CJIM 2003;9(3) : 204—209

EXPERIMENTAL WORK AND RESEARCH

Effect of Acupuncture on Uncoupling Protein 1 Gene Expression for Brown Adipose Tissue of Obese Rats*

LIU Zhi-cheng (刘志诚)[1], SUN Feng-min (孙凤岷)[2], ZHAO Dong-hong (赵东红)[3], ZHANG Zhong-cheng (张中成)[1], SUN Zhi (孙 志)1, WU Hai-tao (吴海涛)[1], XU Bing-guo (徐炳国)[1], ZHU Miao-hua (朱苗花)[1], and LI Chao-jun (李朝军)[3]

ABSTRACT **Objective**: To explore the effects of acupuncture on the expression of uncoupling protein 1 (UCP_1) gene of brown adipose tissue (BAT) in obese rats. **Methods**: The expression of UCP_1 gene of BAT was determined with RT-PCR technique. The changes of body weight, Lee's index, body fat, and the expression of UCP_1 gene of BAT in obese rats were observed before and after acupuncture. **Results**: The body weight, Lee's index, body fat in obese rats were all markedly higher than those in normal rats, but the expression of UCP_1 gene of BAT in obese rats was all lower than that in normal rats. There were negative correlation between the obesity index and the expression of UCP_1 gene in BAT. After acupuncture the marked effect of weight loss was achieved while the expression of UCP_1 gene of BAT obviously increased in obese rats. **Conclusion**: The abnormal reduction for expression of UCP_1 gene of BAT might be an important cause for the obesity. To promote the expression of UCP_1 in obese organism might be an important cellular and molecular mechanism in anti-obesity effect by acupuncture.

KEY WORDS acupuncture, obesity, uncoupling protein, gene

In recent years, studies showed that obesity involves a series of dietary controlling and metabolic disorders caused by specific biochemical factor, and its pathogenesis process is very complicated. At present obesity has already become the main killer threatening human health in place of former illnesses induced by malnutrition and infection. Therefore, its pathogenesis and treatment has become the focus in the concern of current world medicine and a hot point for contemplation. There has been no breakthrough in treatment on obesity, and ideal non-toxic medicine without any adverse reaction is still under exploration. The author established an approach of acupuncture in treating obesity and its complications, the effect of which proved long-lasting, without any toxic and adverse reaction, and was welcomed by the vast obesity patients[1]. This study has explored the effect of acupuncture on the uncoupling protein 1 (UCP_1) gene expression of brown adipose tissue (BAT) in obesity organisms, and now is reported as follows.

METHODS

Experimental Obesity Rat Model Establishment

SD rats, male, 1 month old and weaned, body weight 50—70 g, were provided by Experimental Animal Center of Nanjing Military Region General Hospital, and slightly modified referring to LIU's method of experimental obesity modeling[2].

Grouping of Animals

Rats fed with ordinary whole priced forage were used as normal control group (n=6); and successfully established experimental model rats were randomly divided into two groups: one group without any treatment as model control group (control

* The item is supported by the National Nature Science Foundation (No. 39970923)

1. Nanjing University of TCM, Nanjing (210029); 2. Nanjing Population Administration College; 3. Nanjing Normal University

Correspondence to: LIU Zhi-cheng, Tel: 025—6798143; E-mail: zhicheng-liu168@hotmail.com

group, $n=6$), and the other treated with acupuncture (acupuncture group, $n=6$).

Therapeutic Method

The acupuncture group rats were placed in a fixed container, unilateral "Housanli" (corresponding to Zusanli) and "Neiting" points selected, 32 gauge 1 cun filiform needle applied to insert for 5 mm and 3mm in depth, electroacupuncture (EA, comprehensive therapeutic device G6805-III type, manufactured by Fujian Medical Instrument Factory) connected, frequency 10 Hz, strength 1.5V continuous wave, 10 min/time, once daily, for 14 consecutive days, the point on left and right side used alternatively.

Experiment Method

Each group of rats during experiment period was fed freely with fresh ordinary whole priced rat forage and fresh water every day. While the acupuncture group were undergoing needling, the normal and control group were placed in the fixed rat container for 14 consecutive days, 15 min/day for adaptation. Before and after experiment the rats were observed for their forage intake, amount of drinking water, stool and urine, body weight, body length and Lee's index [$\sqrt[3]{\text{body weight (g)}}/\text{body length (cm)}\times10^3$], etc.

