COMPARISON EFFECT OF CV 12, ST 36 AND ST 40 EA ON SHORT TERM ENERGY BALANCE REGULATION IN HIGH FAT DIET RAT

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ABSTRAK

Penelitian ini bertujuan untuk membandingkan efek elektroakupunktur (EA) pada titik CV 12, ST 36 dan ST 40 terhadap pencegahan peningkatan berat badan melalui mekanisme pengaturan jangka pendek pada keseimbangan energi. Desain penelitian ini adalah penelitian eksperimental. Subjek penelitian adalah tikus (Rattus norvegicus strain wistar) dibagi menjadi 5 kelompok: kelompok kontrol negatif (tanpa perlakuan, n=5), kontrol positif (sham EA/punggung, n=5), EA CV 12 (n=6), EA ST 36 (n=6) dan EA ST 40 (n=7). Subjek diberikan diet tinggi lemak selama 2 minggu bersamaan dengan pemberian EA dilakukan 1 kali perhari, 5x perminggu selama 2 minggu dengan frekuensi 2 Hz selama 10 menit, memakai gelombang kontinyu. Pengukuran antropometri yaitu berat badan, Indeks Massa Tubuh (IMT), lingkar anggota gerak depan dan belakang dilakukan selama penelitian. Pemeriksaan kadar glukosa darah kolesterol dan trigliserida, LDL dan HDL dilakukan di akhir penelitian yang menggambarkan pengaturan jangka pendek pada homeostasis energi. Pada kelompok EA CV 12, ST36 dan ST40 terjadi penurunan berat badan secara bermakna dibandingkan dengan kontrol, penurunan kelompok ST 40 lebih nyata dibandingkan dengan kelompok ST36 dan CV12, begitu pula dengan IMT. Paparan EA tidak mempengaruhi kadar glukosa darah, namun terjadi modulasi pada profil lipid darah. Pada kelompok ST 40 terjadi penurunan yang bermakna pada kolesterol, LDL dan trigliserida. Sebagai kesimpulan, EA pada titik ST 40 memiliki potensi yang lebih kuat untuk mencegah peningkatan berat badan IMT pada tikus yang diberikan diet tinggi lemak dibandingkan pada titik CV 12 dan ST 36, begitu pula dengan penurunan kadar LDL dan trigliserida. Titik ST 40 memiliki peranan pada pengaturan homeostasis energi jangka pendek dan pencegahan dislipidemia. ***(FMI 2016;52:174-179)***

Kata kunci: *elektroakupunktur, homeostasis, kadar glukosa, profil lipid, Indeks Masa Tubuh*

ABSTRACT

The aim of this study was to determine the comparative effects of EA (EA) on the CV12, ST36 and ST40 to weight gain prevention over the short-term regulation of energy balance. The study was conducted with a completely randomized design. Rats were divided into five groups: negative control group (no treatment, n=5), positive control (sham EA/back, n=5), EA CV 12 (n=6), EA ST 36 (n=6) and EA ST 40 (n=7). Rats were exposed to high-fat diet for two weeks and EA was simultaneously performed once daily, five days a week for two weeks with 2 Hz, for 10 minutes with continuous wave. Body weight, BMI, front limb circumference and rear were measured during study. Levels of blood glucose, cholesterol, triglycerides, LDL and HDL were measured at the end of the study; which reflects the short-term regulation of energy homeostasis. For weight loss, EA CV12, ST36 and ST40 group have lost weight significantly compared to the negative and positive control group. The ST40 group has a significant decrease than ST36 and CV12. The most significant decrease in BMI found in the ST40 group. EA did not affect blood glucose levels, but modulated blood lipid profile. In ST 40 group there was a significant decrease in cholesterol, LDL and triglycerides. EA at point ST 40 is potential in preventing increased body weight and BMI in rats exposed to high-fat diet compared to the CV 12 and ST 36. ST 40 is a point with a potential of lowering LDL and triglycerides serum so that it can play a role in the short term regulation of energy homeostasis but also in the prevention of dyslipidemia. ***(FMI 2016;52:174-179)***

Keywords: *electroacupuncture, energy homeostasis, glucose levels, lipid profile, BMI*

