) Apo E, Hemopexin, Ft A, Ft B, IGNT2	↑↓	↓↑
	ITIH3, SERPINA3N, RBP4	↓↑	↑↓
B	***Proteins showing no gender difference but HFD response (Group III)***		
	A1M, ZAG, Adiponectin	↓	↓
	Cp, ITIH4, RBP, TTR tetramer, Resistin	↑	↑
	GSH-Px(p)	↓	↑
	α1-III(p), Complement C3, SERPING1(p), Tf, VDBP	↑	–
	A1AT, TTR monomer, TUBB5	↓	–
	Afamin, SERPINA3K	–	↓
	Carboxylesterase	–	↑


Summary of differential regulation of plasma proteins between male and female rats fed either a low fat diet (LFD) or a high fat diet (HFD). Arrows indicate higher (↑) or lower (↓) levels of each protein between LFD rats (blue) and HFD rats (red), where regulation patterns were combined data from both proteomic and immunoblot analyses. Black arrows refer to HFD-responsive regulation of each protein, and (-) stands for no HFD response. For abbreviation of each protein name, see abbreviations. (p), precursor.

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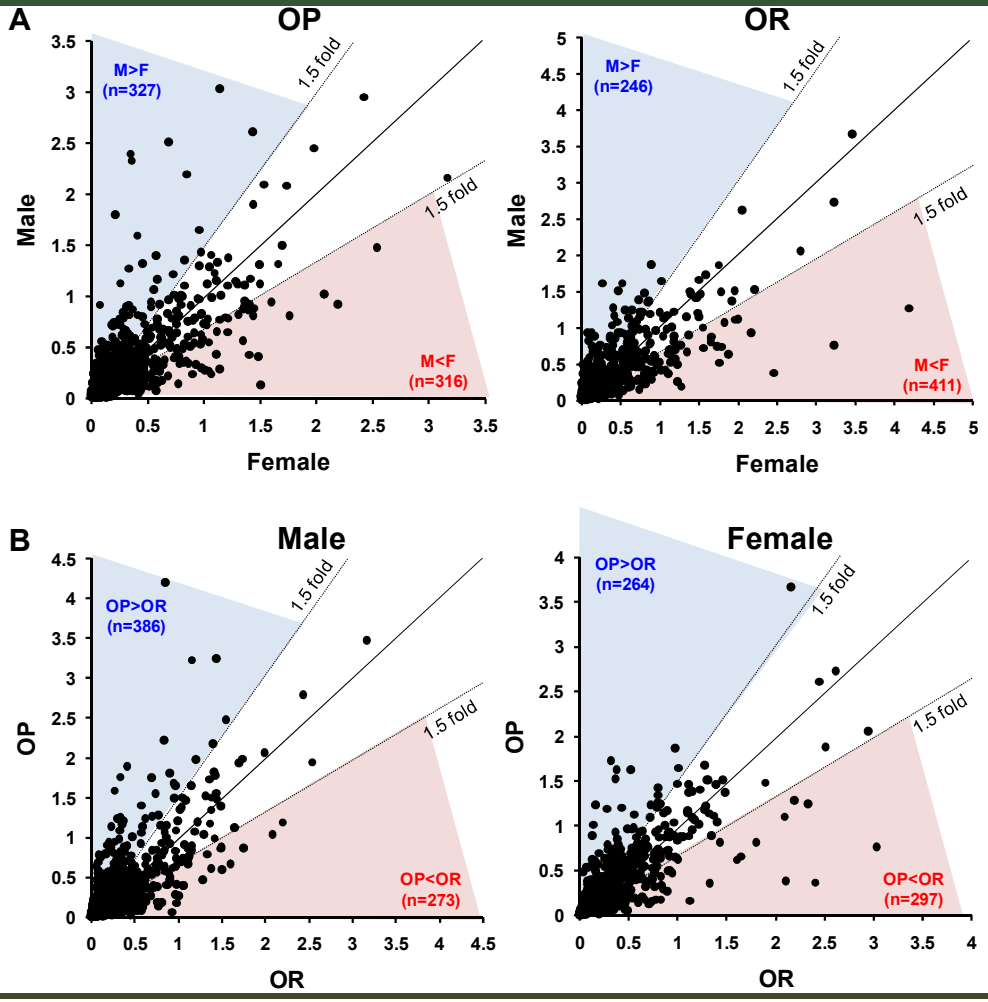
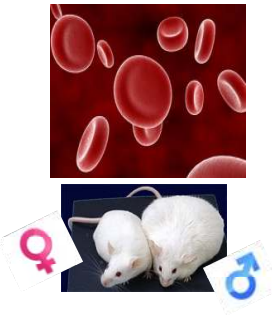
Profiling of gender-specific rat plasma proteins associated with diet-induced obesity susceptibility and resistance





Scatter plots comparing global protein regulation profiles between males vs. females (A) or OP vs. OR rats (B). Regulation of plasma proteins of males or OP rats was plotted against those of the females or OR rats and these plots were drawn using all spots appeared in three gels of each group. Spots with volume (%) of five and over were excluded from scatter plots. Upper and the lower diagonal lines show the 1.5-fold regression lines. X- and Y-axis represent volume (%) of each spot in the normal, OP, and OR groups of males and females..

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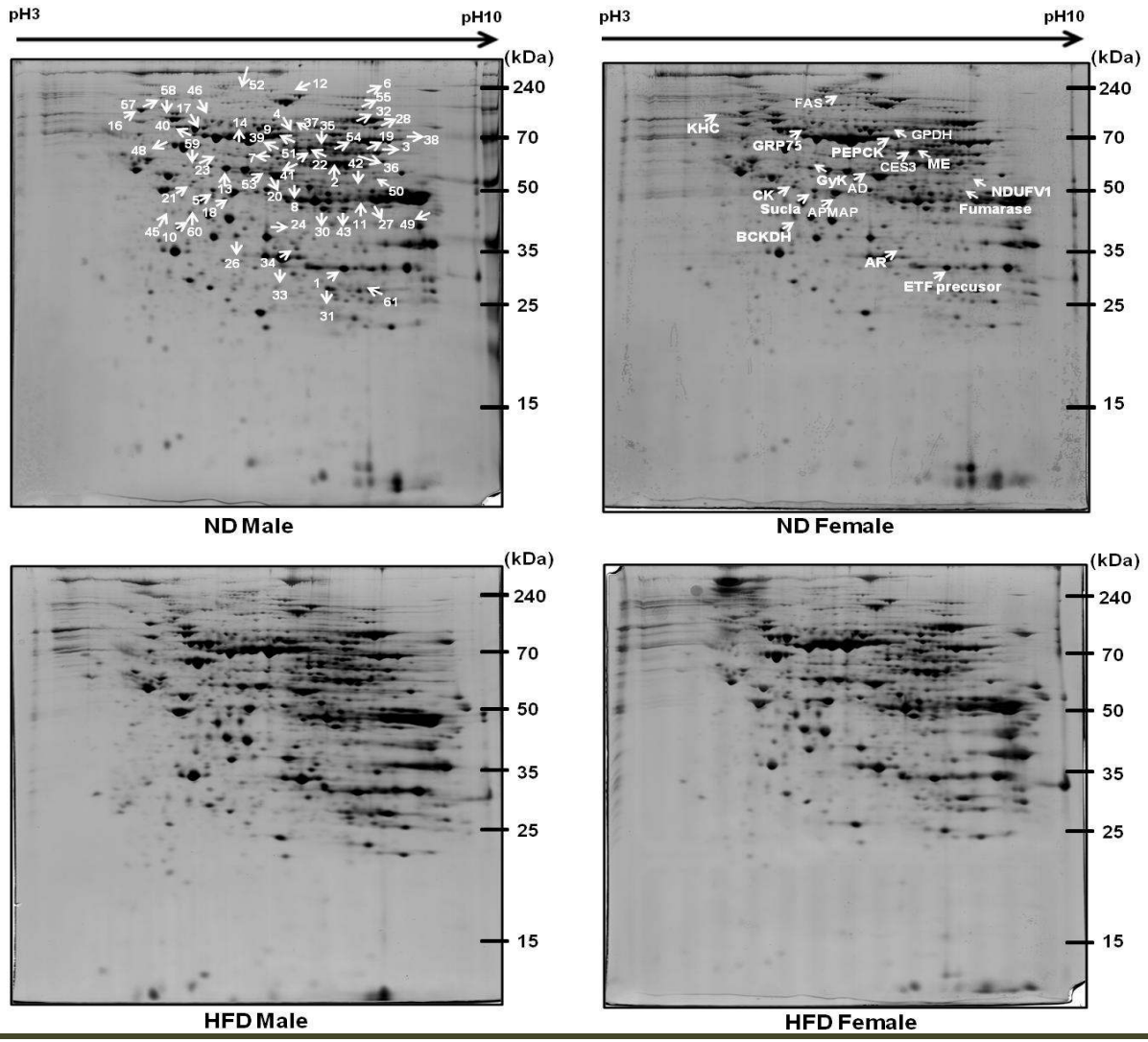
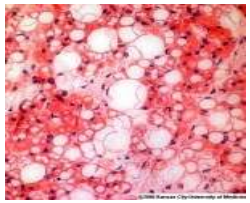
2 BAT proteome of male and female rats





Representative silver-stained 2-DE gel images of rat BAT proteome in ND and HFD rats. Differentially regulated proteins in each group are marked with arrows

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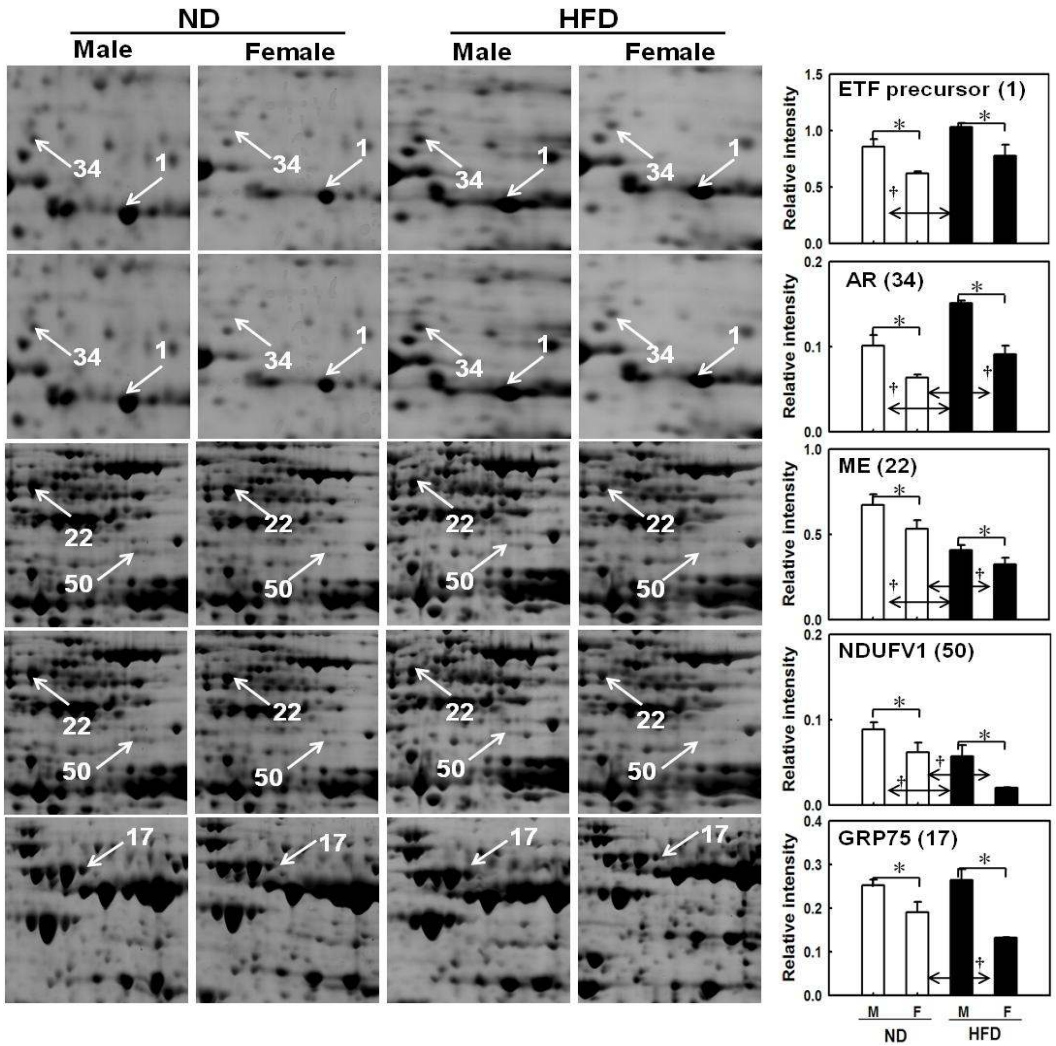
Proteins showing gender-difference in BAT

Group I : Proteins showing gender differences with higher levels in males



Differentially expressed BAT proteins showing gender difference in both ND and HFD rats with identical patterns. These proteins showed higher expression in males. Data are exhibited as mean values ± SEM of volume density (%) of the changed spot in 3 individual gels using pooled BAT from 7 rats per group. These 11 proteins have *p* values < 0.05 when comparing males and females fed ND and/or HFD. Statistical significance between male (M) and female (F) rats was determined by a *t*-test, where *p* values were $^{*}p<0.05$ and $^{**}p<0.01$ and significance between ND and HFD rats was represented by $^{\dagger}p<0.05$ and $^{\dagger\dagger}p<0.01$. Arabic numerals in parenthesis of bar graphs indicate spot numbers in zoon-in gel images.