By the end of experiment, the rats were fasted for a night, and on the next day the rats were decapitated, fat of pericardium, peri-renal and epididymis swiftly isolated, weighed and recorded; at the same time BAT between scapulae and located at the neck-back part was swiftly isolated, washed with pre-cooling phosphate buffer, cut into small pieces in a ice bath of plate, liquid nitrogen poured on the tissue to coagulate it, the coagulant transferred to labeled freezing tube, and preserved in the liquid nitrogen container. The next day place the frozen brown adipose tissue in the mortar to pulverize it and provide it for extraction.

Examination and Determination Method

For reverse transcription polymerase chain reaction (RT-PCR) examination, refer to method reported by Nisoli E, et al[3-5].

The extraction and purification of total RNA: According to the instruction manual of Tripure isolation reagent kit given by German BM Company, a total of 50 μg RNA was isolated from 100 mg BAT and purified; ultraviolet spectrophotometry detection revealed that the A260/A280 ratio of RNA sample is greater than 2.0; formaldehyde agarose gel electrophoresis determination showed the total RNA segment was kept intact and has not been down-regulated, and the 18s and 28s band were clear.

Reverse transcription (RT): 1 μg RNA RT was taken to synthesize cDNA, RT reaction should follow the instruction manual of RT system reagent kit provided by Promega Company, USA for implementation. The total volume of RT reaction system was 20 μl. The chief component concentration of reaction system were: 25 mmol/L $MgCl_2$ 6 μl, 10×RT buffer 2 μl, 10 mmol/L deoxyribonucleoside triphosphate (dNTP) 2 μl, 2.5 μmol/L Oligo $(dT)_{15}$ primer 0.5 μl, 10 unit (U) RNAase inhibitor, 15 U avian myeloblastosis virus (AMV) RT. RT reaction was carried out in DTC 150 type PCR reactor of MJ Research Company, USA. The procedure was: 42℃ 15 min, 99℃ degeneration 15 min, 0—5℃ and incubation 5 min. The RT product was preserved at −70℃ for use.

Design and synthesis of primer: According to UCP_1 cDNA sequence, the primer was designed and synthesized for amplifying UCP_1 cDNA. The primer was synthesized by Shanghai Shenggong Bio-engineering Co. Ltd. The sequence of primer was 5′-GAG TTC GGT ACC CAC ATC AGG-3′; UCP_1 r5′-GCA TAG GAG CCC AGC ATA GG-3′. The amplifying length was 1062 bp.

CJIM 2003;9(3) : 204—209

In order to precisely detect expression level, β-actin cDNA primer was selected for internal reference. β-actin f5′-CGT AAA GAC CTC TAT GCC AA-3′; β-actin r5′-AGC CAT GCC AAA TGT GTC AT-3′; The amplification length was 473 bp.

PCR amplification reaction: It was carried out following the PCR amplifying system provided by Promega Company of USA and its procedure. The PCR reaction system: RT product 12 μl, upper and lower reach of primer each 2 μl, dNTP 2 μl, 10× PCR buffer 2 μl, 25 mmol/L $MgCl_2$ 6μl, Taq DNA polymerase 2.5 U, and the total reaction volume was 30 μl. The amplification was implemented according to following procedure: 95℃ 3 min, 55℃ 40s, 72℃ 1 min, 30 cycles, finally 72℃ prolonged for 7 min.

Analysis of products: Take 5 μl PCR reaction product for electrophoresis in 1.2% agarose aldehyde for 1 hr, the scanning quantitation by GDS-800 gel imaging system of Bio-Rad Company, USA and the grey of video electrophoretic band were applied to affirm the relative amount of amplifying substance. For the analysis of UCP_1 cDNA expression UCP_1 cDNA/β-actin cDNA ratio amplified under the same condition would show the difference between the changes of sample UCP_1 mRNA expression.

Statistical Analysis

SPSS software was used to analyze the experimental data, for the comparison between groups *t* test was adopted, and multivariate analysis was adopted for correlation analysis.

RESULTS

Effect of Acupuncture on Obese Index of Experimental Adipose Rats

See Table 1. From Table 1 we could see that the body weight and Lee's index of model rats were all obviously higher than the level of normal rats, and after the treatment of acupuncture, the body weight and Lee's index obviously reduced, the difference being significant statistically ($P<0.01$).

