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Original article

Trimmed off adipocytes: a source of newly secreted adiponectin

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Summary. Given the beneficial roles of adiponectin on body metabolism and its profound protective effects against metabolic disease, a better understanding of the adiponectin secretion is very important. The objective of this study was to isolate, detect and quantify total adiponectin in trimmed off abdominal adipose tissues from halal meat sources namely chicken, beef and lamb. Abdominal adipose tissues were isolated from the aforementioned sources and delipidation of the tissues were performed by chloroform/methanol extractions. Afterwards, the protein concentration was determined by using Protein Assay Bicinchoninate Kit method. This was followed by quantification of the adiponectin using ELISA kit assay. The experiment was conducted in triplicates and the results are presented as means ± SD. The data was statistically analyzed using SPSS (Version 21.0). One-way analysis of variance (ANOVA) was used and the means were considered statistically different at 95% confidence interval. Results indicate that the extraction of 10 gram subcutaneous adipose tissues from chicken, beef and lamb yielded 0.10 gram, 0.15 gram and 0.15 gram respectively of protein amount which was 1 to 1.5 % from total tissue mass. The protein concentration in abdominal adipose tissue from chicken, beef and lamb were 1.25 ± 0.05, 1.75 ± 0.05 & 2.53 ± 0.07 mg/ml, respectively. The isolated adiponectin concentration in chicken, beef and lamb were 158 ng/ml± 0.05, 24 ± 0.05µg/ml and 37 ± 0.08 ng/ml, respectively. Adiponectin concentration in beef abdominal adipose tissue was significantly ($p<0.05$) higher compared to chicken and lamb. The present study suggests that beef protein have highest amount of adiponectin followed by chicken and lamb. The observed adiponectin proteins in the wasted adipose tissues in meat sources would be promising target for future novel therapeutics in insulin resistance and other metabolic diseases.

Key words: Adipose tissue, protein, adiponectin

Introduction

Adipose tissues in meat are normally trimmed off from meat sources with a view that it is higher in fat contents and carry health hazards as well (1) and

(2). Therefore, lean meat is always preferred for hu-man consumption. Thus, the trimmed off fat is either discarded or used in other formulation. Adipose tis-sue is not just an energy storage depot but now it is regarded to be an active organ in energy homeostasis and physiological functions. It is known to secrete a variety of bioactive proteins known as adipokines

(3). Currently, the secretory functions and adipokines secretion of adipocytes are under intense investigation of researcher involved in study of various health conditions including non-communicable diseases (NCDs). Among these adipokines, adiponectin is receiving greater interest involved in diabetes, obesity or dyslipidaemia. Adiponectin modulate the function as an insulin sensitizing agent in the body thus act like hormones (4). It is well known that individuals that are obese or suffer from metabolic diseases showed a characteristic of imbalance adiponectin levels. This alteration may lead to changes in insulin sensitivity and other biochemical profile which make an individual more prone to metabolic disorders (5).

Given the beneficial roles of adiponectin on body metabolism and its profound protective effects against metabolic disease, a better understanding of the adiponectin secretion is very important (6). The detailed analysis of these adiponectin proteins from wasted adipose tissues in meat sources may provide crucial information which in turn may lead to the development of better methods or production of useful protein for the effective treatment of obesity, type 2 diabetes and its related metabolic diseases. This fact together with the promising results of experimental studies suggests the possibility that adiponectin replacement extracted from wasted abdominal adipose tissues in chicken, beef and lamb might become a new pharmacological or therapeutic approach for treatment/ prevention of insulin resistance and other metabolic diseases (7), (8) and (9). Thus, the objective of this study was to isolate, quantify total protein and adiponectin from trimmed off abdominal adipose tissues from meat sources namely chicken, beef and lamb.

Methodology

Extraction of Protein from Adipose Tissue

Abdominal adipose tissue was removed from chicken, lamb and beef. Delipidation of tissue lysates was performed by chloroform/methanol extractions (10) and (11) with some modification. Homogenization of tissue sample was performed in a ceramic mortar in 500 ml of isolation medium (50 mM Tris, 150 mM NaCl, 0.2 mM EDTA, and protease inhibitors) and 1875 ml of a chloroform/methanol (1:2) mixture. The homogenates were diluted with 625 ml of chloroform and 625 ml of water to change the water/chloroform/methanol ratio from 0.8:1:2 to 1.8:2:2 in the final organic solution. The result was 3 layers, 2 liquid and one interface. The adipose tissue proteins were contained in the interface and upper layer. Proteins were precipitated from aqueous phase by using a 10 % Trichloroacetic Acid (TCA) in acetone at -20 °C overnight followed by centrifugation 7500 x g for 30 min. The acetone was discarded without disturbing the resulting pellet. The pellet was washed with additional ice-cold acetone and dried (12).