ETF, electron transfer flavoprotein subunit alpha; **AR**, aldose reductase; **ME**, NADP-dependent malic enzyme; **NDUFV1**, NADH dehydrogenase (ubiquinone) flavoprotein 1

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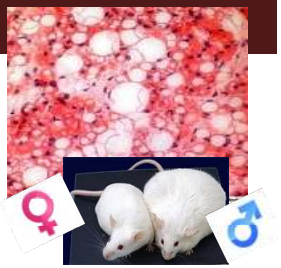
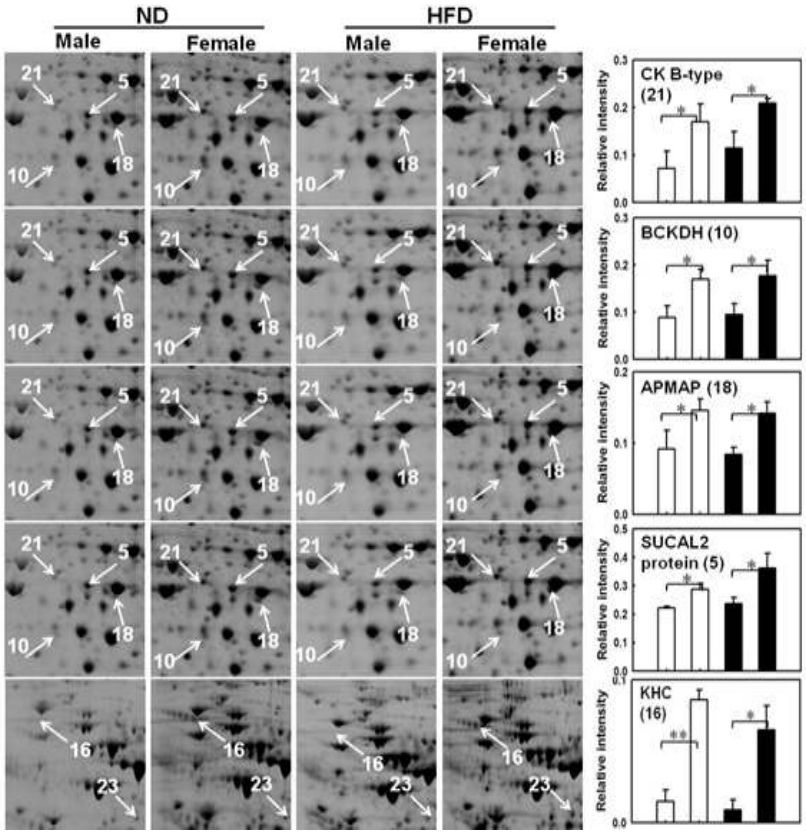
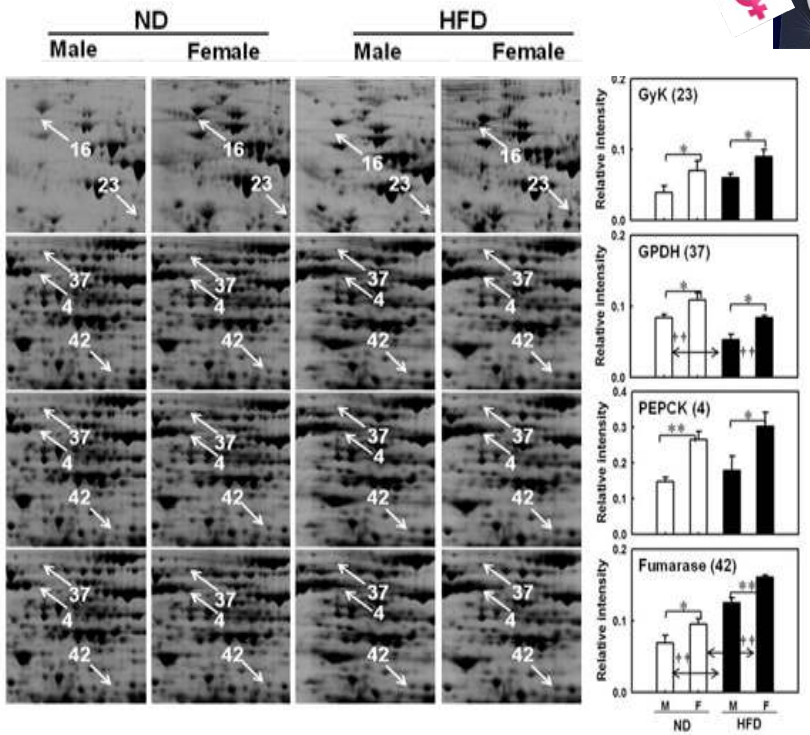
Proteins showing gender-difference in BAT



Group II : Protein showed gender difference which are higher levels in females





Differentially expressed BAT proteins showing gender difference in both ND and HFD rats with identical patterns. Data are exhibited as mean values ± SEM of volume density (%) of the changed spot in 3 individual gels using pooled BAT from 7 rats per group. These 11 proteins have *p* values < 0.05 when comparing males and females fed ND and/or HFD. Statistical significance between male (M) and female (F) rats was determined by a *t*-test, where *p* values were $^{*}p<0.05$ and $^{**}p<0.01$ and significance between ND and HFD rats was represented by $^{\dagger}p<0.05$ and $^{\dagger\dagger}p<0.01$. Arabic numerals in parenthesis of bar graphs indicate spot numbers in zoon-in gel images. **CK B-Type**, creatine kinase B-type; **BCKDH**, branched chain keto acid dehydrogenase E1; **APMAP**, adipocyte plasma membrane-associated protein; **KHC**, kinesin heavy chain isoform 5C; **GyK**, glycerol kinase; **GPDH**, glycerol-3-phosphate dehydrogenase 2; **PEPCK**, phosphoenolpyruvate carboxykinase

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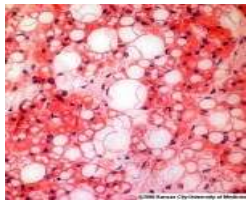
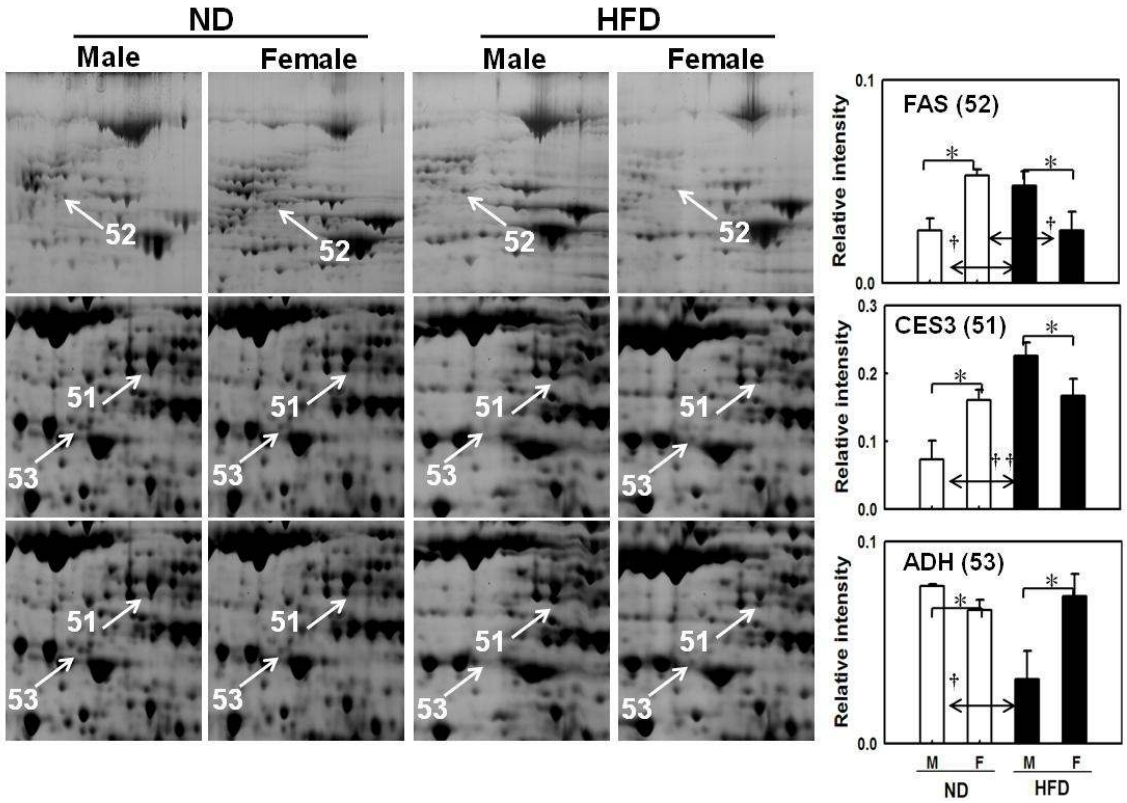
Proteins showing gender-difference in BAT

Group III : Protein showed gender difference both in ND or HFD rats with opposite patterns





Differentially expressed BAT proteins showing gender difference in both ND and HFD rats with opposite regulation patterns. Data are exhibited as mean values ± SEM of volume density (%) of the changed spot in 3 individual gels using pooled BAT from 7 rats per group. Statistical significance between male (M) and female (F) rats was determined by a *t*-test, where *p* values were $^{*}p<0.05$ and $^{**}p<0.01$ and significance between ND and HFD rats is represented by $^{\dagger}p<0.05$ and $^{\dagger\dagger}p<0.01$. Arabic numerals in parenthesis of bar graphs indicate spot numbers in zoon-in gel images. **FAS**, fatty acid synthase; **CES3**, carboxylesterase 3; **ADH**, aldehyde dehydrogenase

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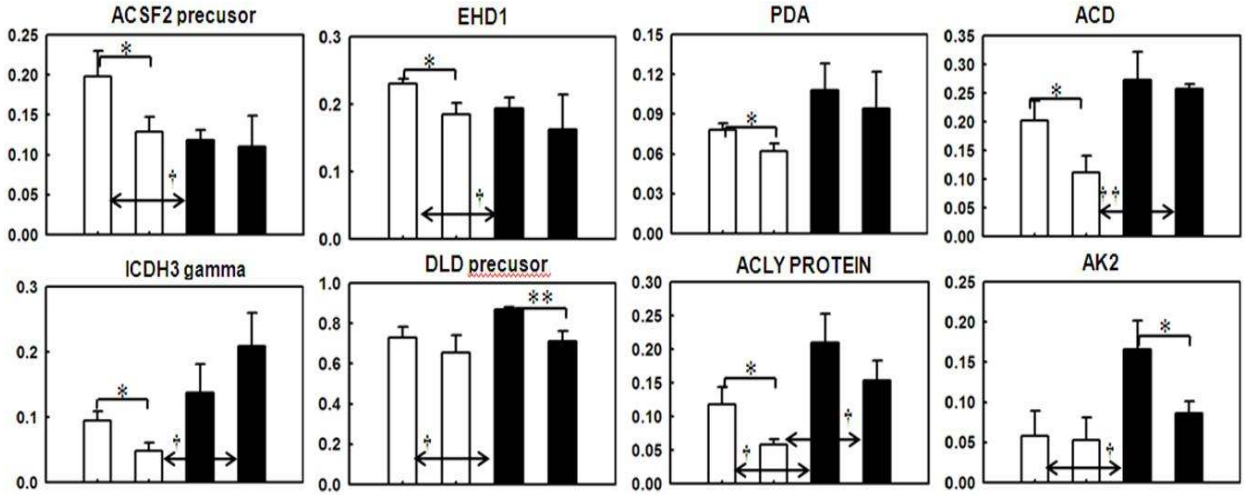
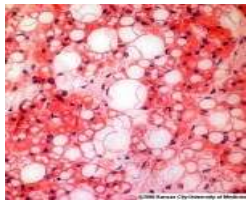
Proteins showing gender-difference in BAT

Group VI : Proteins showing a gender-difference in the regulation patterns in only ND or HFD rats

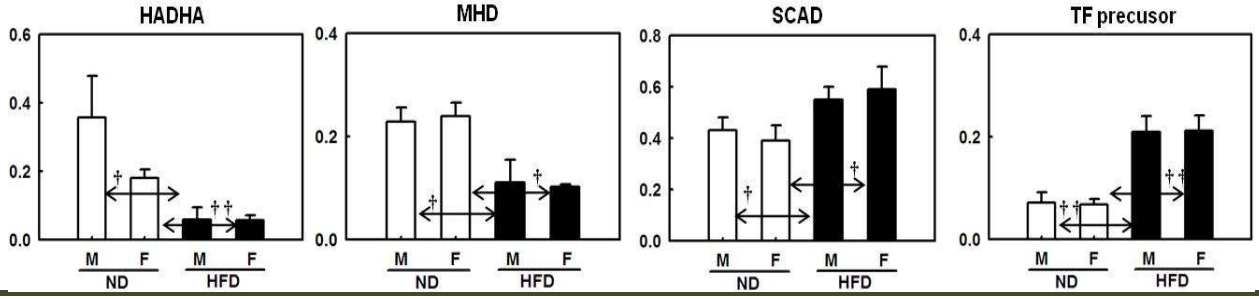
(A)





Group V : Proteins that were HFD-responsive, but not expressed in a gender-dependent

(B)



(A) Proteins showing a gender-difference in the regulation patterns in only ND or HFD rats, and proteins showing no gender-difference and (B) HFD-responsiveness proteins. Data are exhibited as mean values ± SEM of volume density (%) of the changed spot in 3 individual gels using pooled BAT from 7 rats per group. Statistical significance between ND and HFD rats is represented by $^{\dagger}p<0.05$ and $^{\dagger\dagger}p<0.01$. **ACSF2**, acyl-CoA synthetase family member 2; **EHD1**, EH domain-containing protein 1; **PDA**, pyruvate dehydrogenase E1 component subunit alpha; **ACD**, acyl-Coenzyme A dehydrogenase; **ICDH3,** isocitrate dehydrogenase 3 (NAD) gamma; **DLD**, dihydrolipoyl dehydrogenase ; **AK2**, adenylate kinase 2; **HADHA,** mitochondrial long-chain enoyl-CoA hydratase/3-hydroxycyl-CoA dehydrogenase alpha-subunit; **MHD**, malate dehydrogenase; **SCAD,** short-chain specific acyl-CoA dehydrogenase; **TF**, serotransferrin

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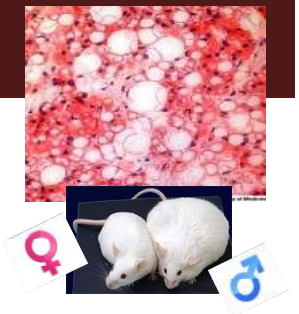
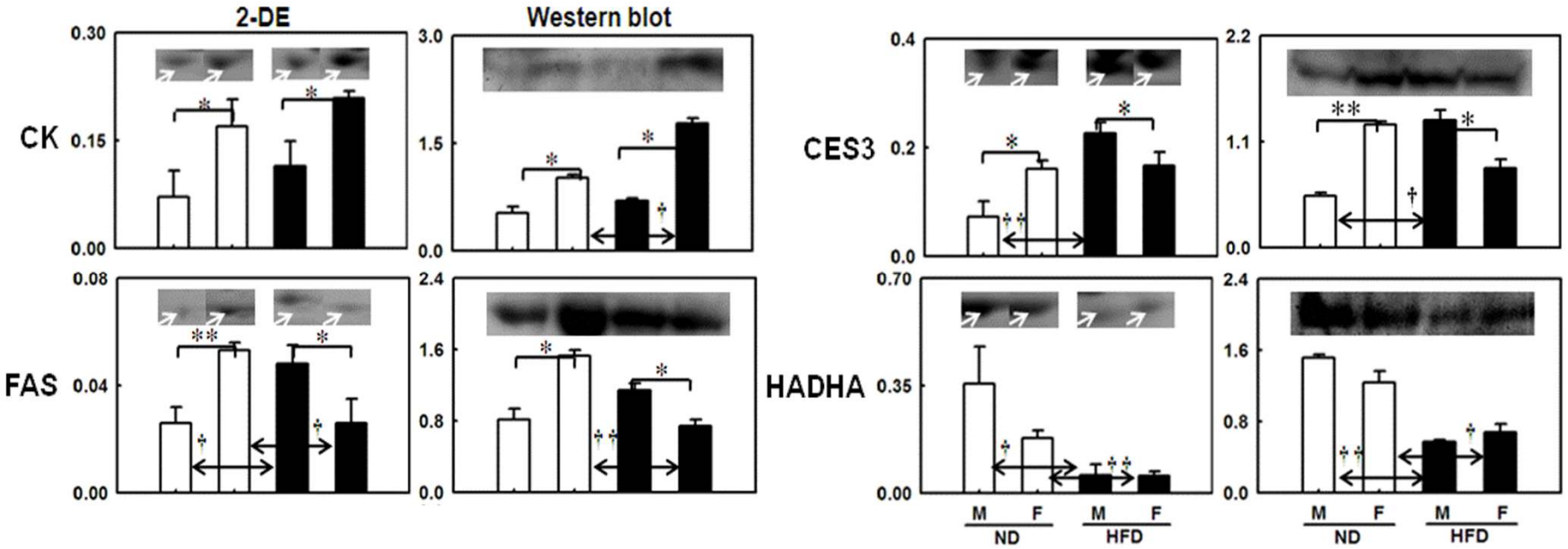
Validation of proteomic data in BAT by Western blot analysis





Validation of differentially regulated BAT proteins in male (M) and female (F) rats in response to HFD by immunoblot analysis. Levels of 4 proteins identified from 2-DE analysis were established using pooled BAT samples from 7 rats per group. Data are representative of 3 independent experiments.