Table 1. Comparison between Body Weight, Body Length and Lee's Index ($\bar{x}\pm s$)

Group		Body weight (g)	Body length (cm)	Lee's index
Normal	BT	382.11±32.55	25.25±0.76	299.18±4.01
(6)	AT	398.66±28.15	24.46±0.71	300.89±4.25
Control	BT	516.17±29.46△	25.11±0.52	319.58±3.66△
(6)	AT	513.15±29.33△	25.21±0.53	317.58±3.75△
Acupun	BT	515.64±28.49△	25.06±0.51	319.99±4.53△
(6)	AT	442.36±26.45*△	25.49±0.39	298.92±4.12*▲

Notes: *$P<0.01$, compared with before treatment of the same group; △$P<0.01$, compared with normal group; ▲$P<0.01$, compared with control group; In ( ) is the number of samples, BT is before treatment, AT is after treatment

Comparison between Amount of Adipose of Rats' Different Locations by Acupuncture

Table 2 showed the adipose amount at the 3 locations of control group rats were all higher than that of normal rats, and that of acupuncture group rats were significantly lower than that of control, the difference being significant statistically ($P<0.01$).

Table 2. Comparison between Adipose Amount of Various Groups and Locations (g, $\bar{x}\pm s$)

Group	*n*	Pericardium adipose	Peri-renal adipose	Epididymus adipose
Normal	6	0.54±0.21	3.52±2.17	2.61±1.07
Control	6	0.82±0.14**	7.22±2.67**	5.11±1.88**
Acupun	6	0.58±0.09*△	4.33±0.75*△	3.31±0.81*△

Notes: *$P<0.05$, **$P<0.01$, compared with normal group; △$P<0.05$, compared with control group

RT-PCR Analysis of Various Experimental Group BAT UCP_1 Gene Expression Level

Table 3 showed that the rats' BAT UCP_1/β-actin ratio of control group was significantly reduced, and when compared with normal group, the difference was significant ($P<0.01$); acupuncture group rats' BAT UCP_1/β-actin ratio was obviously elevated, and when compared with control group, the difference was significant ($P<0.05$); compared with normal group, the difference was also significant ($P<0.05$). It denoted that the effect of acupuncture on obese organism

BAT UCP_1 gene expression was up-regulated.

Table 3. RT-PCR Analysis of Various Experimental Group BAT UCP_1 Gene Expression Level ($\bar{x}\pm s$)

Group	n	UCP_1 (grey value)	β-Actin (grey value)	UCP_1/β-actin
Normal	5	269.67±74.22	134.56±39.46	2.04±0.27
Control	6	230.96±38.22	226.16±80.97*	1.10±0.28**
Acupun	6	183.57±43.33	141.70±46.94△	1.47±0.30△

Notes: One sample was erroneously manipulated in RT-PCR test; * $P<0.05$, ** $P<0.01$ compared with normal group; △ $P<0.05$, compared with control group

Correlation Analysis of BAT UCP_1 Gene Expression Level and Adipose Index

The results showed that the rats' BAT UCP_1 gene expression level with body weight, Lee's index and amount of body fat were −0.706, −0.559, −0.618 respectively, all displaying negative correlation, indicating that the more obese the organism, the lower the BAT UCP_1 gene expression level.

DISCUSSION

Recent study showed that the pathogenesis of obesity is correlated with multiple factors such as hereditary gene, environmental factor, dietary structure, etc. Among them, the chief deciding factor is gene[6]. UCP is a specific expressed mitochondria intimal protein of BAT, with its molecular weight as 32KD[7]. The function of UCP is a transportation carrier of fatty acid ion which carries, from inside the mitochondria to outside mitochondria, the fatty acid ion that can not penetrate the mitochondria intima. The fatty acid ion outside the mitochondria would combine with H^+ and re-enter the mitochondria, and is again re-oxidized to fatty acid ion. Such repetition would eliminate different concentration gradients on both sides of mitochondria intima induced by oxidation, so that ADP cannot be phosphorylated to generate ATP. The final results of respiratory and oxidized phosphorylation uncoupling would bring about the transformation of energy production to heat production[8]. At present there have been discovered 3 kinds of UCP: UCP_1, UCP_2 and UCP_3. UCP_1 is the earliest discovered uncoupling protein, and in BAT expression in rodent animal, it is an important uncoupling protein of non-shivering heat production. UCP_2 not only exists in BAT, but existed widely in various tissues and cells, including white adipose tissue, lung, liver, spleen and inside macrophage. UCP_3 mainly exists in skeletal muscle, and also in BAT. Studies showed that UCP_2 and UCP_3 are uncoupling protein with the same function as UCP_1[9-11]. It is well known that UCP gene expression level plays the deciding role in its own content and BAT heat production, which is called the center of BAT heat production. Heat production is closely related with energy balance, metabolism, and body weight regulation. The important protein participating in metabolism under normal physiologic status, most likely, also participates in the target protein of patho-physiologic metabolic disturbance, and therefore it is inferred that UCPs possibly are related with obesity pathogenesis.