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INTRODUCTION

Obesity has become an epidemic condition throughout our world. Its prevalence has increased rapidly and affected the young children as well as the elderly (Mori et al 2008). Obesity is a condition caused by the disruption of energy regulation and energy homeostasis. Regulation of the energy homeostasis in the human body is achieved through short term and long term mechanism. The short term mechanism includes the increasing amount of glucose, amino acid, fatty acid, and triglyceride level in blood along with the mechanical stretch in gastrointestinal tract. Those certain conditions would reduce the human appetite to consume food (Havel 2001). The long term mechanism involves the increasing level of insulin and leptin in the

blood. The energy homeostasis disturbance is triggered by the increasing calorie intake which is higher than the energy expenditure that will result in positive energy balance. Higher calorie rate in high fat diet is the cause of the weight gain in human. Intervention in the regulation of short term mechanism is a breakthrough that is predicted as an effective way in reducing the obesity prevalence in the world.

Drug therapy is one of the therapy interventions found in the community that regulates short term regulation of energy homeostasis. There are two main drugs that work centrally via hypothalamus, sibutramine and amphetamine. Both drugs decrease the calories intake as they modify the center of hunger-satiety in the feeding center. Unfortunately, these drugs have some side effects including tachycardia, hypertension, fecal incontinence or valvopathy in the heart (Yepuru et al 2010) that force the researchers to find a new cure for obesity. Point CV12, ST 36 and ST 40 are the important points that commonly used in obesity management and some other diseases, but somehow the potency and the comparison of these points in the short term regulation of energy homeostasis still remain a mystery.

Electroacupuncture (EA) is combination between acupuncture and electric stimulation as the substitute of manual manipulation in the needle (Ouyang et al 2002, Ma 2004). EAS is mostly used in the need of research because it can be controlled and quantified effortlessly so that it can be repeated easily. In the traditional medication terminology, acupuncture is believed to work by influencing the level of neurotransmitter in the central nervous system through the peripheral nerves in acupoint (acupuncture point). The stimulated nerves will conduct the signal to the central system which is the medulla spinalis, hypophysis, and midbrain. Central nervous system activation will release the neurochemical substance including endorphin, monoamin, and cortisol (Stux & Pomeranz 1998). In brief, the effect of EA in CV12, ST36 and ST40 point to the short term regulation of energy regulation is interesting to explore further since there is insufficient data to explain the effects.

MATERIALS AND METHODS

Experimental Animal

This research used the male rat (Rattus novergicus galur Wistar) with the age range of 14-16 weeks weighing approximately 150-200 grams obtained from UPPH Universitas Gadjah Mada Yogyakarta. Each of the experimental animals was placed in a cage with 30x40 cm in size, free access to food and water (ad libitum), 12 hours light exposure, and 12 hours dark exposure. The treatment to the experimental animals had been approved by the Ethic Commission on Research Faculty of Veterinary Medicine Universitas Airlangga, Surabaya.

Diet

Diet given in this research was a high-fat diet that had been conducted by Unit Pengujian Veteriner dan Analisis Pakan (Veterinary and Food Analysis Testing Unit) Faculty of Veterinary Medicine Universitas Airlangga with the certificate test number 125/PT.03/SERTF/06/2013. Food composition given to the animals contained 11.052% of ashes, 17.224% of raw protein, 25.29% of raw fat, and 4.922% of raw fiber with total energy of 3,828.13 kkal/kg foods.

Intervention and Experiment Procedure

The 35 rats were acclimatized for 2 weeks and then divided into 5 groups with 7 rats in each group. The intervention was done in two weeks and the groups only consisted of negative control (n=6), positive control (n=6), acupuncture on CV 12 point (n=5), acupuncture on ST 36 (n=5), and acupuncture on ST 40 (n=7). In the negative control group, the rats were not given the EA intervention. Meanwhile in the positive group control, EA was performed in the back of the rats. In CV 12 group, the EA was given at the median point between xyphoid process and umbilicus. In ST 36 group, EA was given at the proximal one-fifth of the craniolateral surface of the leg distal to the head of tibia in a depression between muscles of the cranial tibia and long digital extensor, 5 mm lateral and inferior to the tibial tubercle in rat. While in ST 40 group, EA was given at lateral central line of tibia. EA was given using a stimulator acupuncture tool made by Faculty of Science and Technology Universitas Airlangga, at the frequency of 2 Hz with continuous wave. EA was performed once a day for five days per week at 13.00-15.00 during 2 weeks of experiment. Before EA was conducted, the rat was not administered with any anaesthetic drugs, fixated on the board, and finally the acupuncture needle was inserted and EA was performed with the duration of 10 minutes. To avoid the acute effects of EA, blood samples were taken 24 hours after the last EA.