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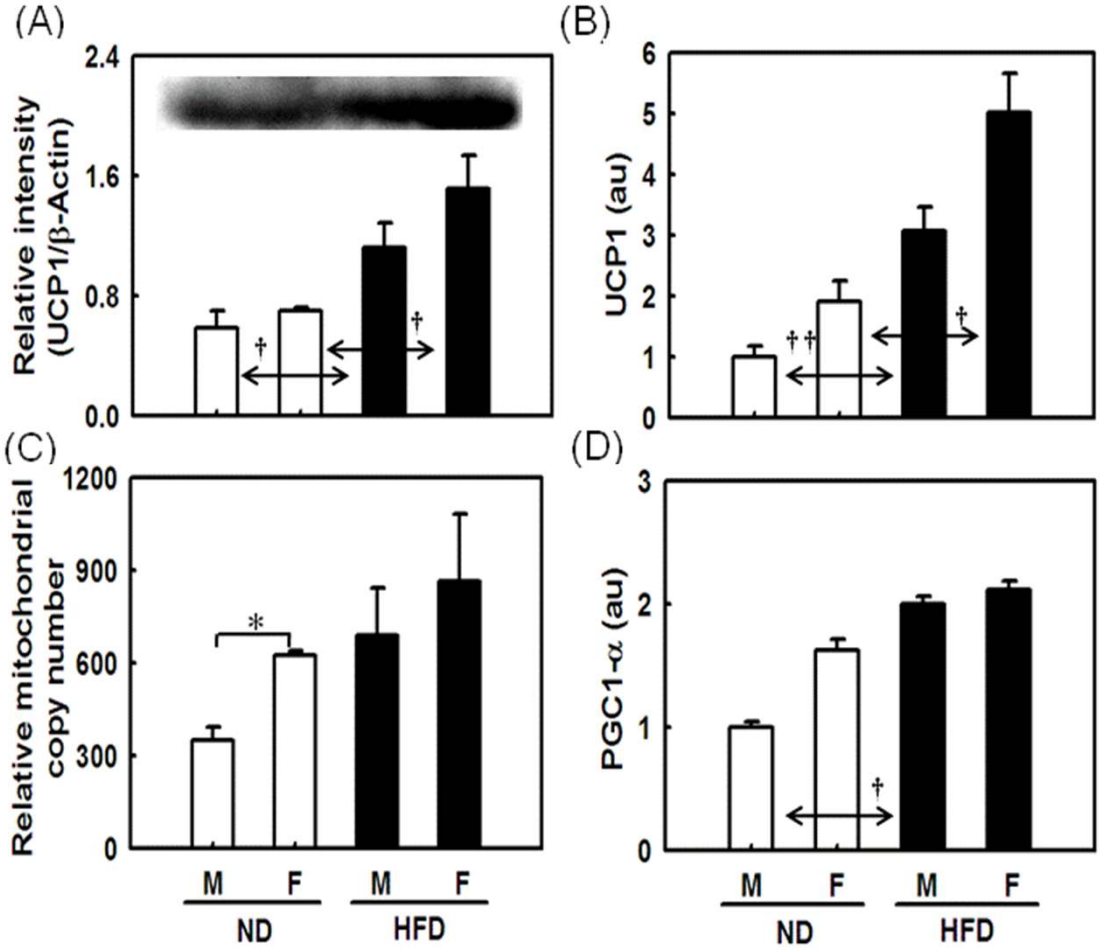
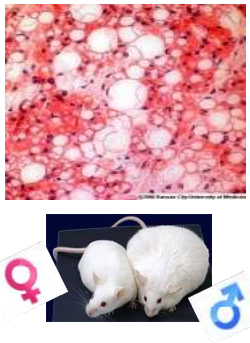
Are females more thermogenic than males ?





Comparison of protein (A) and mRNA levels of UCP1 and(B) PGCα1 (C) as well as mitochondrial content (D) in male (M) and female (F) rats in response to HFD. All were established using pooled BAT samples of seven rats per group. Data are representative of three independent experiments.

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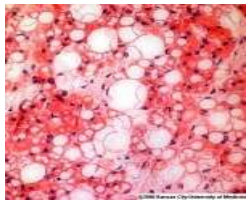
Summary from BAT proteome analysis



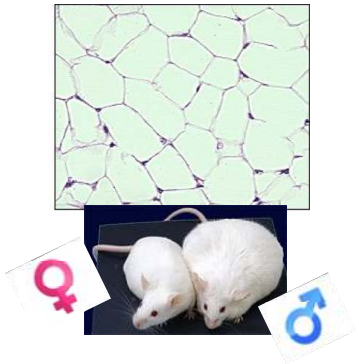
- Greater expression of numerous proteins directly or indirectly involved in thermogenic action in females

- Traits of lower fat synthesis and higher oxidation activity in females were suggested, although the activity of each enzyme activity was not been determined in this study.

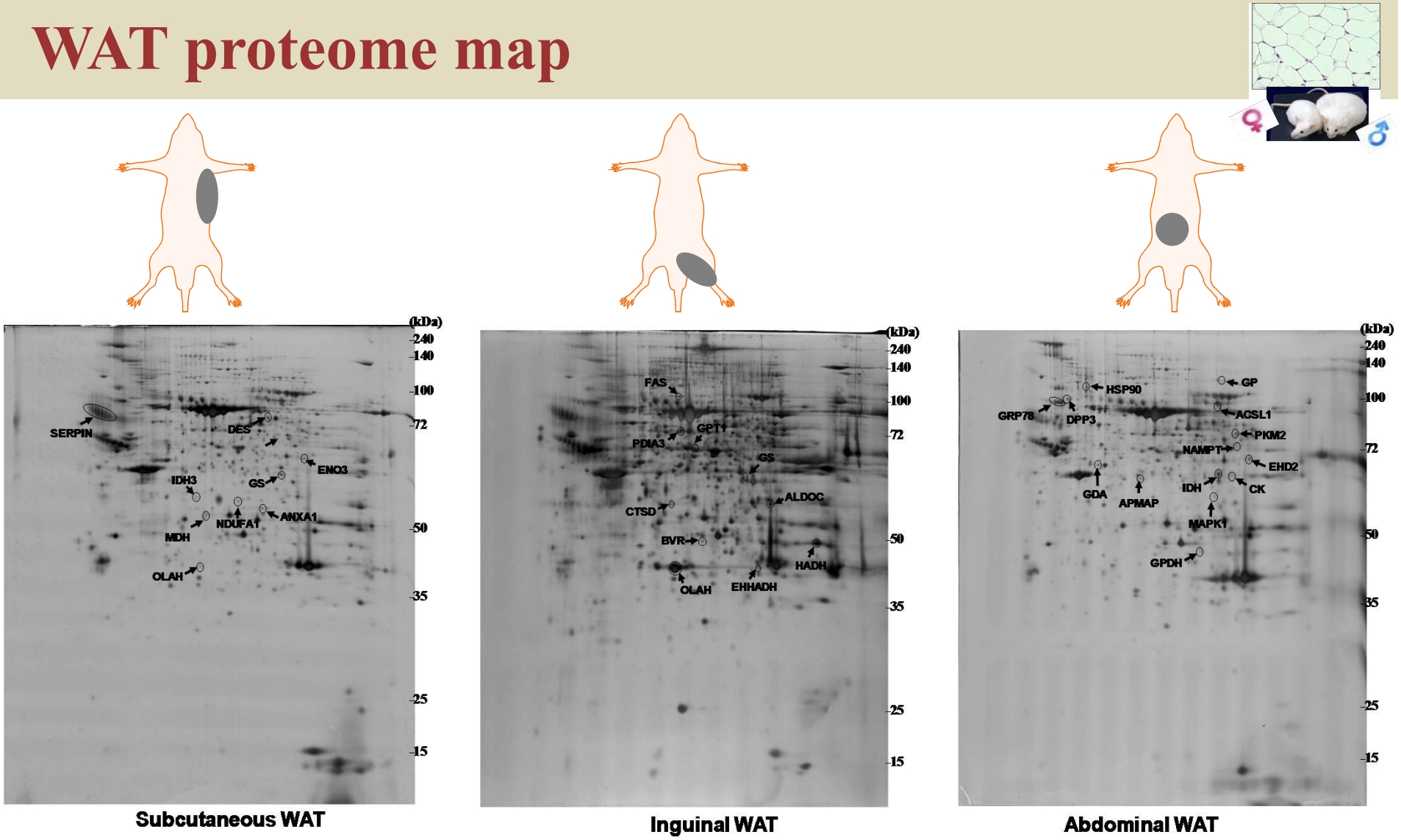
Choi et al. C*ell Physiol Biochem, 28, 933* (2011)

3 Gender-dependent proteome analysis in white adipose tissues (WAT)

Degree of lipogenesis and fat oxidation are closely related with adiposity



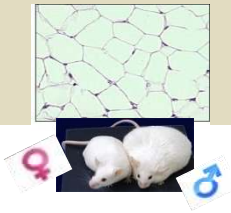
WAT proteome map



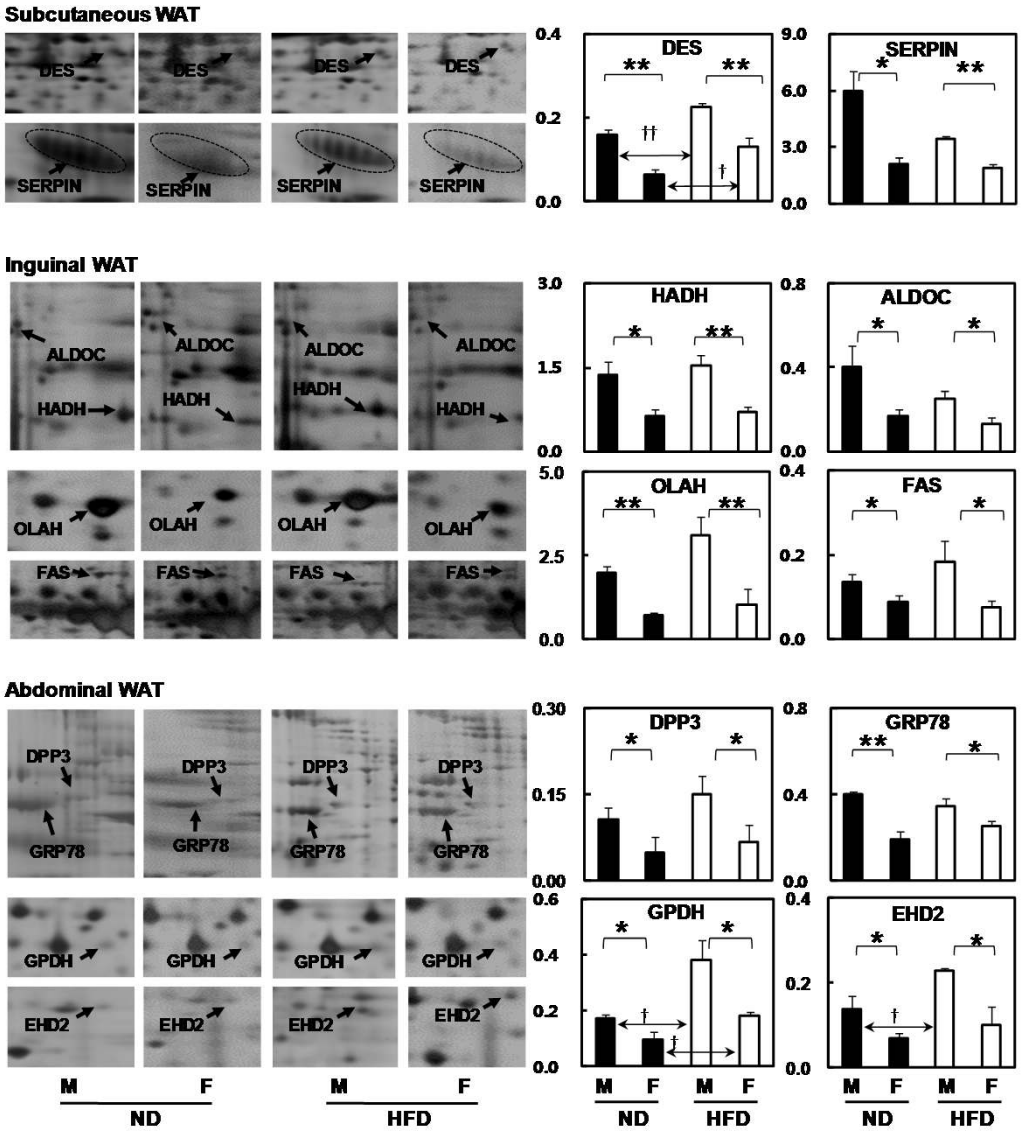
Representative 2-DE gel images of WAT protein homogenate. Proteins were extracted and separated on pH 3–10 IPG strips for the first dimension, followed by 12% (w/v) polyacrylamide gel electrophoresis (PAGE) for the second dimension. Differentially expressed proteins were marked with arrows.

Rajib et al. *Proteomics*, submitted (2012)

WAT proteome analysis



Group I Gender different in both ND and HFD rats with higher protein abundances in males



Differentially expressed proteins showing gender differences in both ND and HFD rats with higher protein abundances in males. Data are expressed as mean values ± SD of the volume density (%) of the changed spot in 3 individual gels using pooled WAT (3 types) from 7 rats per group. These proteins have *p* values <0.05 when comparing males and females fed ND and/or HFD. Statistical significance between male (M) and female (F) rats as well as ND and HFD was determined by ANOVA test, where *p* values were $^{*}p<0.05$ and $^{**}p<0.01$.

