Original Article

Artemisia capillaries Herbal Acupuncture Improves Metabolic Abnormalities in High Fat Diet-induced Obese ICR Mice

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국문초록

인진약침이 고지방식이유도 비만 ICR Mice에서 항비만 및 대사이상 개선에 미치는 영향

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목적 : 인진약침이 고지방식이로 유발된 비만 ICR mice에서 비만 및 동반 대사이상에 미치는 효과와 그 기전을 연구하고자 한다.

방법 : 인진약침의 비만 예방효과를 검증하기 위하여, 4주간 고지방식이를 급여하면서 150mg/kg 또는 300mg/kg의 인진약침을 양측 비수(BL_{20})에 교대로 매일 피하에 시술하였다. 또한 인진약침의 비만 치료효과를 검증하기 위하여, 4주간 고지방식이를 급여한 비만 ICR mice에 추가 4주간 고지방식이를 유지하면서 300 mg/kg 인진약침액과 vehicle control로써 등량의 distilled water를 양측 비수(BL_{20})에 교대로 매일 피하에 약침시술하였다. 인진약침의 항비만효과와 기전을 알아보기 위해, 체중, blood glucose, insulin, total cholesterol, triglyceride, non-esterified fatty acid (NEFA), AST, ALT levels 등 대사지표를 측정하고 부고환조직의 조직학적 관찰을 시행하였으며, AMPK activation과 adipocyte differentiation, fatty acid β-oxidation 및 thermogenesis와 관련된 gene expressions을 평가하였다.

결과 : 인진약침의 치료를 통하여 고지방식이 급여로 인한 체중의 증가가 억제되었을 뿐만 아니라, 비만

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ICR mice의 체중을 감소시켰으며, glucose 및 lipid homeostasis를 개선시켰으며 지방조직의 증식을 억제하였다. AMPK의 phosphorylation과 CPT-1 및 UCP2의 발현을 증가시켰으며, PPAR-γ, C/EBPα, aP2, LPL,FAS, SCD-1의 발현을 억제하였다.

결론 : 인진약침은 고지방식이 유도 동물모델에서 비만 및 동반 대사이상을 개선시키는 효과가 있으며, 이는 식이억제에 의한 2차적 효과라기 보다는 energy expenditure를 증가시키고, pre-adipocyte differentiation 및 proliferation을 억제하며, lipogenesis를 억제하고 lipolysis를 증가시키는 효과에 의한 것으로 사료된다.

핵심 단어 : 인진, 약침, 고지방식이, 비만, 지방축적, 지방합성, 대사증후군

Ⅰ. Introduction

Obesity is a progressive, multifactorial, complex, and metabolic syndrome related to overnutrition, sedentary lifestyle and resultant excess adiposity[1]. On a global scale, obesity has reached epidemic proportions, and it is a major contributor to the global burden of chronic disease and disability. Currently, more than one billion adults worldwide are overweight and at least 300 million of them are clinically obese[2,3]. The overall prevalence of obesity in Korean adults body mass index (BMI) ≥ 25.0 kg/m^2[2] is reported as 30.6%[4], and the prevalence of metabolic syndrome among Korean adults was 15~30% according to various criteria of metabolic syndrome[5].

Obesity is not only a constitutional problem, but it is also one of clustering components that reflect impaired glucose tolerance, insulin resistance, dyslipidemia, and elevated blood pressure associated with other morbidities including the prothrombic state, proinflammatory state, and nonalcoholic fatty liver disease[6,7]. A trend toward increases in blood cholesterol, triglyceride, glucose and insulin are observed with obesity development and lead to exacerbations of cardiovascular risk factors[8,9]. The majority of overweight or obese individuals are insulin resistant[10], especially in the individuals with the combination of obesity, physical inactivity and consumption of an atherogenic diet[11]. In the state of insulin resistance, maintenance of normoglycemia is determined by an increasing β-cell function[12]. Under the condition of insulin resistance and adipose tissue increment, insulin is unable to properly suppress lipolysis from stored adipose tissue triglyceride[13]. Elevated plasma-free fatty acid levels are shown to be account for arising of insulin resistance in obese patients with type 2 diabetes mellitus[14]. Also insulin resistance increases atherogenesis and atherosclerotic plaque instability by inducing proinflammatory activities on vascular and immune cells[15].

Various measures are used for the treatment of obesity including dietary change, exercise therapy, behavior therapy, medicinal therapy and surgical procedures. A number of drugs are developed for reducing intestinal fat absorption through inhibition of pancreatic lipase[16,17], suppression of appetite[18,19], modulating targeting peripheral episodic satiety signals and blocking fat absorption[20,21]. Due to dissatisfaction with high costs and potentially hazardous side-effects including increased blood pressure, dry mouth, constipation, headache, and insomnia[22,23], the potential of natural products for treating obesity is being explored, and this may be an excellent alternative strategy for developing future-effective, safe anti-obesity drugs[24,25]. A variety of natural products, including crude extracts and isolated compounds from plants, can induce body weight reduction and prevent diet-induced obesity[26,27].

The young leaves of *Artemisia capillaris* (AC) have been used as herbal medicine to cure chronic and acute liver cirrhosis and liver cancers[28-31]. The essential oil of *Artemisia capillaries* exhibited bactericidal, insecticidal, and antimutagenic effects[32-35].