Food Consumption Measurement

In the adaptation cage period, average amount of food consumed by the rat was 20 grams/portion sizes/rat/day. In the experimental stage, the animals were given free access to food and drink (ad libitum) daily at 01:00 P.M with the amount of 40 grams and then the leftover was

measured in the following days to determine the exact amount of food per rat per day.

Blood Sampling

After two weeks of intervention, the rats were eventually terminated and their blood samples were obtained from the heart. The blood samples used to illustrate the glucose, HDL, LDL, and triglyceride level.

Anthropometry Measurement

The antropometry measurement included body weight, height, abdomen circumference, front and back leg circumference. All measurements were conducted once a week except for body weight which was measured every day during the two-week intervention. Body height was measured from the mouth until the anal. The abdomen circumference measurement was performed by measuring the central line of the abdomen. The front leg circumference measurement was performed through the left central line of femur. The back leg circumference measurement was performed through the left leg in the central line of the brachius.

Fat Mass Measurement

The fat mass was obtained from the visceral fat of the rat from three different places, namely, retroperitoneal fat, mesentrical fat, and perigonadal fat. Retroperitoneal fat was obtained from the fat attached to the posterior wall of the abdomen, mesenterical fat from the fat covering the intestinal mesenterium, while perigonadal fat from the fat located near the testis.

Statistical Analysis

Data was analyzed using ANOVA test from SPSS 17 version.

RESULTS

General Characteristic

Initial weight and the weight in the end of experimental intervention done in each of 5 groups can be seen in table 1. The measurement of body weight before the intervention was homogeneous statistically. After the exposure of high-fat diet during two weeks and intervention according to each group, there is a body weight gain in the negative control group negative and positive control group, while in the other three groups, the reduction of body weight was eventually shown.

Table 1. Rat Body Weight in Pre- and Post Intervention

Group	Initial Body Weight (gram)	End Body Weight (gram)
Negative Control (n=6)	193 (18	199 (18
Positive Control (n=6)	177 (18	180 (18
CV12 (n=5)	217(17	156(17
ST36 (n=5)	220(25	206(25
ST40 (n=7)	232(25	214(25

The anthropometry results from the five groups can be seen in table 2. From the abdomen circumference, all groups showed a reduction but without a significant difference. The front leg circumference measurement on the other hand showed an increasing rate but without a significant difference. In the measurement of the back leg, the increasing and decreasing rate was found without a significant difference in all five groups.

Prevention Effect of Body Weight Gain Caused by EA in Three Points (CV 12, ST 36, ST 40)

Figure 1A describes the EA on CV 12, ST 36, and ST 40 point has the potency in preventing the body weight gain caused by the exposure of high-fat diet compared to the negative control or the positive control. From the three groups, EA in ST 40 pint has the biggest potency in the prevention of body weight gain because of the high amount of calorie.

Table 2. Anthropometry in pre- and post- intervention

Group	Abdomen Circumference (cm)		Front Leg Circumference (cm)		Back Leg Circumference (cm)	
	Pre-	Post-	Pre-	Post-	Pre-	Post-
Negative Control (n=6)	14.33±1.72	11.72±1.31	2.75±0.42	3.83±0.52	4.50±0.32	4.88±0.38
Positive Control (n=6)	13.08±1.24	11.75±0.99	2.92±0.38	3.80±0.24	4.80±0.26	4.70±0.35
CV 12 (n=5)	12.80±1.60	12.38±1.38	3.70±0.57	3.70±0.27	5.12±0.67	4.70±0.27
ST 36 (n=5)	13.00±0.71	12.60±0.65	4.30±0.45	4.70±0.45	5.50±0.36	4.70±0.27
ST 40 (n=7)	14.29±1.63	12.76±0.48	3.43±0.45	3.64±0.42	4.93±0.38	4.84±0.24