DES, Desmin; **SERPIN**, Serine/cysteine peptidase inhibitor; **HADH**, Hydroxyacyl CoA dehydrogenase; **ALDOC**, Fructose bisphosphate aldolase C; **OLAH**, S acyl fatty acid synthase thioesterase; **FAS**, Fatty acid synthase; **DPP3**, Dipeptidyl peptidase 3; **GRP78**, Glucose regulated protein 78; **GPDH**, Glycerol 3 phosphate dehydrogenase; **EHD2**, EH domain containing protein 2

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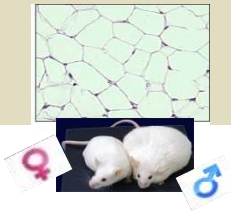
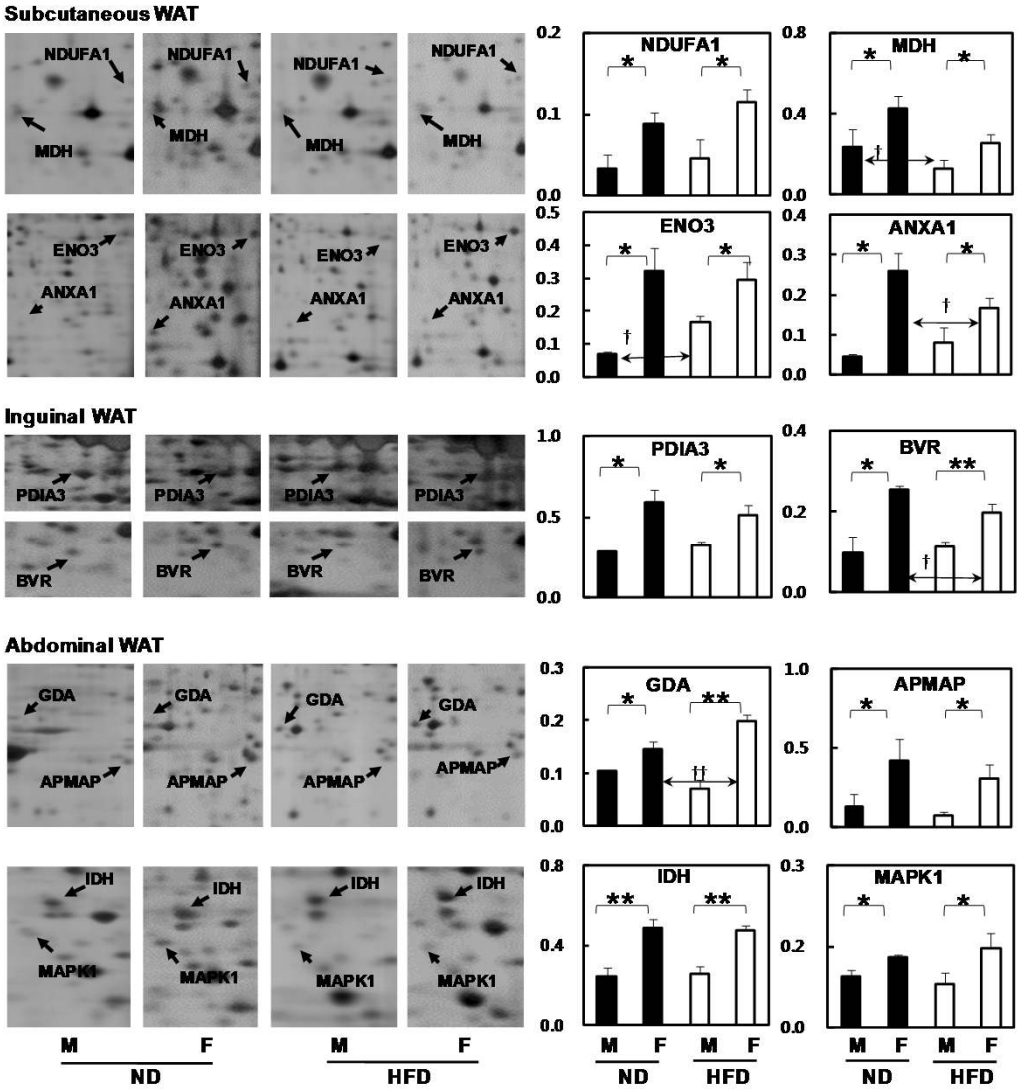
WAT proteome analysis



Group II Gender different in both ND and HFD rats with higher protein abundances in females



Differentially expressed proteins showing gender differences in both ND and HFD rats with higher protein abundances in females. Data are expressed as mean values ± SD of volume density (%) of the changed spot in 3 individual gels using pooled WAT (3 types) from 7 rats per group. These proteins have *p* values <0.05 when comparing males and females fed ND and/or HFD. Statistical significance between male (M) and female (F) rats as well as ND and HFD was determined by ANOVA test, where *p* values were $^{*}p<0.05$ and $^{**}p<0.01$.

NDUFA1, NADH dehydrogenase; **MDH**, Malate dehydrogenase; **ENO3**, Beta enolase ; **ANXA1**, Annexin A1; **PDIA3,** Protein disulfide isomerase associated 3; **BVR,** Biliverdin reductase A; **GDA**, Guanine deaminase; **APMAP**, Adipocyte plasma membrane associated protein; **IDH**, Isocitrate dehydrogenase; **MAPK1**, Mitogen activated protein kinase 1

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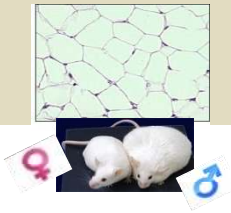
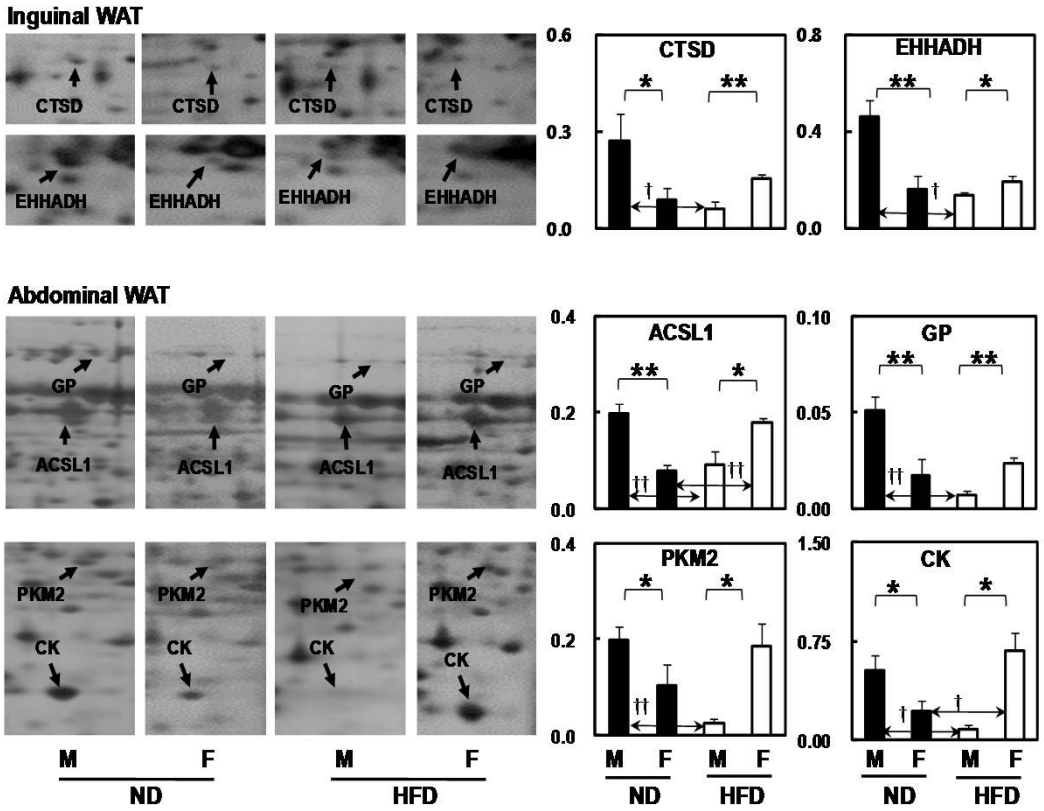
WAT proteome analysis



Group III Gender different in both ND and HFD rats with opposite protein abundances between the genders



Differentially expressed proteins showing gender differences in both ND and HFD rats with opposite protein abundances between the genders. Data are expressed as mean values ± SD of volume density (%) of the changed spot in 3 individual gels using pooled WAT (3 types) from 7 rats per group. These proteins have *p* values <0.05 when comparing males and females fed ND and/or HFD. Statistical significance between male (M) and female (F) rats as well as ND and HFD was determined by ANOVA test, where *p* values were $^{*}p<0.05$ and $^{**}p<0.01$.

CTSD, Cathepsin D; **EHHADH**, Rat enoyl CoA hydratase; **ACSL1**, Long chain fatty acid CoA ligase 1; **GP**, Glycogen phosphorylase; **PKM2**, Pyruvate kinase isozyme M1/M2; **CK**, Creatine kinase M type

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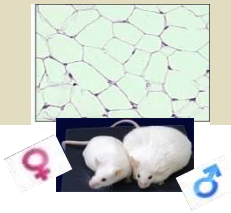
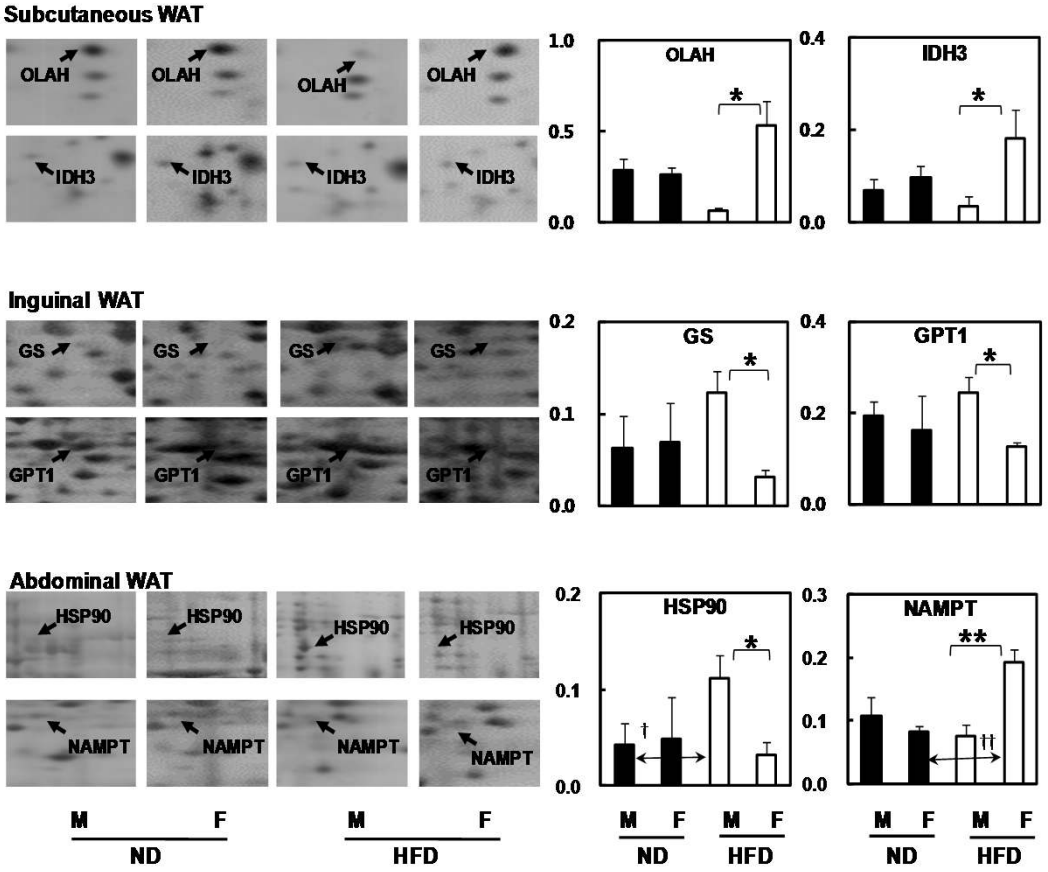
WAT proteome analysis



Group IV Gender-different only in HFD rats

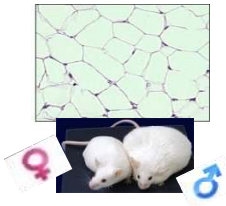


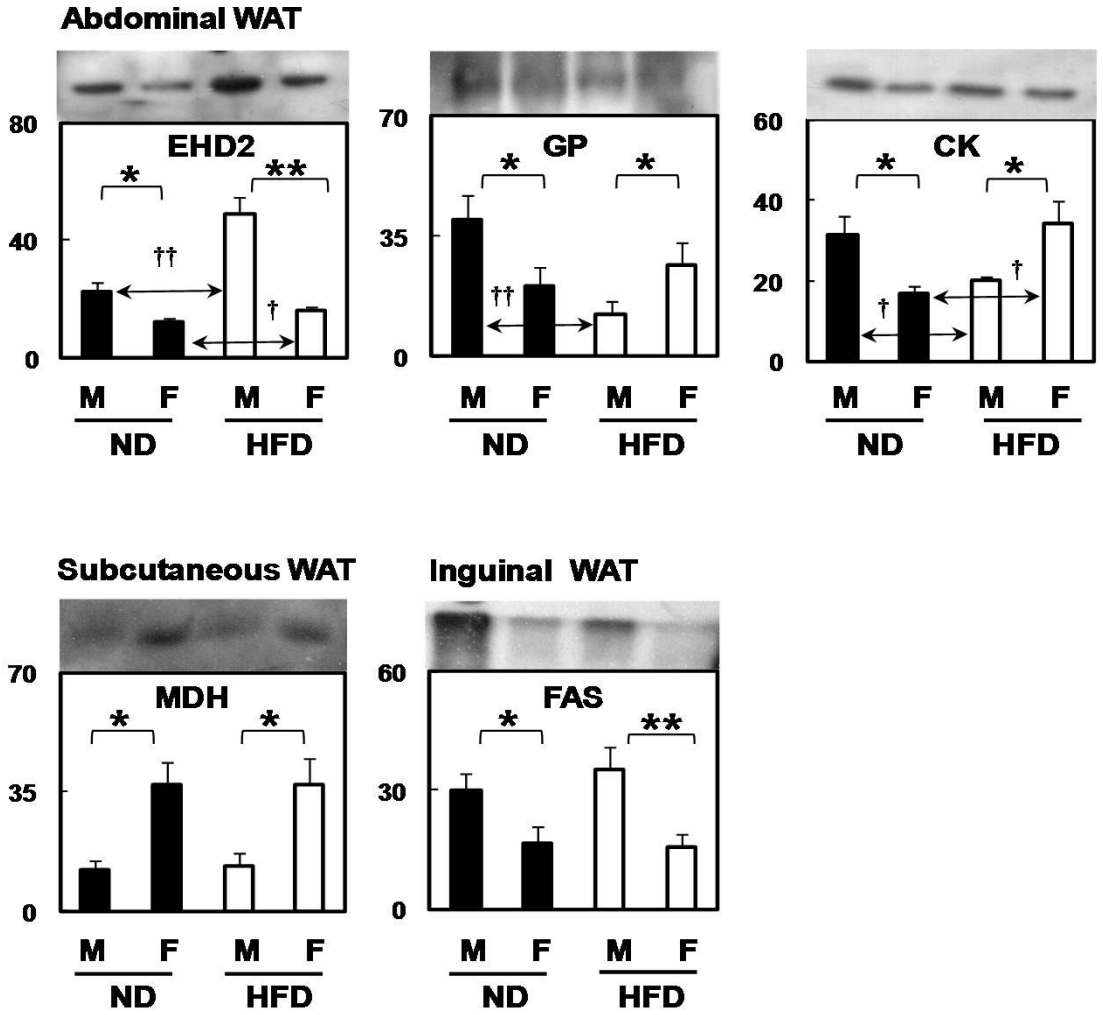
Differentially expressed proteins showing gender differences only in HFD rats. Data are expressed as mean values ± SD of volume density (%) of the changed spot in 3 individual gels using pooled WAT (3 types) from 7 rats per group. These proteins have *p* values <0.05 when comparing males and females fed ND and/or HFD. Statistical significance between male (M) and female (F) rats as well as ND and HFD was determined by ANOVA test, where *p* values were $^{*}p<0.05$ and $^{**}p<0.01$.