Preliminary experiments demonstrated that *Arte-*

misia capillaris (AC) herbal acupuncture was the most effective anti-obese medicinal herb for adipocyte differentiation assay among 33 medicinal herbs tested. In the previous studies, it was reported that oral administration of a mixture of three herbs, *Morus alba*, *Melissa officinalis* and *Artemisia capillaries* improves lipid metabolism, and inhibits body weight gain and adiposity in part through changes in the expression of hepatic peroxisome proliferator0r-activated receptor alpha target genes[36]. And Hong et al[37] reported that oral administration of 0.1 g/kg of body weight of *Artemisia capillaries* ethyl acetate fraction on diet-induced obesity model increases mitochondrial β-oxidation and suppressed the activity of the fatty acid synthase and gylycerol-3-phosphate dehydrogenase activity and lowered hepatic lipid droplet accumulation and adipose tissue weight and size. But the study on the anti-diabetic, anti-steatotic activities and the regulating mechanisms in the adipocyte differentiation related gene expression of the AC herbal acupuncture in the diet-induced obese mice had not been accomplished.

Therefore, in this study, we investigated the anti-obesity effect of AC herbal acupuncture and action mechanisms against obesity in high fat diet-induced obese ICR mice.

Ⅱ. Materials and methods

A. Preparation of *Artemisia capillaries* extract

Artemisia capillaries (AC) was purchased from Kyung Hee herb pharmaceuticals (Seoul, Korea). Following drying in the shade for 1 week, plant extract was prepared by boiling 100 g of AC in 1 liter of distilled water for 6 hr. After filtration and evaporation, the solution was evaporated under vacuum to obtain extract powder. The AC herbal extract powder was dissolved in distilled water as AC herbal acupuncture to 150 mg/kg or 300 mg/kg before use.

B. Animals and diets

Five-week old ICR mice were purchased from Orient Bio (Seoul, Korea). All animals were acclimatized to the laboratory environment for 1 week before the experiment. Mice were allowed free access to water and food (standard rodent chow, lab diets, USA) under constant room temperature (22±2℃) and humidity (50±10%) conditions with an automatic 12 hr light and dark cycle. The care, maintenance and treatment of animals in these

Table 1. Composition of the Experimental Diets

	RD 10% kcal		HFD 45% kcal	
	gm %	kcal %	gm %	kcal %
Protein	19.2	20	24	20
Carbohydrate	67.3	70	41	35
Fat	4.3	10	24	45
Total		100		100
kcal/gm	3.85		4.73	
Ingredient	**gm**	**kcal**	**gm**	**kcal**
Casein, 80 mesh	200	800	200	800
L-cystine	3	12	3	12
Corn starch	315	1,260	72.8	291
Maltodextrin 10	35	140	100	400
Sucrose	350	1,400	172.8	691
Cellulose, BW 200	50	0	50	0
Soybean oil	25	225	25	225
Lard	20	180	177.5	1,598
Mineral mix S10026	10	0	10	0
DiCalcium phosphate	13	0	13	0
Calcium carbonate	5.5	0	5.5	0
Potassium citrate	16.5	0	16.5	0
Vitamin mix V10001	10	40	10	40
Choline bitartrate	2	0	2	0
FD&C yellow dye #5 (RD), Red dye #40 (HFD)	0.05	0	0.05	0
Total	1,055.05	4,057	8,58.15	4,057

RD : regular diet(D12450B. research diets, INC, New Brunswik, NJ, USA).
HFD : high fat diet(D12451. research diets, INC, New Brunswik, NJ, USA).

studies followed protocols approved by the Institutional Animal Ethics Committee of the Kyung Hee University. For prevention mode, mice were randomly divided into four groups: regular diet group (RD, D12450B; Research Diets, INC, New Brunswik, NJ, USA), high fat diet group (HFD, D12451; Research Diets, INC, New Brunswik, NJ, USA) with vehicle control, group fed HFD with 150 mg/kg of AC herbal acupuncture treatment (AC 150) and group fed HFD with 300 mg/kg of AC herbal acupuncture treatment (AC 300). For treatment mode, mice were fed with high fat diet for 4 weeks, and then 300 mg/kg of AC herbal acupuncture or distilled water as vehicle control was subcutaneously administered for additional 4 weeks with continuous high fat diet. During the experimental period, mice were housed in groups and water and food (RD or HFD) were provided ad libitum. Body weight and plasma glucose levels were determined twice a week. The composition of the experimental diet (Table 1) was based on the HFD 45% kcal semi-synthetic diet.

C. Acupuncture treatment

For the herbal acupuncture treatment, *Bisu* (BL_{20}) located at 1.5 B-cun lateral to the same level as the inferior border of the spinous process of the 11th thoracic vertebra (T11)[38] was used. The acupuncture point is widely applied for the treatment of various conditions such as spleen deficiency syndrome, gastrointestinal disorders, abdominal mass, jaundice, ascites, edema, diabetes by spreading, regulating and nourishing spleen qi, and clearing dampness[39]. Animals were subcutaneously injected with 1.0ml syringe (26 gauge needle, Green Cross Medical Equipment, Korea) into both *Bisu* (BL_{20}) everyday alternately with either AC herbal acupuncture (150 mg/kg or 300 mg/kg) or distilled water as vehicle control respectively for 4 weeks.

D. Determination of serum parameters

At the end of the experimental period, the blood samples were collected and the levels of plasma glucose, insulin, triglyceride (TG), total cholesterol (TC), non-esterified fatty acid (NEFA), AST and ALT levels were measured. Plasma glucose concentrations were determined using the glucose oxidase method (Asan pharmaceutical Co, Korea). Plasma insulin concentrations were determined using a mouse insulin enzyme immunoassay kit (Gunma, Japan). Plasma TG and TC concentrations were determined using commercially available kit (Asan pharmaceutical Co., Seoul. Korea). Plasma NEFA levels were determined using enzymatic colorimetric method (Eiken, Tokyo, Japan). Plasma AST and ALT levels were determined using commercially available kit (Stanbio, Boerne, Texas, USA). Homeostasis model assessment was used to calculate an index of insulin resistance (HOMA-IR) as (fasting insulin mU/L) × (fasting glucose mmol/L) / 22.539).