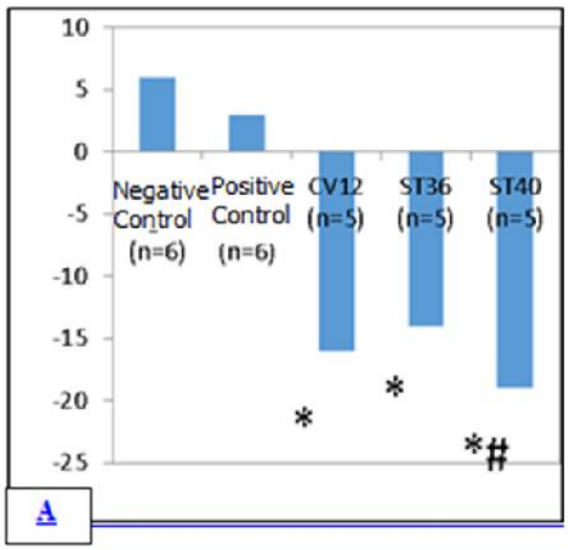

Fig. 1.A. Body weight difference (End Body Weight - Initial Body Weight) on each rat in every group. B. BMI difference (BMIpost-BMIpre) on each rat in every group. Notes: *significant difference was noted ($p<0.005$) between the control group and the group with the intervention. #significant difference ($p<0.005$) between group with the intervention and all other group monitored.

The result from measurement of visceral fat showed the fat mass in the negative control group was 5.55 (0.39 gram, positive control group was 6 (1.23 gram, EA CV 12 was 6.60(1.97 gram, ST 36 was 5.98 (1.33 gram and on ST 40 was 6.09 (0.72. There was not any significant difference according to the statistical analysis done in all five groups based on the visceral fat mass measurement.

EA Effect on the Glucose and Lipid Profile

Glucose and Lipid profile was measured from the blood obtained from the rat heart. Before taking the blood, the rats were put in the fasting condition and the intervention was stopped to avoid the acute effect of acupuncture. The result of the glucose and lipid profile measurement can be seen in Table 3.

The level of glucose, cholesterol, HDL, and LDL was found to be relatively the same and there is not any big gap from all five groups. On the other hand, triglyceride level in EA on ST 40 is shown to be the lowest in number and proven to have a significant difference from other four groups (31.57±8.63 with $p<0.005$).

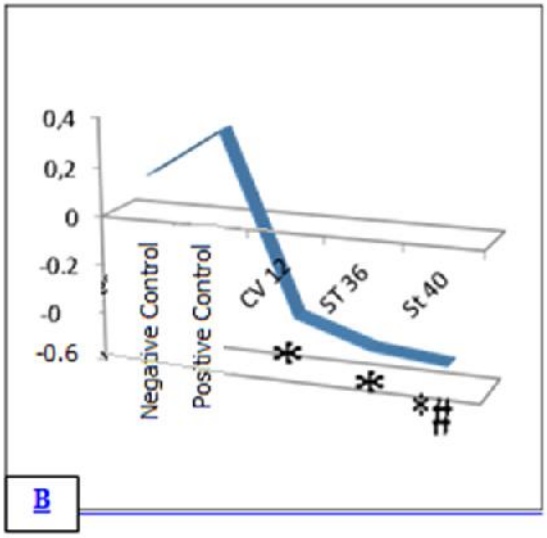

Fig. 1B describes the reduction of Body Mass Index (BMI) in all groups that were given the EA compared to the control group. The most significant BMI reduction was found in the EA on ST 40 point compared to the EA done in CV 12 or ST 36.

DISCUSSION

Rattus male was chosen to eliminate the involvement of hormonal estrogen disturbance that has a role in the reproduction cycle in female rattus. The range of 14-16 weeks age of the rat was chosen because this range is the young adult age for rat as the increasing prevalence of obesity in human is in the adolescence until adult age. High-fat diet was used to initiate more calorie intake that triggered energy balance to the positive direction to make us easier see the EA role in the prevention of weight gain because of the high-fat diet used in the research.

Acupuncture is a complex therapy that had been used in China since 2000 years ago. According to the Chinese Traditional Medicine methods, the puncture through the acupuncture sites has a specific effect through the local effect or long distance effect through the acupuncture meridian system. Chinese Traditional concept has a paradigm in which a disease is an imbalance of qi in the body. Based on the theory, to actually treat a disease, there is a method needed to balance the energy inside the body. The energy balance can be achieved by stimulating certain acupuncture sites according to the certain disrupted qi system. The EA is used to set the standard measurement by setting the frequency and the type of wave used in order to make it easier when repetition is needed.