OLAH, S acyl fatty acid synthase thioesterase; **IDH3**, Isocitrate dehydrogenase 3 (NAD^+); **GS**, Glutamine synthetase; **GPTI**, Glutamic pyruvic transaminase 1; **HSP90**, Heat shock protein 90; **NAMPT**, Nicotinamide phosphoribosyltransferase

Rajib et al. *Proteomics*, submitted (2012)

Validation of WAT proteomic results by Western blot analysis





Validation of proteomic data using immunoblot analysis. Levels of five proteins identified from 2-DE analysis were established using the pooled WAT (3 types) samples from 7 rats per group. Data are representative of three independent experiments. Statistical significance between male (M) and female (F) rats as well as ND and HFD was determined by ANOVA test, where *p* values were $^{*}p<0.05$ and $^{**}p<0.01$.

Rajib et al. *Proteomics*, submitted (2012)

Summary from WAT proteome analysis

- Some important lipogenic enzymes like FAS, OLAH, GS, GPDH were present in higher concentrations in males, irrespective of dietary groups and WAT tissue types.

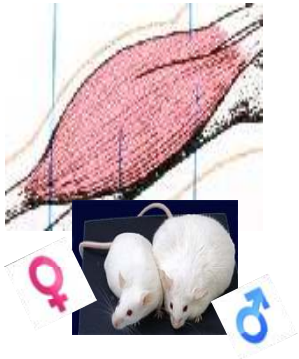
- Females had a higher concentration of numerous mitochondrial enzymes including NDUF1, MDH, IDH3 and PC, which may lead to a higher energy homeostasis or energy dissipation capability in females when compared to males.

- The male have higher weight gain because of higher expression of lipogenic proteins as well as the male exhibits slower rate of fat oxidation when compared to female. These two phenomenon work together for excess fat accumulation in male.

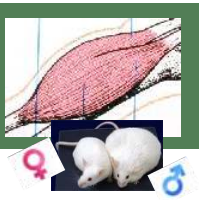
Rajib et al. *Proteomics*, submitted (2012)

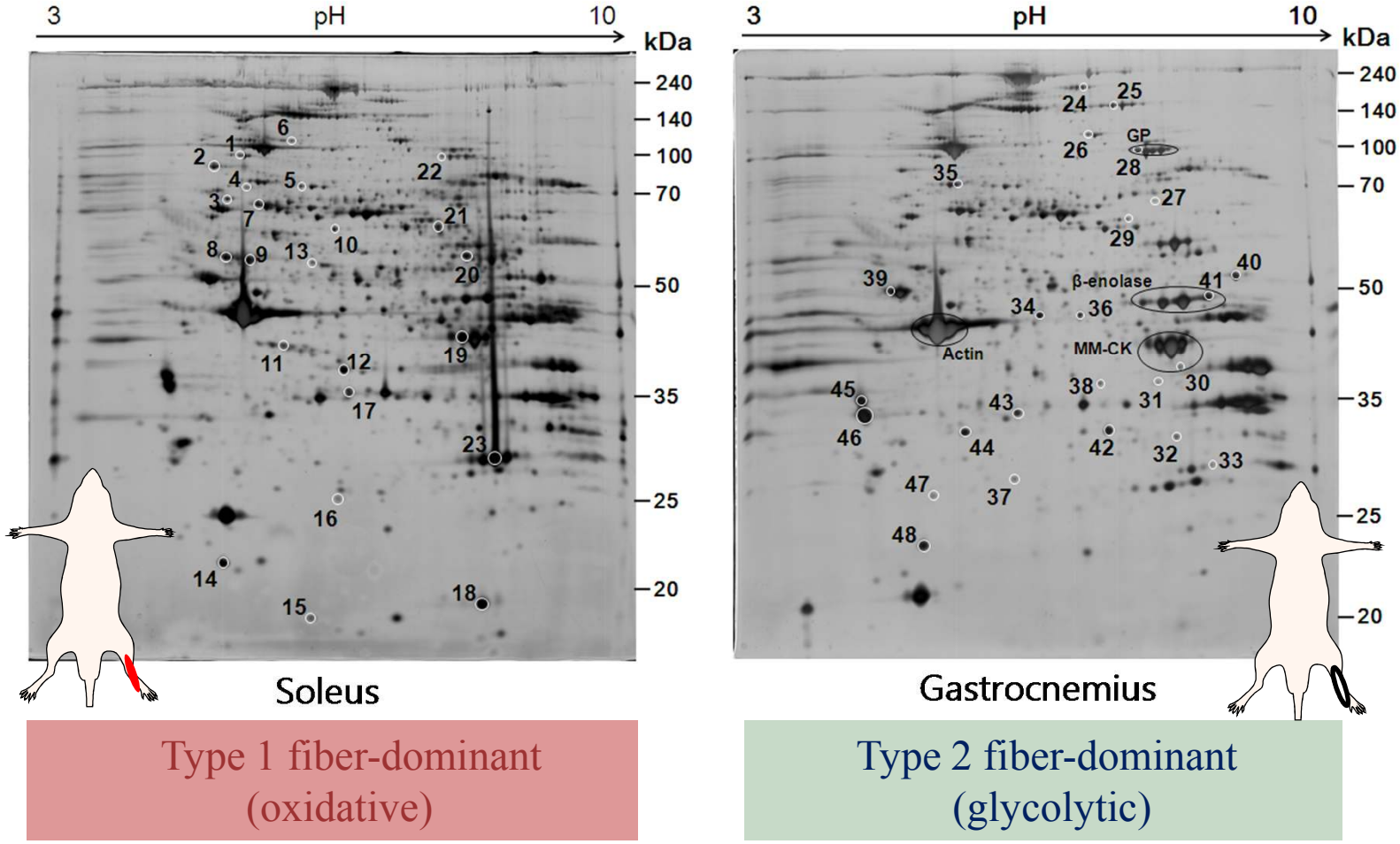
4 Gender-dependent proteome and genome analysis in skeletal muscle

Inefficient muscle lipid utilization may be associated with development of obesity



Skeletal muscle proteome map





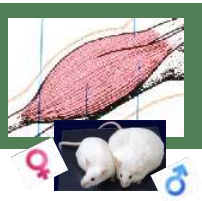
Type 1 fiber-dominant (oxidative)

Type 2 fiber-dominant (glycolytic)

Representative silver-stained 2-DE gel images of rat muscle proteome in soleus and gastrocnemius muscles. Differentially regulated proteins in each group are marked with circles.

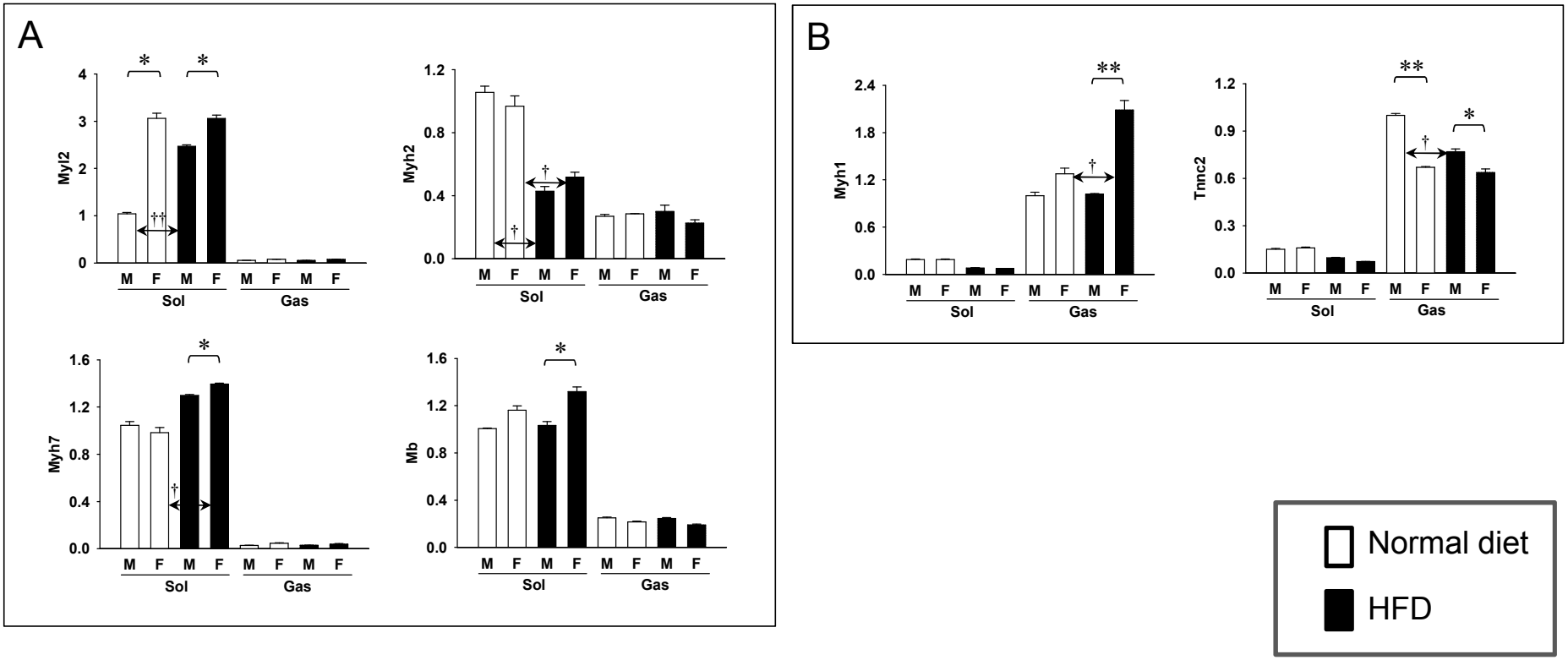
Oh et al. *Cell Physiol Biochem,* 28(5), 2011

Skeletal muscle proteome analysis



Type 1 fiber-specific genes

Type 2 fiber-specific genes



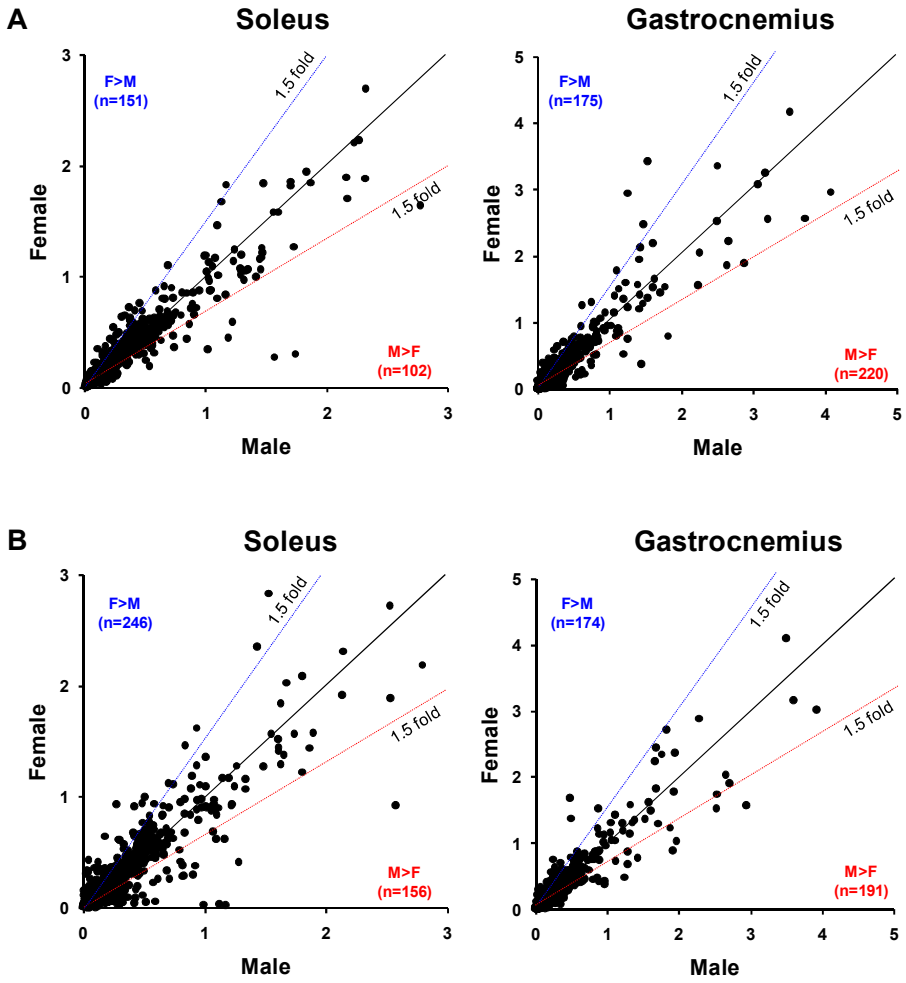
Comparison of muscle type-specific gene expression between the genders (males vs. females) and diets (ND vs. HFD). White bars are for normal diet and black bars are for HFD. Panel A shows the type 1 fiber-specific genes and panel B shows the type 2-specific genes. Data are presented as mean values ± SD of relative mRNA expression levels using pooled muscle cDNA from 7 rats per group. Statistical significance between male (M) and female (F) rats was determined by a *t*-test, where *p* values were $^{*}p<0.05$ and $^{**}p<0.01$ and significance between ND and HFD rats was represented by $^{\dagger}p<0.05$ and $^{\dagger\dagger}p<0.01$. **Myh**, myosin heavy polypeptide; **Myl**, myosin light polypeptide; **Mb**, myoglobin; **Tnnc**, troponin C type