E. Histological analysis

The epididymal fat tissue was removed and fixed in 10% neutral buffered formalin, embedded in paraffin and sectioned with 5 μm thickness (Leica, Wetzlar, Germany), followed by staining with hematoxylin-eosin for microscopic assessment (Olympus, Tokyo, Japan).

F. RNA preparation and Reserve Transcription-Polymerase Chain Reaction (RT-PCR)

Total mRNA was isolated from epididymal fat using an Easy-Blue kit (Intron Biotechnology Inc, Seoul, Korea) according to the manufacture's instruction. From each sample, total RNA (10 μg) was reverse transcribed into cDNA using Moloney murine leukemia virus transcriptase and Oligo (dT) 15 primers (Promega, Madisan, USA) as primers. The cDNA fragment was amplified by PCR using the specific primers (see Table 2). Primer was added to the 2 $\mu\ell$ of reaction solution containing 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 0.5 mM dNTP, 5 $\mu\ell$ cDNA and 2.5 unit of Taq DNA

Table 2. Characteristics of Specific Primers Used for RT-PCR Analysis

Gene	Forward primer (from 5′ to 3′)	Reverse primer (from 5′ to 3′)	Annealing temperature (℃)
PPAR-ɣ	GCGCTACCGGTCTTCTATCA	TGCTGCCAAAAGACAAGCG	57
C/EBPα	GATCCTGGAACGAGAACAC	AGACTCGTGGAACACGGTGGT	57
aP2	CGAGGGTTGGTTGTTGATCTGT	ATAGCACTGTTGGCCCTGGA	57
FAS	GGTAGTGGATACTCTGTCGTCCA	CATCAGCAACATCATTCGGT	66
SCD-1	CCCTGAACATCGAGTGTCGA	CTTGCCCAGAGATTTGAGGTCCT	57
CTP 1	CCTGGGCATGATTGCAAAG	ACAGACTCCAGGTACCTGCTCAC	55
UCP 2	GCAAGCTCAATGTTGGTGTCTT	ACTCTGCAGATAGACAGGCCTG	50
β-actin	GTCGTACCACTGGCATTGTG	GCCATCTCCTGCTCAAAGTC	57

polymerase. PCR was initiated a thermal cycler programmed at 95℃ for 5 min, 95℃ for 30 sec, 57℃ for 30 sec, 72℃ for 30 sec, and amplified for 30 cycles. The RT-PCR products were electrophoresed on 1% agarose gels and visualized by 0.5 ㎍/ml ethidium bromide staining and scanning densitometry was performed with I-MAX Gel Image analysis system (Core-Bio, Seoul, Korea). β-actin was amplified as a control gene.

G. Western blot analysis

After sacrificed, epididymal fat was immediately removed and instantly soaked in liquid nitrogen and stored at -70℃. Protein extracts were prepared using a protein extraction kit (Intron Biotechnology Inc., Seoul, Korea). Lysates (40 ㎍) were electroblotted onto a nitrocellulose membrane following separation on a 8% SDS-polyacrylamide gel electrophoresis. Blotted membranes were incubated for 1 hr with blocking solution (tris-bufferd saline/Tween 20, TBST) containing 5% skim milk (w/v) at room temperature, followed by incubation overnight at 4℃ with 1 : 2,000 diluted AMP-activated protein kinase (AMPK), phospho AMP-activated protein kinase (p-AMPK), acetyl-coA carboxylase (ACC), phospho-ACC (Cell signaling, USA) and β-actin primary antibodies (Santacruz Biotechnology, Santacruz, USA). Membranes were washed four times with 0.1% TBST and incubated with 1 : 3,000 diluted horseradish peroxidase-conjugated goat anti-rabbit or donkey anti-rabbit IgG (Santacruz Biotechnology, Santacruz, USA) secondary antibody for 1 hr at room temperature. Membranes were washed four times in TBST and then developed by ECL (Amersham, Upsala, Sweden).

H. Statistical analysis

Results were expressed as mean±S.E.M and differences between groups were analyzed using Student's *t*-test. Statistical significance was considered at $p<0.05$.

Ⅲ. Results

A. Prevention mode experiment

1. Effects on body weight

Fig. 1 shows the pictures of whole body, opened abdomen and body weight change during the 4-week period. As shown in panel A, high fat diet fed group (HFD control) gained weight, especially in epididymal fat area when compared to that of regular diet fed group (RD). The HFD control group gained weight rapidly from the beginning and body weight changes were markedly different from RD group since 10th day of administrating high fat diet. While body weight in the HFD control group was increased by 263% compared to that of RD group, AC herbal acupuncture groups (AC 150 and AC 300) prevented weight gain by

