

Identification and Quantification of Leptin in Serum by using a Disposable electrochemical Immunosensor

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ABSTRACT

Obesity has already become one of the most common medical condition in the world. Leptin has been reported that it has essential role in obesity. The identification and quantification procedure of hormone leptin using disposable electrochemical immunosensor is described in this paper. Serum has been prepared from diet-induced obesity (DIO) model (C57BL/6J, male). DIO model is induced by high fat feed for over 2 months with above 30% of body weight. We used immune- assay method involving in the target analyte (leptin) and polyclonal anti-leptin antibody which has been immobilized on disposable electrodes by chemical linkers, 4-mercaptobenzoic acid (4-MBA), N-hydroxysuccinimide (NHS) and N-ethyl-N'-(3-dimethyl aminopropyl) carbodiimide (EDC). A linear calibration plot has also suggested with a specific concentration range between 100pg/mL and 100ug/mL by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The usefulness of the immune sensor was evaluated by comparing with enzyme-linked immunosorbent assay (ELISA). This attractive method with the simplicity and miniaturization of the required instrumentation would be promising alternative in the development of portable devices for clinical analysis.

1. INTRODUCTION

Leptin is a peptide hormone made mostly by white adipose cells that participate in regulating energy balance, appetite, metabolism and sexual maturation. (1,2) In mammals, it is reported that leptin level in blood would control food intake and energy expenditure at the hypothalamic level. Ultimately, leptin involve with negative feedback process of body weighting. Many studied proved that leptin inhibits appetite and it leads to decrease of food intake. Normal level of leptin of mouse (C57BL/6J) reported 2-4 ng/ml in male and 3-5 ng/ml depending upon their age. Comparably its level has been observed much higher in obese models. In this study, we prepared diet-induced obesity (DIO) mouse model

as obesity model. Also we arranged them with 130% of average body weight difference compared with control group by feeding specially-designed feed. By using disposable electro- chemical immunosensor which is commercially available, we could evaluate leptin level from serum from two different models.

2. MATERIALS

2.1 Animals and Experimental diet process

Male C57BL/6 mice, 4 weeks old, were purchased from Hyo-Chang Science. (Daegu, Korea). As included weight-matched process, mice were caged in animal-care facility. (4 mice/cage). They were fed with mouse standard diet feed from Harlan. for a week. Mice were divided into 2 groups; standard feed group (control) and high-fat feed group at 6 weeks of age. Mice were fed with each types of feed for 14 weeks. (Fig. 1) Also, in this process, NIH guidelines were strictly followed and all procedures involved with experiments of animal were approved by the Committee of the Keimyung University and Institutional Laboratory Animal Care.

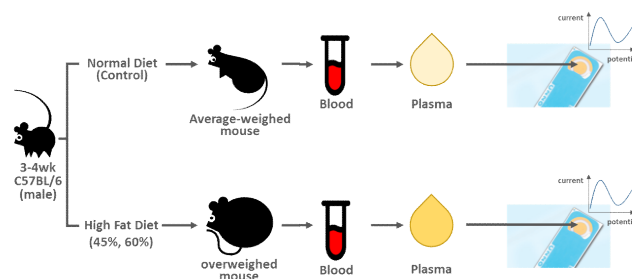


Figure 1. Experimental scheme of this study with electrochemical measurement method

2.2 Blood serum collection

Blood was collect from anesthetized mice by facial vein bleeding method with EDTA-treated tubes and serum was obtained by centrifugation at 15000 rpm for 15 minutes at 4°C. Serum were separated for measurement and stored at -20°C. To terminate of this study, mice were killed by cervical dislocation.

2.3 Apparatus and electrodes

All the electrochemical measurements were performed on a CHI700 electrochemical workstation (CH Instruments, China). Screen-printed carbon electrodes consisting of a carbon working electrode flat-coated gold with 4 mm in diameter, a silver pseudo-reference electrode, and a carbon counter electrode were purchased from DropSens. All the electrochemical measurements were performed at room temperature.

2.4 Reagents and solutions

Mouse monoclonal anti-leptin antibody (L3160, Sigma), Human Leptin (L4146, Sigma) were used as immuno-reagents. Bovine serum albumin (BSA, Fraction V), potassium ferrocyanide, EDC, NHS and 4-MBA were purchased from Sigma-Aldrich. BSA and potassium ferrocyanide was diluted with 0.01M PBS solution and prepared by 1% BSA solution and 1mM potassium ferrocyanide solution.