Table 3. Glucose and Lipid Profile Post-Intervention

Group	Glucose (mg/dL)	Cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	TG (mg/dL)
Negative Control	47.60±3.90	61.16±36.12	22.83±12.28	12.50±8.71	30.38±18.24
Positive Control	53.67±9.93	72.67±14.07	31.00±6.26	12.83±3.43	68.83±37.97
CV 12	49.50±7.72	50.25±14.24	21.00±5.48	10.50±3.70	45.75±12.33
ST 36	66.80±24.84	77.60±6.15	24.00±1.41	18.24±4.27	35.20±8.18
ST 40	51.71±7.60	48.28±9.70	22.00±5.29	9.43±2.76	31.57±8.63*

*P<0.005

Body weight is the easiest macro indicator used to evaluate the balance of energy inside our body. The weight gain is found both in the negative control and positive control group after 2 weeks of intervention. This finding supports the previous research which has successfully proven that high-calorie diet (with 31% of fat) would increase the rate of weight gain, increasing amount of adipose tissue mass that reached 121%, as well as associated with higher level of leptin (Irani et al 2007). Other finding on the other 3 groups that were given the EA on CV12, ST 35, and ST 40 point, showed reduction of the weight that previously had proven the capacity of this EA to prevent the development of obesity caused by the high-fat diet. EA on ST40 point was proven to have the greatest potency in preventing the weight gain. Body mass index (BMI) is considered as a more objective indicator in assessing the nutrition status and energy balance than the weight that was also used. Along with the reduction of weight, there was also a significant reduction of BMI measured in the groups given the EA on ST40 point.

Long term high-calorie diet also causes central resistance to anorexia hormone including leptin, in the lean rat or in the obese rat (Tulipano et al 2004). While short term high fat diet decreases the leptin level in the circulated blood and results in the weight gain (Ainslie et al 2000). On the other side, high-fat diet also causes the increasing intake of fat to the body as well as increases the adipose tissue mass. The increasing of adipose tissue mass will also proportionally increase the level of IL6 and TNF alpha (Senn et al 2003). In short, there is an urgency to investigate this finding furthermore on how the effect of acupuncture to the pro-inflammatory cytokine that is also believed as one of the obesity triggers.

Experiment in human has showed that high-fat diet will increase the total calorie intake. In a healthy woman subject, who was manipulated by the hidden high-fat diet, there is increasing daily amount of calorie intake as big as 15.4% during the high-fat diet (45-50% of energy from fat) while compared to the medium-fat diet(30-35% of energy from fat) in two weeks. The increasing of calorie intake also causes the weight gain in the monitored subject (Lacey et al 2003). Extremity circumference shows the growth of subcutaneous fat and muscle that is not influenced by the condition of body fluid compared with the body weight. The growth of the arm circumference is slower and not a precise indicator in assessing the alteration of nutrition status. This finding also found in the five groups since there is not any significant changes in the front and back circumferences.

Based on the experiment results, after high fat-diet intervention given during two weeks, the glucose level in all five groups did not show any significant differences. This finding appeared probably because two weeks are still not enough to change the homeostasis of glucose level in the blood. The lipid profile, both in the negative control group and positive control group, was actually higher than the other 3 groups given the EA intervention. The result is matched as the previous experiment conducted which also shows that the rats given the high-fat and calorie diet tend to have the increasing level of triglyceride, total cholesterol, low-density lipoprotein cholesterol (LDL-C), and lipid hydroperoxide (LH) higher than the rats given the normal diet. The level of high-density lipoprotein cholesterol (HDL) will also be reduced in the rats with high-fat diet. An interesting finding is found in the EA groups on ST 40 point because there is a reduction in the LDL and triglyceride level, and even for triglyceride, proven to have a significant reduction level, compared to the point ST 36 or CV 12. This interesting result supports the previous experiment conducted by Zhang et al (2012) which concluded that EA on ST 40 pint has a potency in regulating the blood lipid profile and the gene expression that is related in the regulation of lipid in blood. Thus, EA on ST 40 point has an important role not only in preventing the body weight gain caused by the high-fat diet intake but also in preventing the condition of dyslipidemia

CONCLUSION

EA on ST 40 point has a stronger potency in preventing the body weight gain and BMI elevation in the rat exposed by the high-fat diet compared to the EA in the CV 12 and ST 36 point. The three sites or points of acupuncture do not seem to affect the glucose level but

affect the regulation of lipid profile in the blood. ST 40 is the point that has a potency in decreasing the LDL and triglyceridelevel in the blood. In short, EA on ST 40 point acts as the regulator of short term energy homeostasis as well as regulator in the prevention of dyslipidemia.

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