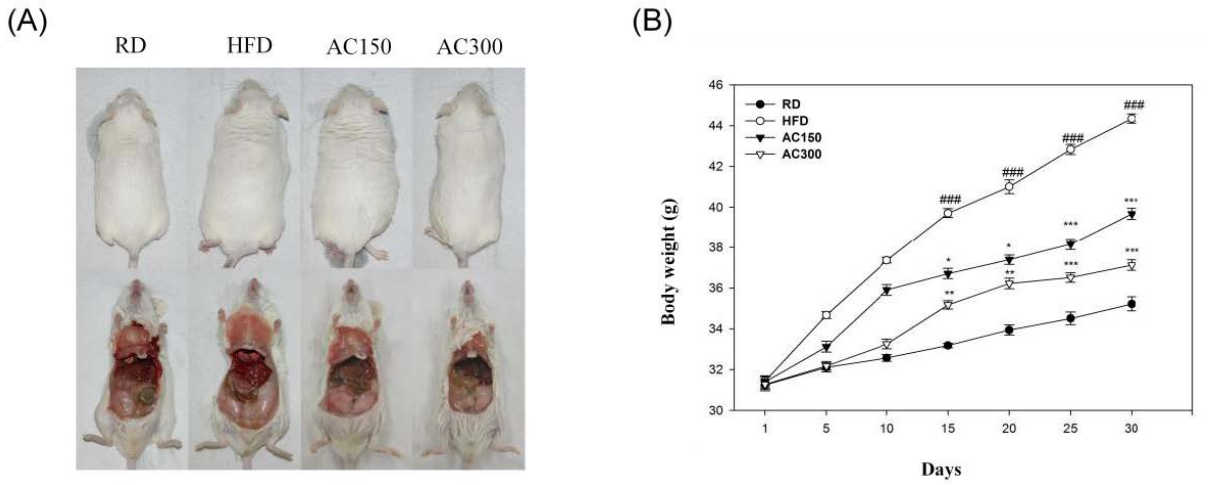

Fig. 1. Gross appearance of whole body and abdomen (A), and comparison of body weight change during 4-week treatment of *Artemisiacapillaries* (AC) herbal acupuncture (B)
RD : regular diet group. HFD : high fat diet group. AC : high fat diet group treated with AC herbal acupuncture.
Animals were subcutaneously injected daily into both *Bisu* (BL_{20}) alternately with either distilled water as vehicle control or AC herbal acupuncture (150 mg/kg or 300 mg/kg) respectively for 4 weeks.
Values represent the mean±SE (n=6). ### : $p<0.001$ vs RD. * : $p<0.05$. * : $p<0.01$. * : $p<0.001$ vs HFD.

Table 3. Effect of AC Herbal Acupuncture on Body Weight and Weight Gain

Group	Body weight (g)		Weight gain (g)
	initial	final	
RD	31.8±0.9	35.9±1.1	4.1±0.8
HFD	32.9±0.9	43.7±2.5†††	10.8±1.7††
AC 150	32.4±1.3	39.7±2.3**	7.3±3.4*
AC 300	32.3±0.4	38.7±1.6***	6.5±1.7*

RD : regular diet group. HFD : high fat diet group.
AC : high fat diet group treated with AC (150 mg/kg or 300 mg/kg) herbal acupuncture.
Values represent the mean±SE (n=6).
†† : $p<0.01$. ††† : $p<0.001$ vs RD. * : $p<0.05$.
** : $p<0.01$. *** : $p<0.001$ vs HFD.

32.5 and 39.9%, respectively, when compared to that of the HFD control group (Table 3).

2. Effects on metabolic parameters

Table 4 shows the effects of AC herbal acupuncture on metabolic parameters in the HFD-induced obesity mice. Compared to the HFD control group, plasma glucose levels were significantly decreased by 37.6% ($p<0.01$) in AC 150 and 44% ($p<0.01$) in AC 300 group. Plasma insulin levels were also dose-dependently decreased by 12% ($p<0.01$) in AC 150 and 36.1% ($p<0.001$) in AC 300 group compared to the HFD control group. With decreased plasma glucose and insulin levels, insulin resistance index (HOMA-IR) values for AC herbal acupuncture groups were markedly decreased by 45.1% ($p<0.01$) in AC 150 and 64.3% ($p<0.001$) in AC 300 group compared to the HFD control group. As shown in Table 5, NEFA levels of AC herbal acupuncture groups were also markedly reduced compared to the HFD control group, by 26.5% in

Table 4. Preventive Effect of AC Herbal Acupuncture on Plasma Glucose, Insulin and Homeostasis Model Assessment Values for Insulin Resistance (HOMA-IR)

Group	Glucose (mM)	Insulin (μU/ml)	HOMA-IR
RD	4.9±2.0	48.3±3.2	10.5±1.6
HFD	12.5±3.2†††	101.2±11.7†††	56.2±2.8†††
AC 150	7.8±2.6**	89.1±6.2**	30.9±4.3**
AC 300	7.0±1.3**	64.7±4.6***	20.1±2.7***

RRD : regular diet group. HFD : high fat diet group.
AC : high fat diet group treated with AC (150 mg/kg or 300 mg/kg) herbal acupuncture.
Homeostasis model assessment was used to calculate an index of insulin resistance (HOMA-IR) as (fasting insulin mU/L) × (fasting glucose mmol/L) / 22.5.
Values represent the mean±SE (n=6).
††† : $p<0.001$ vs RD. ** : $p<0.01$. *** : $p<0.001$ vs HFD

Table 5. Effect of AC Herbal Acupuncture on Plasma Lipid Levels

Group	RD	HFD	AC 150	AC 300
NEFA	1,285.3±44.7	2,628.6±200.0†††	1932.6±131.4*	1325.8±147.7**
TC(mg/dL)	151.5±12.7	191.8±4.1††	164.0±19.2	157.0±27.0
TG(mg/dL)	78.7±11.7	183.7±11.0†††	121.6±16.1*	106.0±11.7**
AST(U/L)	10.2±12.3	60.4±14.9†††	59.6±19.2	44.8±13.5*
ALT(U/L)	20.6±12.6	82.1±7.2†††	33.5±5.6***	28.1±6.5***