Protein Concentration Determination

The Bovine Serum Albumin (BSA) range 0 to 2 mg/ml were used as a standard. 25 µl protein samples and 25 µl of standard was pipetted to 96 well plate. 200 µl of working solution was added to each well. Deionized water was used as a blank. The solution was mixed well for 30 seconds. This solution was incubated at 37°C for 30 minutes. The absorbance was measured at 562 nm.

Adiponectin Concentration Determination

Chicken, beef and lamb adiponectin concentrations were measured using commercially available specific Chicken, Bovine and Sheep Adiponectin (ADP)

ELISA kits from Cusabio Biotech Co. (Wuhan, China) according to the manufacturer's protocol.

Result and Discussion

Protein Extraction

Protein isolation, purification and analysis in white adipose tissues is very challenging because of its higher lipid contents and hydrophobic structure. In the present study, the extraction of 10 grams of subcutaneous adipose tissue from chicken, beef and lamb yielded 0.10 gram, 0.15 gram and 0.15 gram respectively of protein which is about 1 to 1.5 % from the total fat mass (Figure 1). Another study on adipose tissues, (12) has shown that the isolation of white adipose tissue protein yield less than 2 % of total tissue which was similar with this study. These highly hydrophobic proteins are extremely difficult to solubilize in aqueous solutions during the protein extraction process. The process often requires the presence of detergents to maintain their solubility and stability of the protein due to high content of lipid. Thus, larger amount of detergents is required to break down the lipid-protein complex and increase protein solubility. High concentration of detergents had been used over the past several years for most of the methods for sample preparation of membrane protein. However, detergent may lead to the protein loss and poor protein yields (13) and (14). Other than that, most of detergents were not compatible with proteomic approaches which was based on mass spectral analysis (15). Thus, detergents free method was chosen for this study.

In this study, chloroform was used as a lipid dissolvent as the lipid dissolves efficiently in the chloroform phase, while protein precipitates at the chloroform/methanol interface. Then, acetone and TCA was used to further precipitate proteins and dissolve most lipids. Protein were precipitate between solvent and lipid known as protein disk (Figure 1).

Protein Determination

As mentioned earlier, the concentration of protein from chicken, beef and lamb abdominal adipose tissue were measured by Protein Assay Bicinchoninate (BCA) Kit. A trichloroacetic acid (TCA) acetone precipitation protocol was used prior to protein determination assay to remove any interfering substances from samples. The protein concentration in abdominal adipose tissue from chicken, beef and lamb were 1.25 ± 0.05, 1.75 ± 0.05 and 2.53 ± 0.07 mg/ml respectively (Figure 2). Preliminary results indicate that, among these three halal meat used, lamb gave a highest protein content followed by beef and chicken.

Protein purification often requires a rapid and sensitive method for the quantitation of protein. The easiest way to remove interfering non-proteinaceous substances from protein preparations was to precipitate the protein prior to the assay. TCA acetone precipitation was the easiest method and the most compatible with most assays. Bicinchoninic acid forms the basis of an analytical method for the determination of protein. This method of protein determination was re-

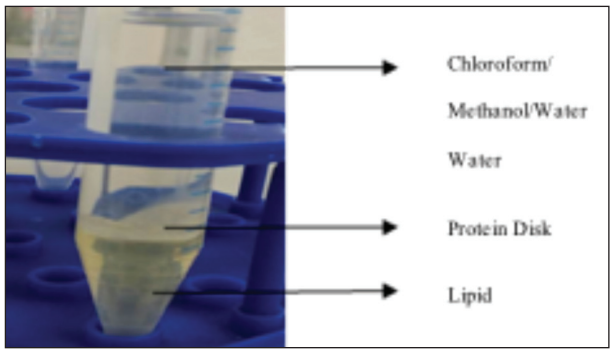

Figure 1. Protein Disk between lipid and solvent (Chloroform/ Methanol/Water)

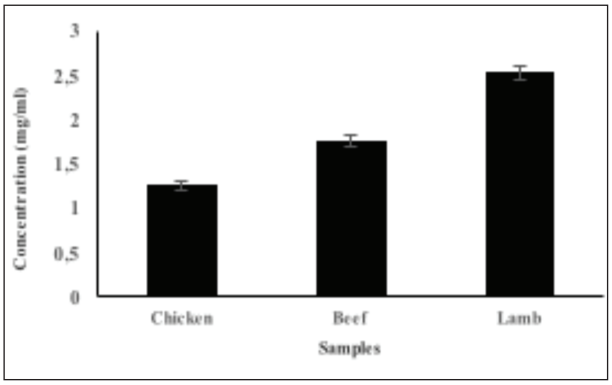

Figure 2. Concentration of protein from chicken, beef and lamb abdominal adipose tissue were measured by Protein Assay Bicinchoninate Kit. Data are represented as mean ±SD (n = 3).

ported to have greater tolerance to many commonly interfering compounds, when compared to the Lowry technique (16).