Oh et al. *Cell Physiol Biochem,* 28(5), 2011

Skeletal muscle proteome analysis

ND

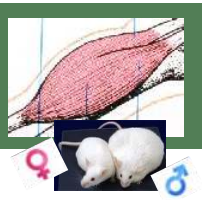
HFD



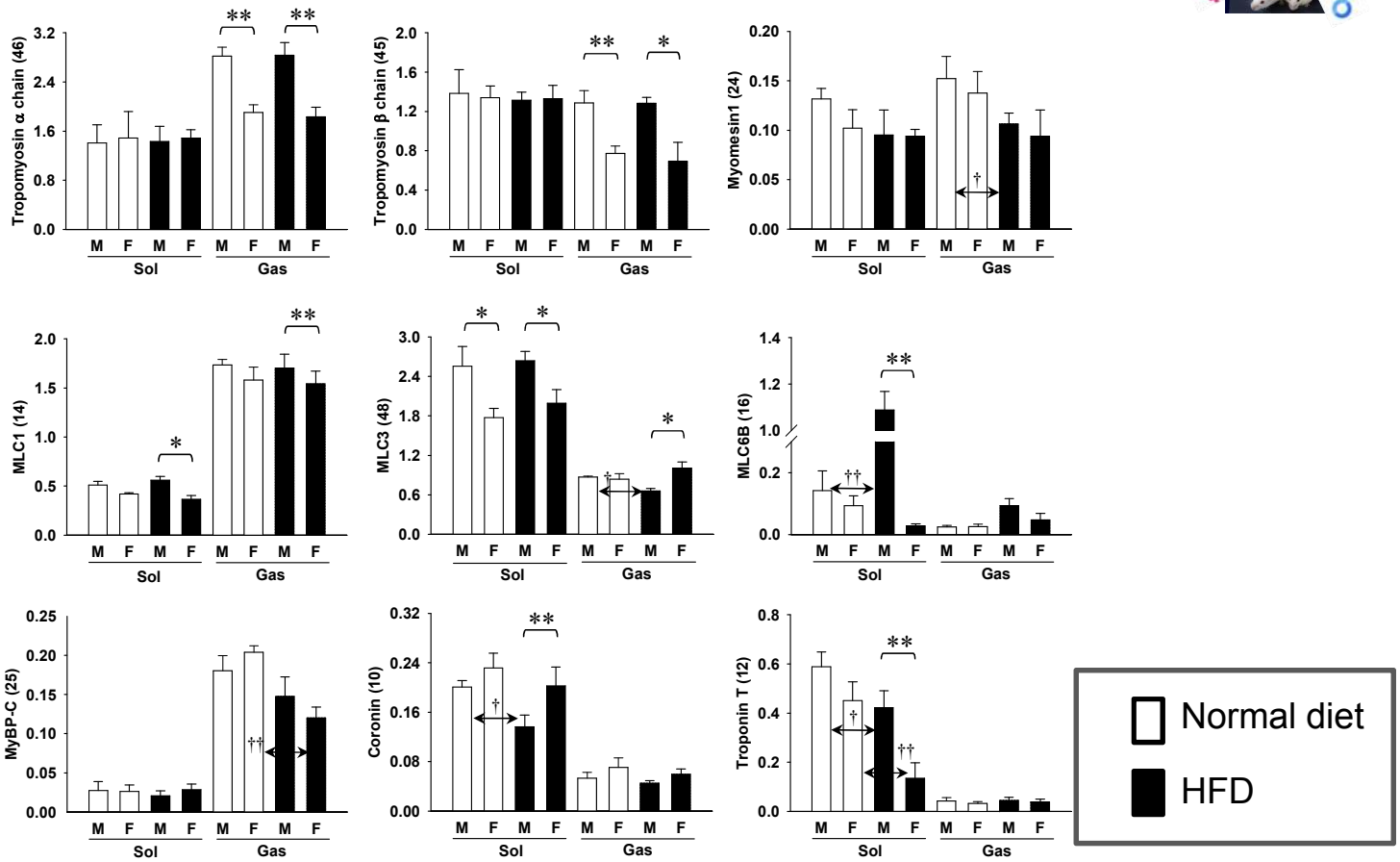
Scatter plots comparing global type-specific muscle protein expression profiles between males vs. females in ND (A) and HFD (B). White bars are for normal diet and black bars are for HFD. Expression of muscular proteins of male and female rats were plotted against soleus and gastrocnemius muscles, and these plots were created using all spots that appeared in the three gels of each group except for actin (3 spots). The upper and lower diagonal lines show the 1.5-fold regression lines. X- and Y-axis represent volume (%) of each protein spot.

Oh et al. *Cell Physiol Biochem,* 28(5), 2011

Skeletal muscle proteome analysis



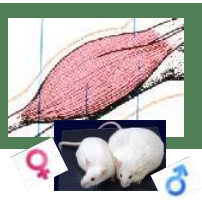
Contractile proteins



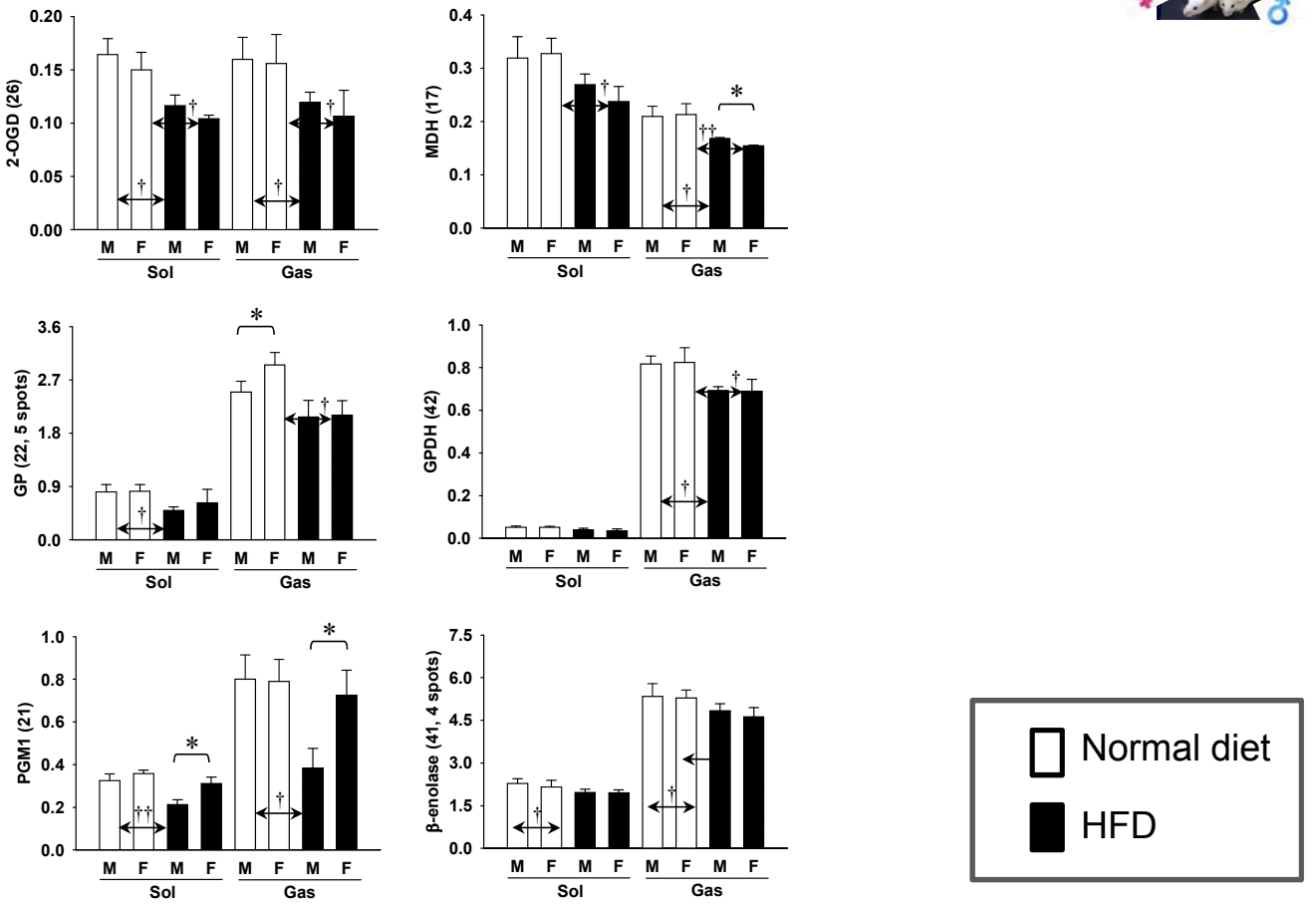
Graphical presentation of the statistical evaluation of contractile protein abundance of soleus (sol) and gastrocnemius muscles (gas) between the genders (males vs. females) and diets (ND vs. HFD). White bars are for normal diet and black bars are for HFD. Data are presented as mean values ± SD of volume density (%) of the changed spot in 3 individual gels using pooled muscle tissue from 7 rats per group. These nine proteins have *p* values< 0.05 when comparing males and females fed ND and/or HFD. Statistical significance between male (M) and female (F) rats was determined by a *t*-test, where *p* values were $^{*}p<0.05$ and $^{**}p<0.01$ and significance between ND and HFD rats was represented by $^{\dagger}p<0.05$ and $^{\dagger\dagger}p<0.01$. **MLC**, myosin light chain; **MyBP-C**, myosin-binding protein C

Oh et al. *Cell Physiol Biochem,* 28(5), 2011

Skeletal muscle proteome analysis



Metabolic proteins



Graphical presentation of the statistical evaluation of metabolic protein abundance of soleus (sol) and gastrocnemius muscles (gas) between the genders (males vs. females) and diets (ND vs. HFD). White bars are for normal diet and black bars are for HFD. Data are presented as mean values ± SD of volume density (%) of the changed spot in 3 individual gels using pooled muscle tissue from 7 rats per group. These 6 proteins have p values<0.05 when comparing males and females fed ND and/or HFD. Statistical significance between male (M) and female (F) rats was determined by a t-test, where p values were $^{*}p<0.05$ and $^{**}p<0.01$ and significance between ND and HFD rats was represented by $^{\dagger}p<0.05$ and $^{\dagger\dagger}p<0.01$. **2-OGD**, 2-oxoglutarate dehydrogenase; **MDH**, malate dehydrogenase; **GP**, glycogen phosphorylase; **GPDH**, glycerol-3-phosphate dehydrogenase; **PGM1**, phosphoglucomutase-1

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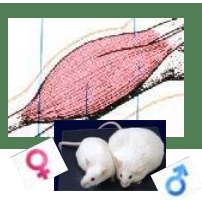
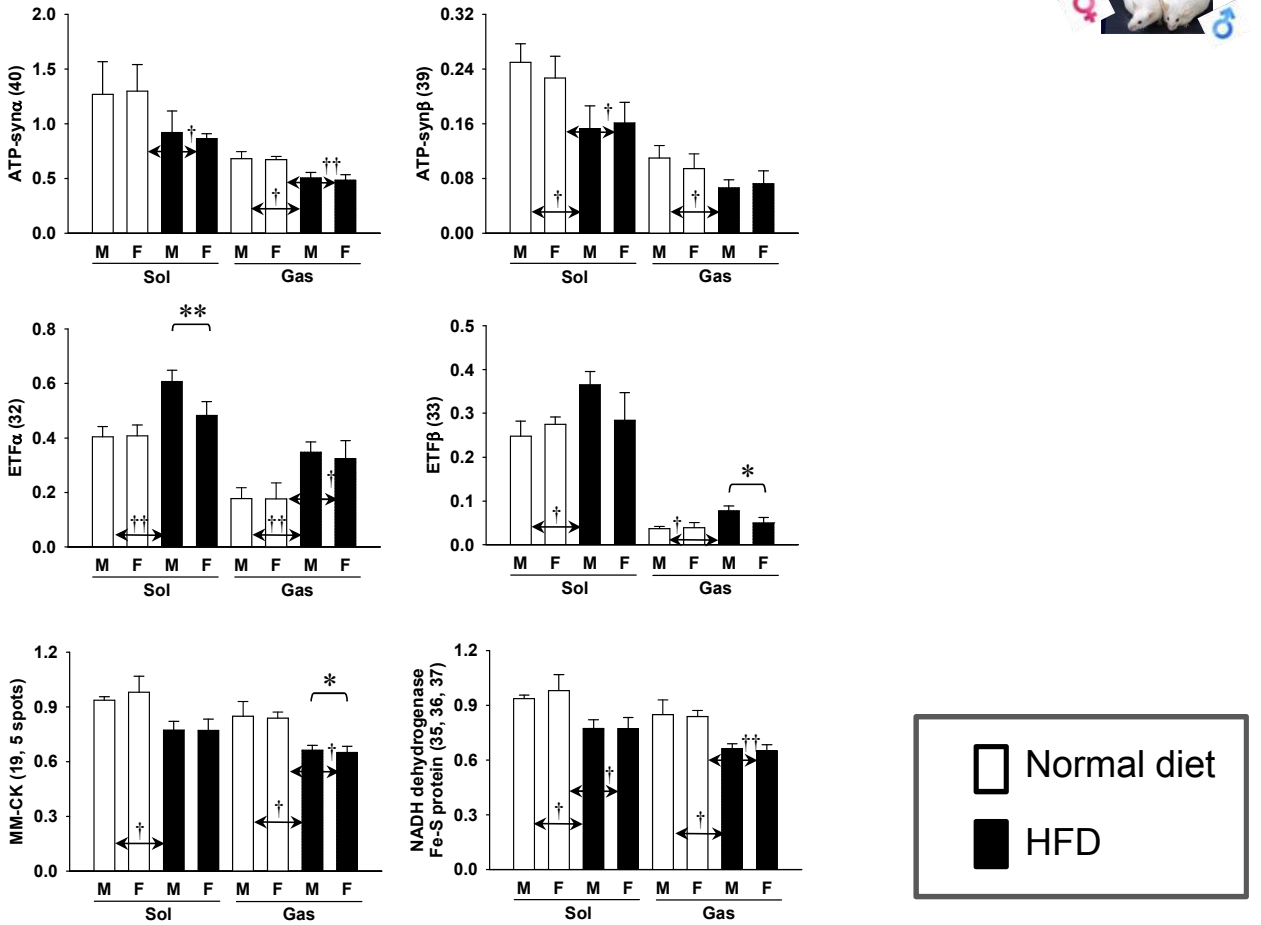
Skeletal muscle proteome analysis



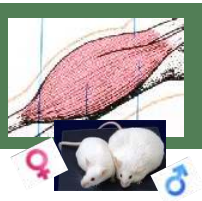
Oxidative and energy-producing proteins



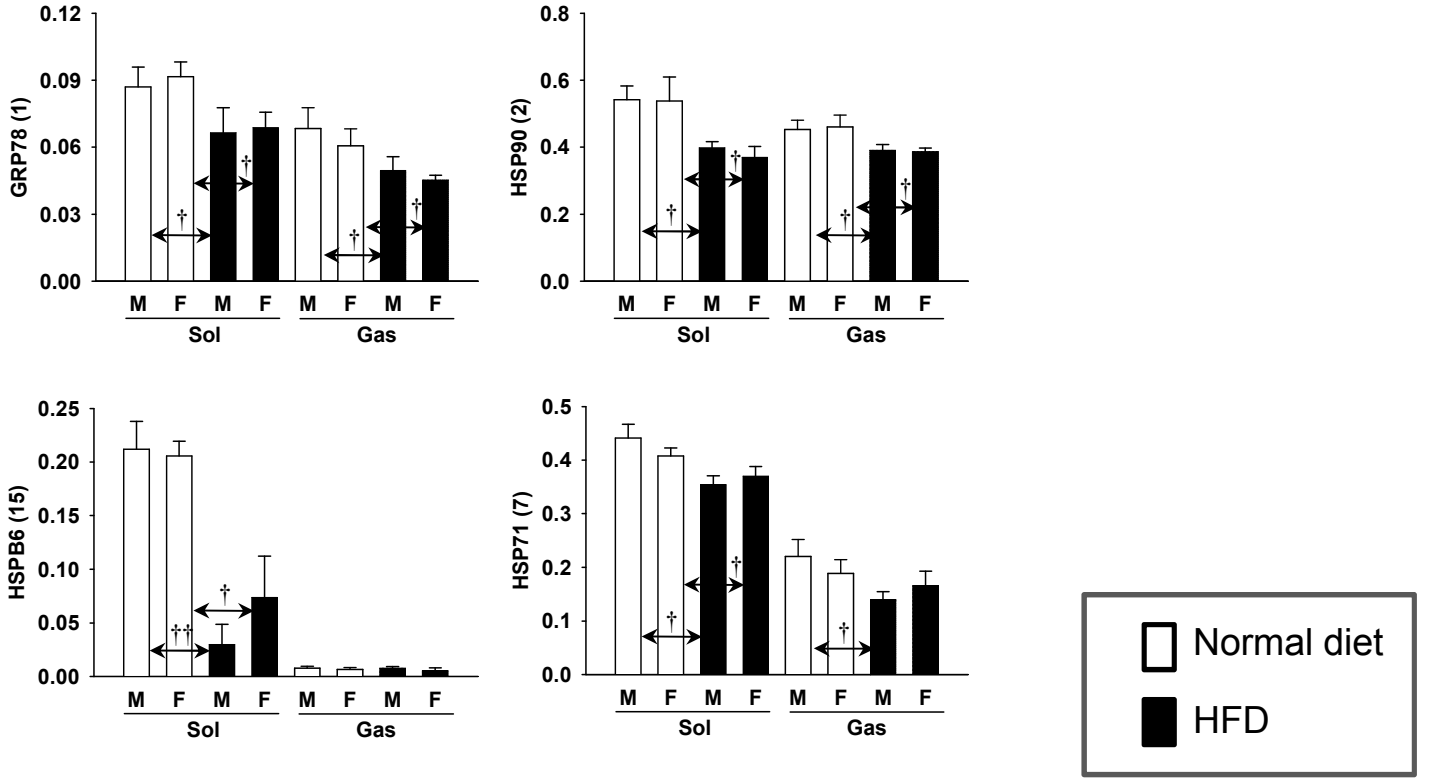
Graphical presentation of the statistical evaluation of oxidative and energy-producing protein abundance of soleus (sol) and gastrocnemius muscles (gas) between the genders (males vs. females) and diets (ND vs. HFD). White bars are for normal diet and black bars are for HFD. Data are presented as mean values ± SD of volume density (%) of the changed spot in 3 individual gels using pooled muscle tissue from 7 rats per group. These 6 proteins have p values<0.05 when comparing males and females fed ND and/or HFD. Statistical significance between male (M) and female (F) rats was determined by a t-test, where p values were $^{*}p<0.05$ and $^{**}p<0.01$ and significance between ND and HFD rats was represented by $^{\dagger}p<0.05$ and $^{\dagger\dagger}p<0.01$. **ATP-syn**, ATP synthase subunit; **ETF**, electron transfer flavoprotein; **MM-CK**, muscle type creatine kinase