NEFA : non-esterified fatty acid. TC : total cholesterol. TG : triglyceride. AST : aspartate aminotransferase.
ALT : alanine aminotransferase RD : regular diet group. HFD : high fat diet group.
AC : high fat diet group treated with AC(150mg/kg or 300mg/kg) herbal acupuncture.
Values represent the mean±SE(n=6).
†† : p<0.01. ††† : p<0.001 vs RD. * : p<0.05. ** : p<0.01. *** : p<0.001 vs HFD

AC 150 and 49.6% in AC 300 group. While total cholesterol level in the HFD control group was increased by 26% compared to RD group, cholesterol levels in AC 150 and AC 300 groups were decreased by 14.5% and 18.2%, respectively. Triglyceride levels in AC 150 and AC 300 groups were also dose-dependently decreased by 33.9% and 42.3%, respectively compared to the HFD control group. To evaluate liver function, AST and ALT activities were compared between groups, and those levels in AC herbal acupuncture groups were both significantly lowered compared to those in the HFD control group.

3. Histological observation

Fig. 2 shows the effect on white adipose tissue (epididymal fat) morphology using hematoxilin-eosin staining method. As shown in panel A, fat

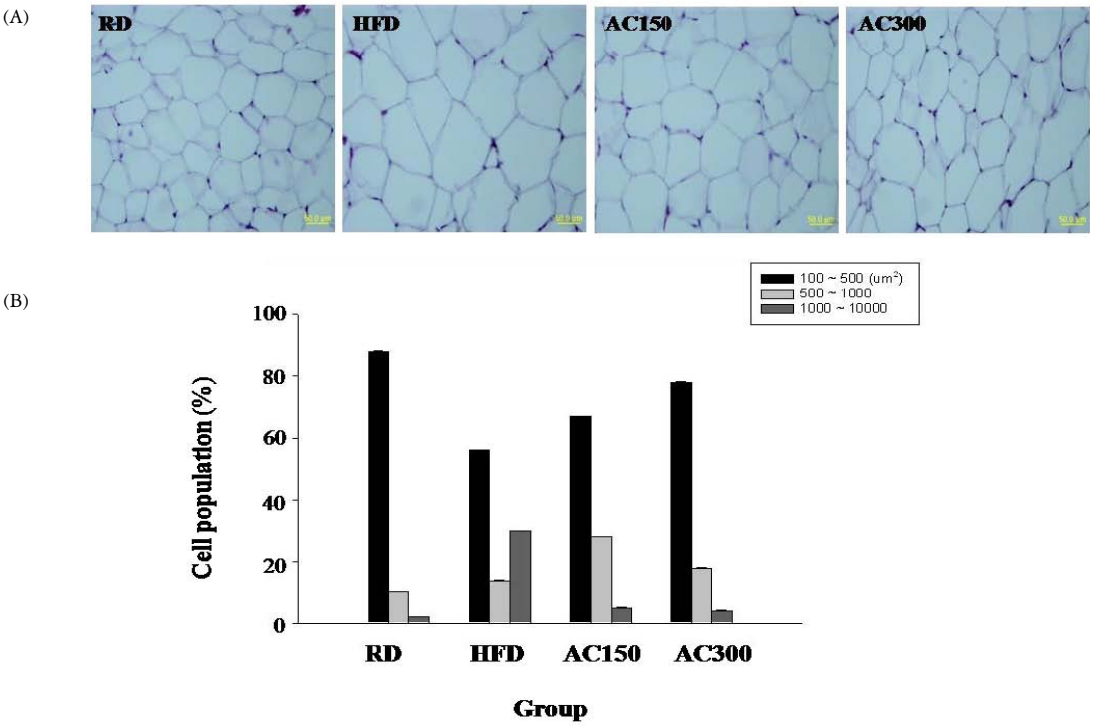

Fig. 2. Effect of AC herbal acupuncture on morphology of the epididymal adipose tissue (A), and number of adipocytes between different ranges of the cell area were determined using an image analysis program (B)
RD : regular diet group. HFD : high fat diet group.
AC : high fat diet group treated with AC (150 mg/kg or 300 mg/kg) herbal acupuncture.

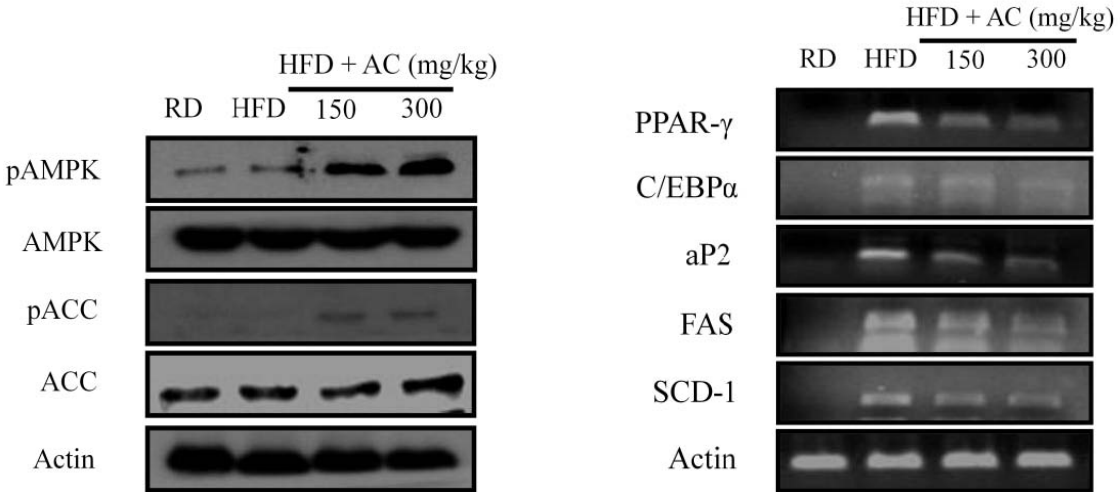

Fig. 3. Effect of AC herbal acupuncture on phosphorylations of AMPK, ACC and gene expressions for adipocytes differentiation and their target molecules in epididymal tissue
RD : regular diet group. HFD : high fat diet group.
AC : high fat diet group treated with AC (150 mg/kg or 300 mg/kg) herbal acupuncture.