The results of the present study showed that obese rats displayed characteristics of overeating, overdrinking, and their heavy weight, Lee's index and body fat were obviously higher than normal. At the same time, the obese rats' BAT UCP_1 gene expression level showed lower than that of normal rats, which is to say, the obese rats' BAT UCP_1 transcription level was abnormally reduced. Correlation analysis revealed that the obese rats' BAT UCP_1 gene expression level and body weight, Lee's index and amount of body fat showed negative correlation. This result showed that BAT UCP gene expression was abnormally decreased, its heat production and energy consumption weakened, which is sure to cause obesity.

It is known that UCPs gene expression is regulated by multiple factors such as certain central nervous system nucleus group, sympathetic nerve, endocrine factors etc.

CJIM 2003;9(3) : 204—209

Noradrenaline, thyroxin, corticosteroid, leptin (LP) and insulin (INS), etc. could stimulate the fat of adipose cell to disintegrate and produce heat. This function mainly would be realized through β_3-adrenergic receptor (β_3-AR). NA and β_3-AR combined together could precisely regulate UCP_1 gene expression and transform pro-adipose cell into BAT cell. Owing to the increase of mitochondria and cell count, UCP_1 could increase. NA's promotion of UCP_1 gene expression also can elevate the synthesis of cAMP related to G coupling protein, the latter could activate the proteinkinase (PKA), which in turn acted on the target protein including corticosteroid (CS) sensitive lipase. When this enzyme was activated by phosphorylation, it could inhibit the storage of triglyceride, so as to disintegrate the fat and increase the free fatty acid; the target protein of PKA also includes cAMP reaction element protein, and it might promote the transcription of UCPs gene to raise the heat production and energy consumption[12—15].

Our previous research work revealed that the balance index of vegetative nerve of obese patients is abnormally reduced, with their sympathetic mediator NA, adrenaline and dopamine (DA) concentration extremely low, and their parasympathetic nerve function index [salivary amylase (S-Am) and blood acetylcholinesterase (AchE)] extraordinarily elevated. At the same time, obese patients plasma cAMP, thyroxin, corticosteroid, noradrenaline, adrenaline, and adrenocorticotropic hormone content were all very low; and serum LP and INS content abnormally raised. Animal experiments indicated that the obese rats' BAT cell volume increased, full, fatty drops large, mitochondria in the cytoplasm obviously reduced in size, mitochondria matrix density increased, the crista unable to be clearly displayed, and interstitial capillary decreased, showing that BAT heat production was abnormally decreased.

Another experiment displayed that the serum LP and INS content of obese rats abnormally increased, which denoted that there existed leptinemia and hyperinsulinemia as well as leptin resistance (LR) and insulin resistance (IR) in obese organisms. At the same time the hypothalamic LP and INS content of obese rats were abnormally low, denoting that the LP blood brain transportation abnormality was likely to be one of the important factors of LR. Experiment showed that the number of RBC's insulin receptor (INSR) of high affinity and low volume and that of low affinity and high volume in obese rats was all decreased, but the dissociation constant in INSR of high or low affinity was abnormally elevated, which denoted that obese organisms' INSR abnormality and INS blood brain transportation abnormality were possibly one of the important factors of IR[1,16]. The above-mentioned data showed that the abnormal lowering of obese organisms' BAT UCPs gene expression level was possibly related with above-mentioned regulating factors.

The present study showed that after acupuncture the obese rats' body weight, Lee's index and body fat all markedly reduced, denoting that acupuncture does have beneficial fat reducing effect. At the same time, the BAT, UCP_1 gene expression level markedly increased, i. e., acupuncture could up-regulate BAT UCP mRNA level, and elevate the effect of UCP_1, and therefore the energy consumption and heat production increased, finally obtaining the fat reduction efficacy.

In summary, abnormal decrease of biologic organisms' BAT UCP_1 gene expression level is possibly the important cause of obesity. The promotion of obese organisms' BAT UCP_1 gene expression by acupuncture is possibly one of the cellular biological mechanisms in weight-reducing by acupuncture.