The bicinchoninic acid method has become widely used to quantitate protein since it was originally described by (16). The greater tolerance of this method to many commonly used buffers and detergents is a significant advantage over the other protein assay procedure (17).

Chicken, Bovine and Sheep Adiponectin (ADP) Elisa Assay

Adiponectin concentrations in chicken, beef and lamb abdominal adipose tissues were 158 ± 0.05 ng/ml, 24 ± 0.05 μg/ml and 37 ± 0.08 ng/ml, respectively (Table 1). Previous study on chicken adiponectin revealed that the concentration ranged from 4 - 10 μg/ml in chicken plasma (18). This is a very high value when compared to this study. This may have been due to the application of plasma samples by the previous study, in where adiponectin circulates at a higher concentration in plasma compared to adipose tissues (19). In addition, it has been reported elsewhere (20) that the concentrations observed in serum samples from dairy cows were between 20 - 40 μg/ml, while the value for protein extracted from adipose tissue were 10 - 28.8 μg/ml. This is consistent with the result from the present study. However, study from another group (21) observed that adiponectin concentrations for dairy cows was 8 - 16 ng/ml that were 1000 times lower when compared to aforementioned study (20). Yet another research group (22) also observed 10 – 100 times lower concentration compared to a study reported (20). This range is far lower than ranges in other species that have been reported such as humans (1.9 -17.0 μg/ml) (23), wild-type mice (10-30 μg/ml) (24), and horses (1.3-2.0 μg/ml) (25) and (26). It appears that plasma adiponectin concentrations vary between species. Moreover, adiponectin in bull samples, ranging from 0 - 40 ng/ml, were similar to cows, the adiponectin concentration ranged from 1-2 μg/ml which is no different than in horses (27). Nevertheless, there is still lack of data study on lamb adiponectin as well as on adiponectin concentration from sources of abdominal adipose tissue. In general, reported (28) that the circulating adiponectin concentrations in the μg/ml range, accounting for about 0.01% of total plasma protein. However, the adiponectin concentrations assessed in animal samples before and after repeated freezing/thawing cycles remained at the same value, indicating that adiponectin is particularly stable and not affected by environmental factors (20).

Several studies shown that obesity and insulin resistance were happened accompanied by decreased in adiponectin levels. Administration of recombinant adiponectin results in improved (hepatic) insulin sensitivity, increased insulin secretion and have a beneficial effect on body weight and hyperglycemia (29). Adiponectin replacement under experimental settings could reduce insulin resistance and body weight. Several clinical studies showed the levels of adiponectin was decreased in obese people compared to lean subjects (23), (31) and (32). In this study (33), there were marked variations in adiponectin levels among obese subjects which was the concentration varied from 1.9 to 17 μg/ml, lower compared to a normal range of human adiponectin (33). Since convincing evidence has been reported on the association of decreased adiponectin levels with obesity and insulin resistance, several laboratories have tried to explore the possibility that hypoadiponectinemia plays a significant role in these diseases by testing the effects of adiponectin replacement. One study in mice consuming a high-fat/high-sucrose diet revealed that the administration of the globular domain of adiponectin was accompanied by a weight loss and decrease of plasma glucose, free fatty acids and triglycerides (34). Another study in normal and diabetic rats treated with recombinant adiponectin reduced serum glucose without stimulating insulin secretion and significantly enhanced the

Table 1. Adiponectin concentration in chicken, beef and lamb abdominal adipose tissue were determined by Adiponectin (ADP) ELISA Assay. Data are represented as mean ± SD (n = 3).

Sample (n=3)	Chicken Adiponectin	Beef Adiponectin	Lamb Adiponectin
Mean Concentration	158±0.05 ng/ml	24 ± 0.05 μg/ml	37 ± 0.08 ng/ml

ability of insulin to suppress glucose production by isolated hepatocytes (4).

The significance of adiponectin concentration in chicken, beef and lamb abdominal adipose tissues are currently not known and it was unique and needs an additional investigation due to knowledge of the insulin-sensitizing effects of adiponectin. Adiponectin produced in these tissues may supplement circulating adiponectin levels in the blood for health supplementation due to many convincing evidences that had been reported from previous studies in various field that can improved metabolic disease as well as cardiovascular diseases.

Conclusion

The present study suggests that beef protein had the highest amount of adiponectin followed by chicken and lamb. Adiponectin proteins extracted from wasted adipose tissues in halal meat can be one of the future novel pharmacological and therapeutic strategies for insulin resistance and other metabolic diseases.

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Conflict of interest

With this submission, I undertake and declare that the contribution of the authors as mentioned in the authorship of this research paper has directly participated in the planning, execution, or analysis of this study. All authors of this paper have read and approved the final version submitted. The authors involved in this research project declare and agreed to publish this article in the present sequence of authorship. Furthermore, there is competing interest among the authors and the fund provider of this research project.

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