cell size of the HFD control group is much larger than that of RD group. In contrast, when cell populations categorized in three different sizes were compared between groups, cell populations having smaller area were increased in AC herbal acupuncture groups, compared to those in the HFD control group (panel B of Fig. 2).

4. Effects on AMPK activation and gene expressions responsible for adipocyte differentiation

AMPK is a potential target of obesity and type Ⅱ diabetes, and plays a key role in regulating carbohydrate and lipid metabolism. Activation of AMPK in fat tissue results in inhibition of acetyl-CoA carboxylase leading to increased fatty acid oxidation, ketogenesis and simultaneous inhibition of fatty acid and triglyceride synthesis. Thus, we evaluated AMPK activation in the fat tissue. As shown in left panel of Fig. 3, AC herbal acupuncture significantly phosphorylated AMPK and ACC when compared to those in RD and the HFD control groups. With activation of AMPK, the expressions of target genes responsible for adipocyte differentiation were examined by RT-PCR. As shown in right panel of Fig. 3, gene expressions for transcription factors, peroxisome proliferator-activated receptor γ (PPAR-γ) and CCAAT/enhancer binding protein α (CEBPα), and their target genes like fatty acid binding protein (aP2), fatty acid synthase (FAS), stearoyl-CoA desaturase-1 (SCD-1) were compared between groups. While expression levels for these genes in the HFD control group were dramatically enhanced compared to those in RD group, AC herbal acupuncture groups suppressed the gene expressions in dose-dependent manners.

B. Treatment mode experiment

1. Effects on body weight

Fig. 4 shows the body weight change during 4-week period. While body weight change of the HFD control group for 4-week period was insignificant, body weight of AC 300 group started to decrease from the beginning and continued weightloss was observed. At the end of treatment, body weight of AC 300 group was decreased by 18.7% ($p<0.001$) compared to that of the HFD control group.

2. Effects on metabolic parameters

Table 6 shows the effects of AC herbal acupuncture on plasma glucose and insulin levels in the HFD-induced obesity mice. Compared to the HFD

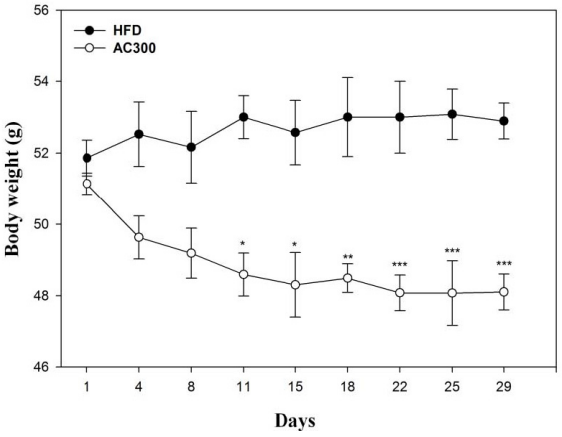

Fig. 4. Body weight change during the 4-week administration of AC herbal acupuncture

HFD : high fat diet group.

AC 300 : high fat diet group treated with AC (300 mg/kg) herbal acupuncture.

Animals were subcutaneously injected daily into both *Bisu* (BL_{20}) everyday alternately with either distilled water as vehicle control or AC herbal acupuncture res- pectively for 4weeks.

Table 6. Therapeutic Effect of AC Herbal Acupuncture on Plasma Glucose, Insulin and Homeostasis Model Assessment Nalues for Insulin Resistance (HOMA-IR)

Group	Glucose(mM)	Insulin(μU/ml)	HOMA-IR
HFD	19.5±2.3	120.1±12.2	104.1±1.6
AC 300	5.3±0.1**	80.9±10.5**	19.1±3.2***

HFD : high fat diet group.

AC 300 : high fat diet group treated with AC (300 mg/kg) herbal acupuncture.

Homeostasis model assessment was used to calculate an index of insulin resistance (HOMA-IR) as (fasting insulin mU/L) × (fasting glucose mmol/L)/22.5.

Values represent the mean±SE (n=6).

** : $p<0.01$. *** : $p<0.001$ vs HFD.

Table 7. Effect of AC Herbal Acupuncture on Plasma Lipid Levels

Group	HFD	AC 300
NEFA	593.0±37.3	249.0±19.5***
TC(mg/dL)	153.3±9.5	65.1±3.1***
TG(mg/dL)	90.9±5.6	37.6±3.2***
AST(U/L)	155.7±10.6	32.4±2.4***
ALT(U/L)	55.1±4.3	19.6±9.6***

NEFA : non-esterified fatty acid. TC : total cholesterol. TG : triglyceride. HFD : high fat diet group.

AC 300 : high fat diet group treated with AC (300 mg/kg) herbal acupuncture.

Values represent the mean±SE (n=6).