3. RESULTS

3.1 Body weight and its ratio with control group and high-fat group represent DIO mouse model properly

Male C57BL/6J mice (n=24) were maintained for 13 weeks with two different condition; control group with standard diet feed and high-fat group with specially-designed feed which is consisted with 60% of fat. DIO mouse model have its effective value when they are 30% heavier than control group. In this study, our high-fat group model has its value to be considered with DIO model. (Fig. 2,3)

3.2 Identification of calibration plot of leptin

Before leptin level has been calculated from blood serum, we established calibration plot with CV (cyclic voltammetry) method which is one of the measurement method of electrochemistry field. By using leptin antigen solution, we set concentration of solution from 7 ng/mL, 10 ng/mL, 25 ng/mL, 50 ng/mL, 75ng/mL, 100ng/mL. In CV graph, currents were getting lower depending upon incensement of concentration of leptin solution. (Fig. 4)

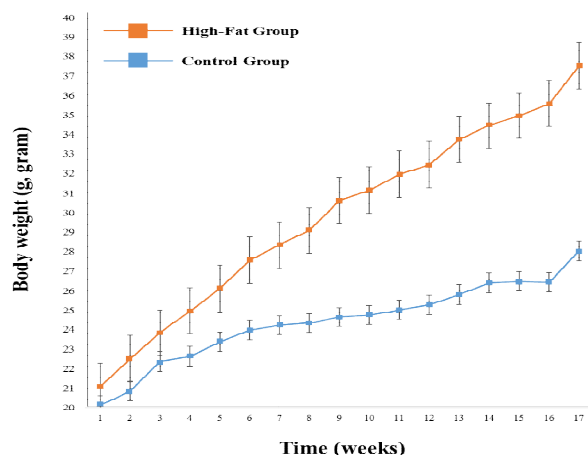


Figure 2. Body weight comparison between control group and high-fat group

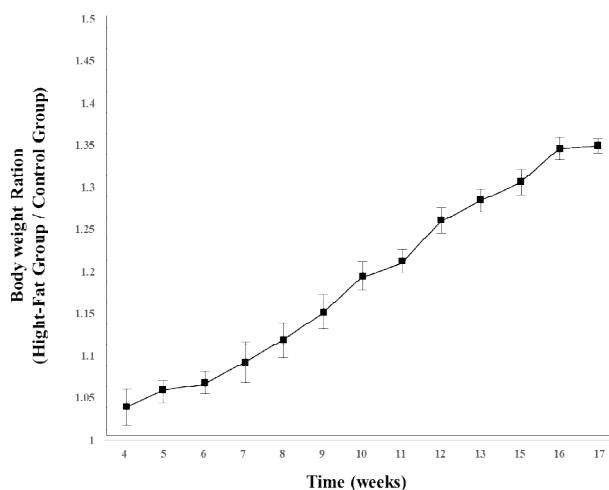


Figure 3. Body weight ratio between control group and high-fat group

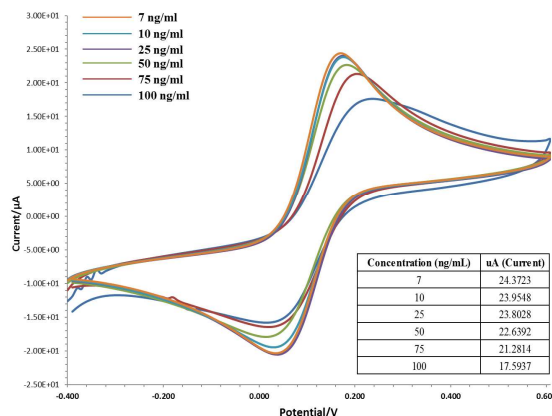


Figure 4. Calibration plot established for leptin solution by CV method by disposable immunosensor. (7 – 100 ng/mL)

4. DISCUSSION

Immunosensor was evaluated with two different mouse serums; control group and high-fat group. There were several unidentified problems. It was extremely unstable to calculate leptin level from blood serum from both groups. We identified two major problems. (1) There are multiple and various numbers of molecules including leptin in blood. Centrifugation step would help and sort most of molecules except leptin but it did not work well. To solve this problem, it is needed to apply much faster speed and spinning time. (2) New technology would be essential to arrive to more successful results. For example, magnetic beads or specially-designed metal molecules could be applied for more accurate and acute data and those lead to more extensive experimental designs.

5. CONCLUSION

The immunosensors could be used as a brilliant analytical tool with high sensitivity, reproducibility of measurement and relatively low detection limit compared with different immunoassays. They would possess a high analytical versatility because it is useful and stable to quantify leptin in a variety of real samples such as human serum. Therefore, the immunosensor could be a powerful analytical tool to meet in relevant problems related with obesity and obesity-related diseases.

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