Oh et al. *Cell Physiol Biochem,* 28(5), 2011

Skeletal muscle proteome analysis



Cellular stress proteins



Graphical presentation of the statistical evaluation of cellular stress protein abundance of soleus (sol) and gastrocnemius muscles (gas) between the genders (males vs. females) and diets (ND vs. HFD). White bars are for normal diet and black bars are for HFD. Data are presented as mean values ± SD of volume density (%) of the changed spot in 3 individual gels using pooled muscle tissue from 7 rats per group. These 4 proteins have p values<0.05 when comparing males and females fed ND and/or HFD. Statistical significance between male (M) and female (F) rats was determined by a t-test, where p values were $^{*}p<0.05$ and $^{**}p<0.01$ and significance between ND and HFD rats was represented by $^{\dagger}p<0.05$ and $^{\dagger\dagger}p<0.01$.

GRP, glucose-regulated protein; **HSP**, heat shock protein

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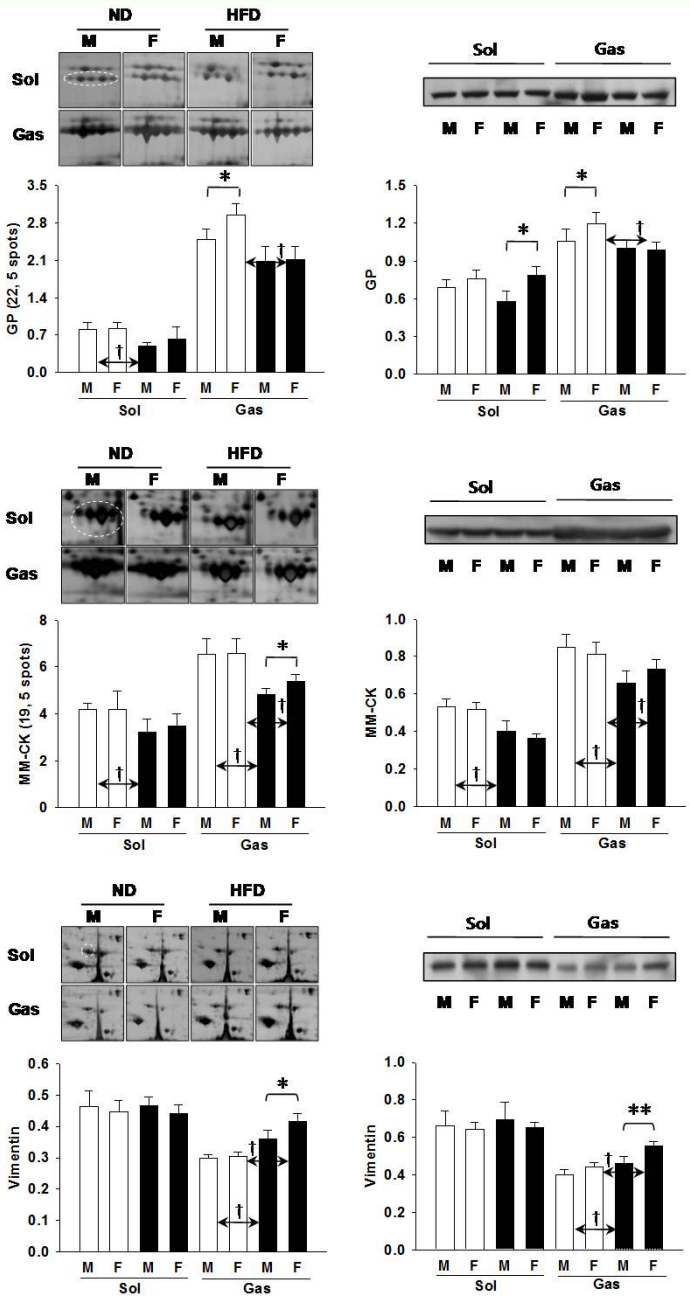
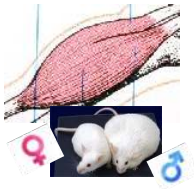
Validation of skeletal muscle proteomic results by Western blot analysis





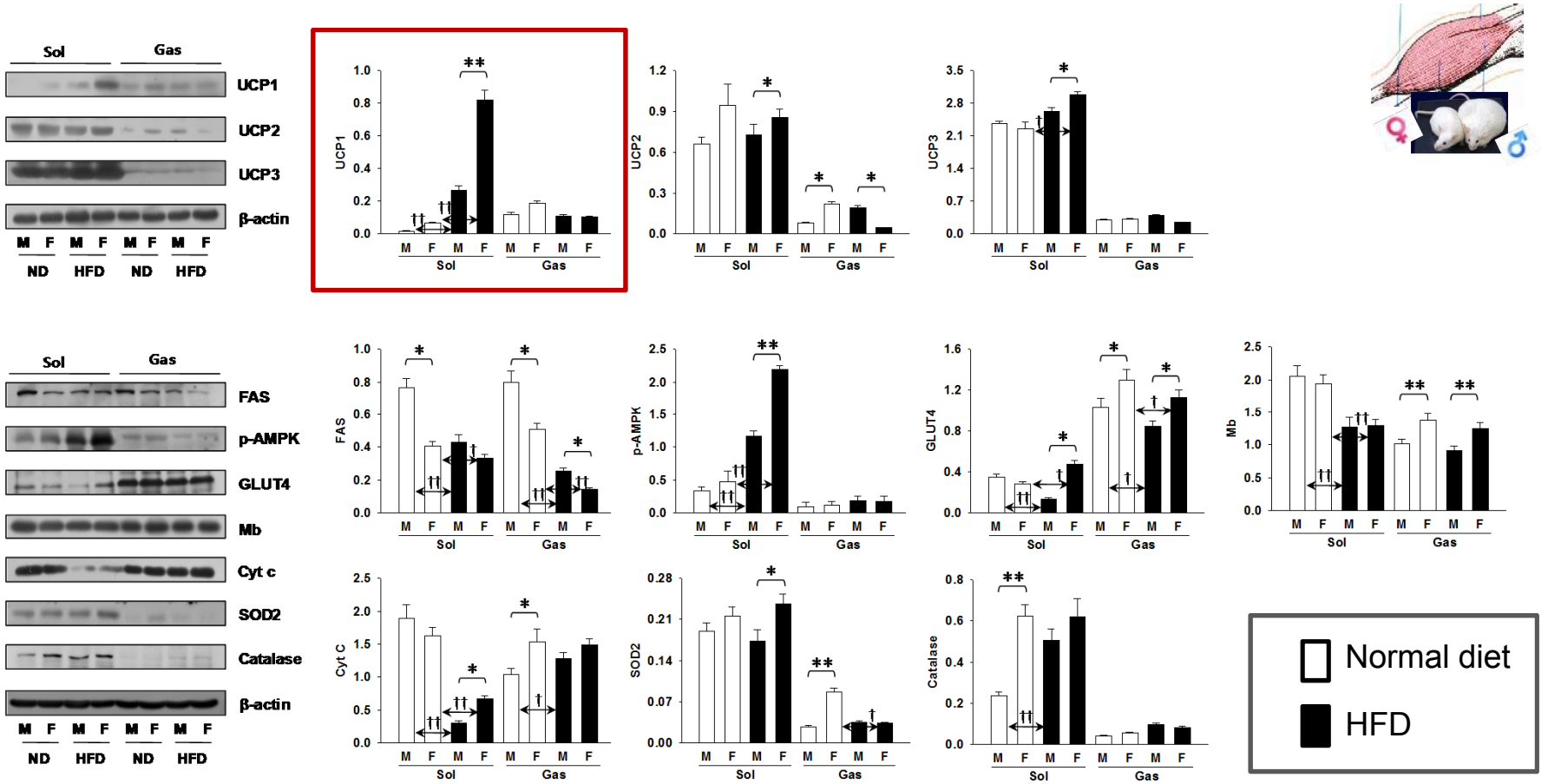
Validation of differentially regulated muscle proteins of soleus (sol) and gastrocnemius muscles (gas) in male (M) and female (F) rats in response to HFD by immunoblot analysis. White bars are for normal diet and black bars are for HFD. Levels of three proteins identified from 2-DE analysis were established using pooled muscle samples from 7 rats per group. Data are representative of three independent experiments. The Y-axes of the bar graphs for the Western blot analysis refer to protein density normalized by β-actin. Statistical significance between male (M) and female (F) rats was determined by a *t*-test, where *p* values were $^{*}p<0.05$ and $^{**}p<0.01$ and significance between ND and HFD rats was represented by $^{\dagger}p<0.05$ and $^{\dagger\dagger}p<0.01$.

Normal diet

HFD

Oh et al. *Cell Physiol Biochem,* 28(5), 2011

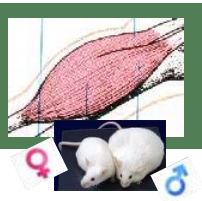
Gender-dependent expressions of metabolic skeletal muscle proteins : Western blot analysis



Differentially regulated muscle proteins of metabolic importance in male (M) and female (F) rats in response to HFD by immunoblot analysis. Sol: soleus muscle, Gas: gastrocnemius muscle. White bars are for normal diet and black bars are for HFD. Levels of 10 important muscle proteins were established using pooled muscle samples from 7 rats per group. Data are representative of three independent experiments. The Y-axes of the bar graphs refer to protein density normalized by β-actin. Statistical significance between male (M) and female (F) rats was determined by a *t*-test, where *p* values were $^{*}p<0.05$ and $^{**}p<0.01$ and significance between ND and HFD rats was represented by $^{\dagger}p<0.05$ and $^{\dagger\dagger}p<0.01$.

Oh et al. *Cell Physiol Biochem,* 28(5), 2011

Summary from muscle proteome analysis



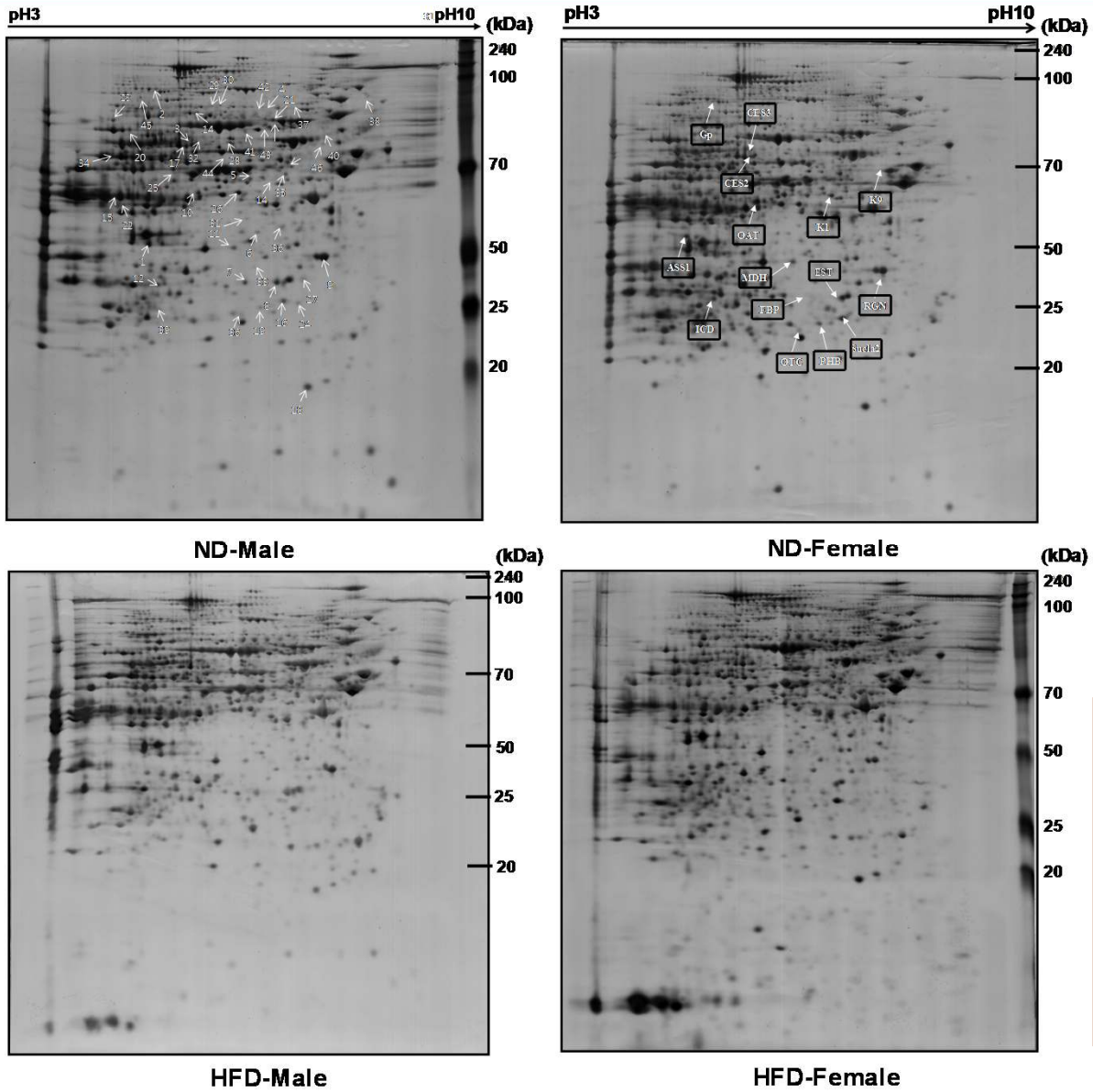
- Down regulation of myofibrillar regulatory proteins may favor decreased contractile capacities when exposed to HFD in both genders.