*** : $p<0.001$ vs HFD.

control group, plasma glucose levels were markedly decreased by 72.9% ($p<0.01$) in AC 300 group. Plasma insulin levels also decreased by 32.7% ($p<0.01$) in AC 300 group compared to the HFD control group. Having decreased plasma glucose and insulin levels, the insulin resistance index (HOMA-IR) values for AC 300 group were profoundly decreased by 81.7% ($p<0.001$) compared to the HFD control group. As shown in Table 7, NEFA, cholesterol, TG, AST and ALT levels in AC 300 group were all markedly reduced by 58.1%, 57.6%, 58.7%, 79.3% and 64.5%, respectively, compared to the HFD control group.

3. Histological observation

Fig. 5 shows the effect on white adipose tissue (epididymal fat) morphology using hematoxilin- eosin staining method. As shown in panel A, when cell populations categorized in three different sizes were

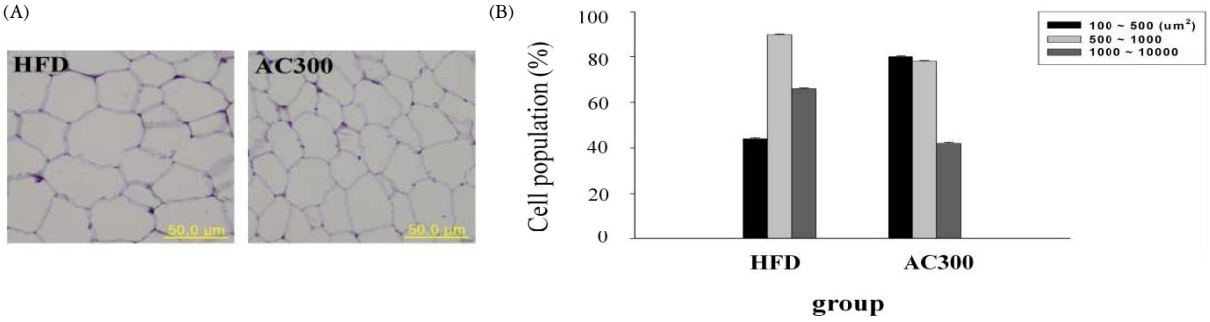

Fig. 5. Effect of AC herbal acupuncture on epididymal adipose tissue morphology

HFD : high fat diet group. AC 300 : high fat diet group treated with AC (300 mg/kg) herbal acupuncture.

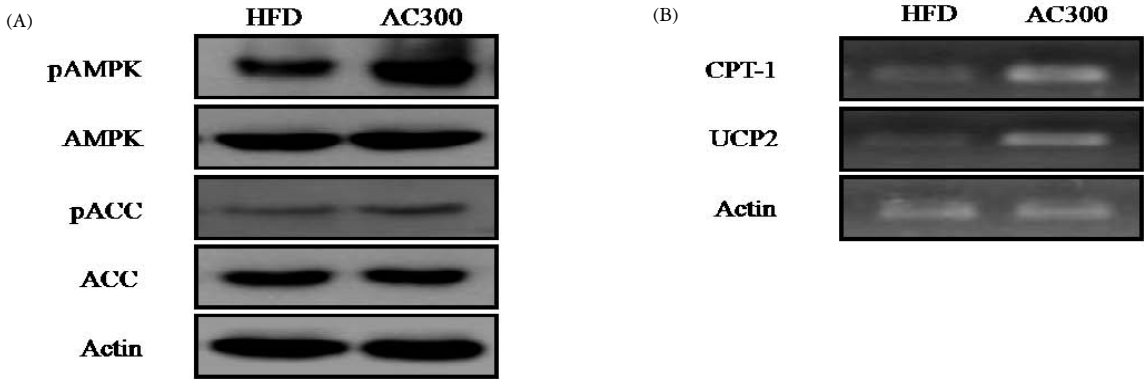

Fig. 6. Effect of AC herbal acupuncture on AMPK activation and lipolysis genes in epididymal tissue
HFD : high fat diet group. AC 300 : high fat diet group treated with AC (300 mg/kg) herbal acupuncture.

compared between groups, cell populations having smaller area were significantly increased in AC 300 group, compared to those in the HFD control group (panel B of Fig. 5).

4. Effects on AMPK, CPT-1 and UCP 2 expressions

Similar to the results in prevention mode experiment, 300 mg/kg of AC herbal acupuncture significantly phosphorylated AMPK and ACC, and also enhanced gene expression responsible for fatty acid β-oxidation and thermogenesis, CPT-1 and UCP 2, respectively, when compared to those in the HFD control group (Fig. 6).

Ⅳ. Discussion

Physiological studies on the dietary fat consumption and development of obesity demonstrated that macronutrients differ in their effects on energy balance[40]. The diets composed with high fat ratio promote a passive overconsumption by less satiating characteristics of energy from fat, a positive energy balance and weight gain[41]. High fat diets induce much lower thermogenesis than that of high carbohydrate diets and lead to energy imbalance[42]. In addition to the differences of thermogenic effects between fat and carbohydrate, fat is more effectively absorbed from gastrointestinal tract and less fecal energy loss than carbohydrate[43].

The relationship between body weight and the risk of diabetes is successive and graded[44]. It is energy intake that matters in relation to the development of obesity, and energy intake is often high when HFD are consumed in large amounts[40]. Obesity in rodent mice is developed by feeding HFD, and the obese mice have the characters of hyperglycemia, insulin resistance and hepatic steatosis[45-47]. HFD-induced hyperglycemia in ICR mice could rely upon the development of obesity that follows protracted access to the HFD. Although many kinds of animal model, like a Lepob, Leprdb and ZDF, are used to assess the efficacy and safety of treatments for obesity and type 2 diabetes, most of all show serious obesity and hyperglycemia, and are suitable to investigation for treating obesity and type 2 diabetes[48]. On the other hand, HFD-induced animal models show mild obesity and hyperglycemia, and are appropriate to develop the preventive agent for obesity and type 2 diabetes.