REFERENCES

1. LIU ZC, SUN FM. Clinical and experimental study on acupuncture in treating simple obesity. China J Integr Med 2001;1(4) : 16—18.
2. LIU ZC, SUN FM, HAN Y, et al. Experimental study

of acupuncture in treating simple obesity. Acupuncture Res 1998;23(1) : 69—75.
3. Nisoli E, Tonello C, Curruba MO. Nerve growth factor, β-adrenoreceptor and uncoupling protein expression in rat brown fat during postnatal development. Neurosci Lett 1998;246 : 5—8.
4. Teruel T, Valverde AM, Navarro P, et al. Inhibition of PI 3-kinase and ras blocks IGF-1 and insulin-induced uncoupling protein 1 gene expression in brown adipocytes. J Cell Physiol 1998; 176 : 99—109.
5. Krook A, Oigby J, O'Rahilly S, et al. Uncoupling protein 3 is reduced in skeletal muscle of NIDDM patients. Diabetes 1998;47 : 1528—1531.
6. SUN ZJ, HUANG ZY. Progress on obesity study. Physiol Sci Prog 2001;32(1) : 39—44.
7. Klaus S, Casteilla L, Bouilaud F, et al. The uncoupling protein UCP: a membranous mitochondrial ion carrier exclusively expressed in brown adipose tissue. Int J Biochem 1991;23 : 781—801.
8. Lowell BB, Flier JS. Brown adipose tissue, β_3-adrenergic receptors, and obesity. Annu Rev Med 1997;48 : 307—316.
9. Boss O, Samec S, Paolini-Giacobino AN, et al. Uncoupling protein-3: a new member of the mitochondrial carrier family with tissue specific expression. FEBS Lett 1977;408 : 39—42.
10. Shimabukaro M, Zhou YT, Lee Y. Induction of uncoupling protein 2 mRNA by troglitazone in the pancreatic islets of zuker diabetic fatty rats. Biochem Biophys Res Commun 1997;237 : 359—361.
11. Vidal Puig A, Solanes G, Gruji CD, et al. UCP_3: An uncoupling protein homologue expressed preferentially and abundently in skeletal muscle and brown adipose tissue. Biochem Biophys Res Commun 1997;235 : 79—82.
12. Larose M, Cassard Doulcier AM, Fleury C, et al. Essential cis-acting elements in rat uncoupling protein gene and in an enhancer containing a complex retinoic acid response domain. J Biol Chem 1996;271 : 31533—31542.
13. Gong DW, He Y, Karas M, et al. Uncoupling protein-3 is a mediator of thermogenesis regulated by thyroid hormone, β_3-adrenergic agonistic, and leptin. J Biol Chem 1997;272 : 24129—24132.
14. Hernandez A, Obregon MJ. Triiodothyronine amplifies the adrenergic stimulation of uncoupling protein expression in rat brown adipocyte. Am J Endocrinol Metab 2000;278(5) : E769—777.
15. Klein J, Fasshauer M, Benito M, et al. Insulin and the beta 3-adrenoreceptor differentially regulated uncoupling protein-1 expression. Mol Endocrinol 2000;14(6) : 764—773.
16. LIU ZC, SUN FM, ZHAO DH, et al. Effect of acupuncture on simple obesity leptin and insulin resistance. Acad J TCM 2003; 23(1) : 15—18.

(Received June 5, 2003)

Preliminary Study on Clinical Efficacy of Integrative Chinese and Western Medicine in Treating Severe Acute Respiratory Syndrome

WANG Rong-bing (王融冰), LIU Jun-min (刘军民), JIANG Yu-yong (江宇泳),
WU Yun-zhong (吴云忠), WANG Xiao-jing (王晓静),
CHI Pin-pin (池频频), and SUN Feng-xia (孙凤霞)
Ditan Hospital, Beijing (100011)

Objective: To explore the treatment of severe acute respiratory syndrome (SARS) by integrative Chinese and western medicine (ICWM), for elevating the clinical therapeutic level of SARS. **Methods**: By adopting the randomized controlled method, the clinical efficacy of treatment of patients with SARS, hospitalized from April 10, 2003 to May 20, 2003 in Ditan Hospital, Beijing was evaluated. All the patients were treated by western medicine according to the therapeutic program recommended by the SARS Coordinating Group, the Chinese decoction for clearing heat and removing dampness was given additionally to the 35 patients in the treated group, but it was not given to the 30 patients in the control group. The therapeutic effect in the two groups was analyzed and compared. **Results**: The efficacy of treatment in the treated group was superior to that in the control group, in apects of promoting the absorption of pulmonarty inflammation and alleviating the inhibitory state of lymphocytes. **Conclusion**: Efficacy of SARS treatment with ICWM is better than that with western medicine alone.

Original article on CJIM (Chin) 2003;23(7) : 492