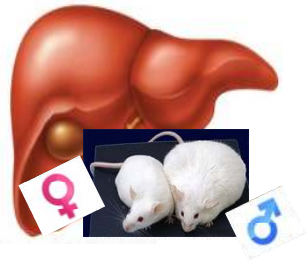
- Female rats oxidized significantly more lipid and less carbohydrate and protein compared with males during exercise.

- HFD intake increases muscle fatty acid uptake and oxidation, leading to excessive production of reactive oxygen species (ROS) as well as impaired antioxidant defenses. This tendency was likely more severe in males than females.

Oh et al. *Cell Physiol Biochem,* 28(5), 2011

5 Liver proteome of male and female rats



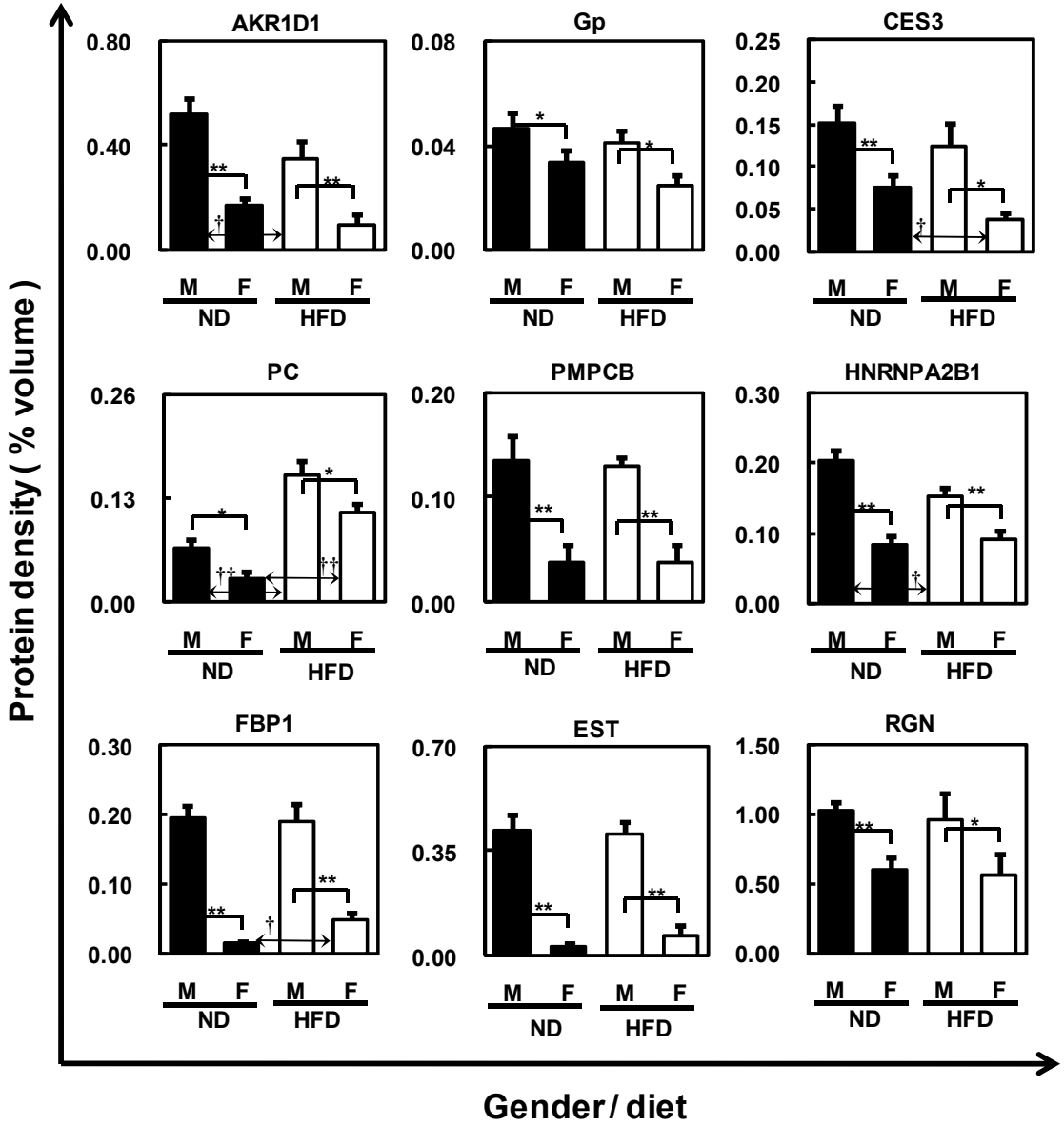


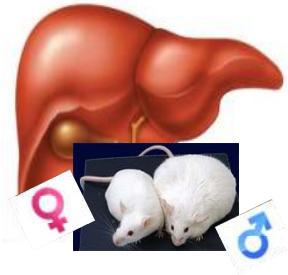
Representative silver-stained 2-DE gel images of liver proteome of male and female rats fed ND or HFD. Differentially regulated proteins in each group are marked with arrows and proteins of numbers in gel are listed in Table 3.

Wang et al. *Proteomics*, 12(2) (2012)

Proteins showing gender-difference in liver

Group I: Proteins showed gender-difference which are higher levels in males





Differentially expressed liver proteins showing gender difference in both ND and HFD rats with higher protein levels in males. Data are exhibited as mean values ±SEM of volume density (%) of the changed spot in three individual gels using pooled liver samples of seven rats per group. These 10 proteins have *p* values below 0.05 between males and females fed a ND and/or a HFD. Statistical significance between male (M) and female (F) rats was determined by an ANOVA, where *p* values were $^{*}p<0.05$ and $^{**}p<0.01$ and significance between ND and HFD rats was represented by $^{\dagger}p<0.05$ and $^{\dagger\dagger}p<0.01$.

AKR1D1, 3-oxo-5-beta-steroid 4-dehydrogenase; **GP**, Glycogen phosphorylase; **CES3**, Carboxylesterase 3; **PC**, Pyruvate carboxylase, Mitochondrial; **PMPCB**, Mitochondrial-processing peptidase subunit beta; **HNRNPA2B1**, Heterogeneous nuclear ribonucleoprotein A2; **FBP1**, Fructose-1,6-bisphosphatase 1; **EST**, Estrogen sulfotransferase isoform 3; **RGN**, Regucalcin

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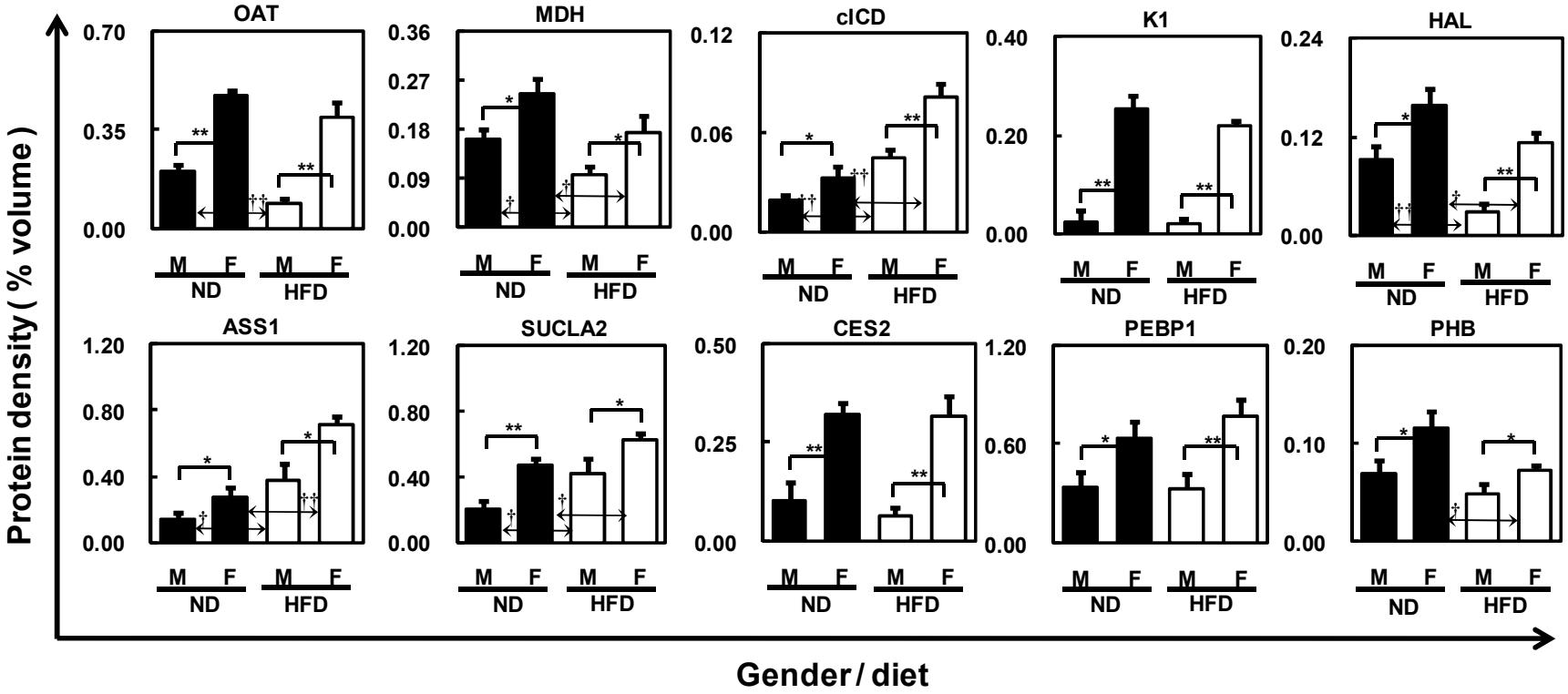
Proteins showing gender-difference in liver

Group II: Proteins showed gender-difference which are higher levels in females



Differentially expressed liver proteins showing gender difference in both ND and HFD rats, with higher protein levels in females. Data are exhibited as mean values ±SEM of volume density (%) of the changed spot in three individual gels using pooled liver samples of seven rats per group. These 9 proteins have *p* values below 0.05 between males and females fed a ND and/or a HFD. Statistical significance between male (M) and female (F) rats was determined by an ANOVA, where *p* values were $^{*}p<0.05$ and $^{**}p<0.01$ and significance between ND and HFD rats was represented by $^{\dagger}p<0.05$ and $^{\dagger\dagger}p<0.01$. **OAT**, Ornithine aminotransferase, mitochondrial; **MDH**, Malate dehydrogenase cytoplasmic; **cICD**, Isocitrate dehydrogenase [NADP] cytoplasmic; **K1**, Keratin, type II cytoskeletal 1; **HAL**, Histidine ammonia-lyase; **ASS1**, Argininosuccinate synthetase 1; **SUCLA2**, Sucla2 protein; **CES2**, Carboxylesterase 2A; **PEBP1**, Phosphatidylethanolamine-binding protein 1; **PHB**, Prohibitin

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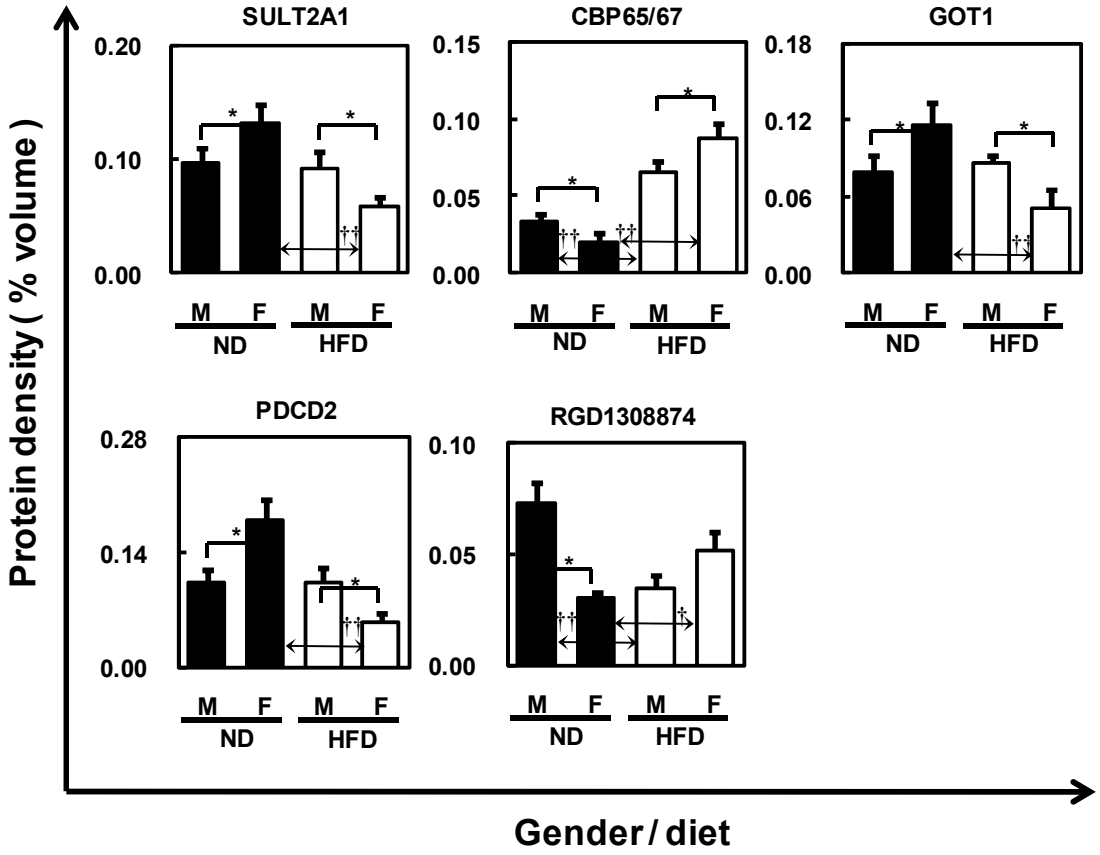
Group III: Proteins showed gender-difference with opposite expression pattern in both ND and HFD rats



Differentially expressed liver proteins showing opposite gender difference between ND and HFD rats. Data are exhibited as mean values ±SEM of volume density (%) of the changed spot in three individual gels using pooled liver samples of seven rats per group. These 5 proteins have *p* values below 0.05 between males and females fed a ND and/or a HFD. Statistical significance between male (M) and female (F) rats was determined by an ANOVA, where *p* values were $^{*}p<0.05$ and $^{**}p<0.01$ and significance between ND and HFD rats was represented by $^{\dagger}p<0.05$ and $^{\dagger\dagger}p<0.01$.

SULT2A1, Alcohol sulfotransferase; **CBP65/67,** Calcium-binding protein 65/67; **GOT1,** Cytosolic aspartate aminotransferase; **PDCD2,** Programmed cell death 8, isoform CRA-a; **RGD1308874,** Adipocyte plasma membrane-associated protein

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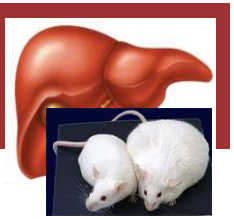
Summary from liver proteome analysis



- Male rats undergo slower fat oxidation, thereby leading to excessive accumulation of dietary fat in liver tissues, compared with female rats.

- Females down regulate lipogenic enzymes and shift partition of FFA toward oxidation, away from TG storage by activation of AMPK and PPAR.

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- 연구참여: 최정원박사, 김동현, 주정인, **Xia Wang, M. Rajib** 오태석, 최덕권, **Hao Liu**

감사합니다 !