As a result of feeding HFD to ICR mice for 4 weeks, HFD control mice developed a pre-diabetic state characterized by obesity, hyperglycemia, hyperinsulinemia, insulin resistance and dyslipidemia. However, AC herbal acupuncture treated mice prevented the subsequent development of obesity and hyperglycemia in spite of continued access to the HFD in the prevention mode experiment. AC herbal acupuncture significantly suppressed the deterioration of the homeostasis of plasma glucose and lipid in

HFD-induced hyperglycemic and hyperlipidemic mice. AC herbal acupuncture was able to increase insulin sensitivity by decreasing blood glucose and insulin levels at a fasting state. Also, in ICR mice with obesity induced by HFD feeding for 4 weeks, AC herbal acupuncture decreased body weight, improved hyperglycemia and hyperlipidemia under the consistent HFD feeding for another 4 weeks. In histological observation on epididymal fat tissue, fat cell populations having larger area were decreased by AC herbal acupuncture treatment in both prevention and treatment mode experiment. With enhanced phosphorylation of AMPK and ACC, suppression of transcription factors and their target gene expressions responsible for adipocyte differentiation (PPAR-γ CEBPα and aP2, FAS, SCD-1) and the increment of gene expressions responsible for fatty acid β-oxidation and thermogenesis (CPT-1 and UCP-2) were seen when AC herbal acupuncture was administered to the obese ICR mice fed with HFD.

The function and survival of all organisms is dependent on the dynamic control of energy metabolism, when energy demand is matched to the energy supply. The AMP-activated protein kinase (AMPK) αβγ heterotrimer has emerged as an important integrator of signals that control energy balance through the regulation of multiple biochemical pathways in all eukaryotes[49,50]. During fasting, post-absorptive conditioned lipids are the predominant substrate for the maintenance of whole body energy metabolism[51]. The ability to efficiently store fuel in the form of energy-dense lipids and their mobilization during times of low carbohydrate availability was an essential development in evolution, allowing organisms to survive periods of famine and prolonged fasting. This coordinated release of free fatty acids from adipose tissue combined with the ability to actively fine-tune the gradient bet- ween fat and carbohydrate metabolism in meta- bolically active tissues in response to a number of dynamic physiological stimuli requires an integrated metabolic system of control. This synchronized regulation of metabolism occurs acutely, as well as through transcriptional control by AMPK. AMPK controls the fate of fatty acids in the cell by controlling rates of uptake by inducing the translocation of CD36 to the plasma membrane. AMPK suppresses malonyl-CoA content by phosphorylating and inhibiting ACC1 and therefore suppressing fatty acid synthesis and increasing mitochondrial β-oxidation, respectively. Futile cycling of fatty acids is suppressed by AMPK inhibition of TG synthesis and TG hydrolysis through the phosphorylation of GPAT and HSL, respectively. AMPK also reduces FA synthesis by inhibiting the transcription factor SREBP1c, which controls the entire synthesis pathway or by directly inhibiting the activity of FAS[50].

In the fat tissue, AMPK coordinates the changes in the activity of enzymes of the lipid metabolism and regulates the partitioning of fatty acids between oxidative and biosynthetic pathways. Acetyl-CoA carboxylase (ACC) is an important rate-controlling enzyme for the synthesis of malonyl-CoA, a critical precursor for biosynthesis of fatty acids and a potent inhibitor of mitochondrial fatty acid oxidation. Phosphorylation and inhibition of ACC by AMPK leads to a fall in malonyl-CoA content and a subsequent decrease in triglyceride synthesis concomitantly with an increase in β-oxidation. This was evidenced by the decrease in plasma triglyceride levels during AC herbal acupuncture administration in high fat diet-induced obese mice.

Previous studies implicated that obesity and its related metabolic syndrome is believed as a partial contribution of deterioration of hepatic metabolic function, and in the clinical practice of traditional medicine, Artemisia capillaris is one of the widely prescribed medicinal herbs for the purpose of amelioration of hepatic function and elimination of dampness-heat[52]. And the preliminary experiments demonstrated that Artemisia capillaris exerted most effective anti-obese properties on adipocyte differentiation assay among 33 medicinal herbs tested. Although the anti-obese activity of Artemisia capillaris observed from the present study could be attributed to many chemical constituents, such as scopoletin, capillarisin, 6, 7-dimethylesculetin, caffeic acid, chlorogenic acid, phenol, cresol, eugenol, and

ethylphenol, previously reported in Artemisia capillaris[53,54], future study to identify active component(s) and their dosage for obesity will be needed. Also, more research on the method of administering herbal acupuncture and selection of the acupuncture points of which will be applied is necessary.

In summary, AC herbal acupuncture not only prevented body weight gain in prevention mode experiment, but also significantly lowered body weight as shown in treatment mode experiment. AC herbal acupuncture exerted beneficial effects on glucose and lipid homeostasis that were not secondary to its ability to decrease food intake but its specific effects on fatty acid β-oxidation, adipocyte differentiation and thermogenesis. Taken together, results obtained in this study suggest that anti-obesity effect of AC herbal acupuncture can be arranged into three categories based on its distinct mechanisms; (1) increased energy expenditure, (2) decreased pre-adipocyte differentiation and proliferation, and (3) decreased lipogenesis and increased lipolysis.

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