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Preparation and Evaluation of Novel Anti-Obesity Immunoglobulins for Immunoprophylaxis and Therapy

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Abstract— Obesity is one of the largest and fastest growing public health problems in the world. The pharmacological options for obesity treatment remain quite limited. Recently, one of the potential exciting research areas is the development of innovative therapeutic molecular vaccines and immunoglobulins. Here, we developed novel immunoglobulins against obesity containing anti-ghrelin O-acyltransferase (GOAT) IgY to block the activity of the appetite-stimulating hormone "ghrelin". Its preliminary pre-clinical evaluation was applied into 3 mice groups, (A) was fed standard pellet chow, (B) was fed a high-fat diet with metabolizable energy contents of 13% kcal from fat and (C) was fed a high-fat diet with metabolizable energy contents of 45% kcal from fat. Oral immunization with this biologics successfully induced beneficial responses that attenuated body weight gain by decreasing food intake and increasing energy expenditure. Anti-GOAT IgY is a promising approach for the treatment of obesity by oral administration but further studies are still required before entry into clinical trials as its effect on physical activity and visceral adipose tissue.

Keywords— Obesity, Immunoglobulins, IgY, Pharmacotherapy, Ghrelin, Ghrelin O-acyltransferase, Oral immunization.

I. INTRODUCTION

Obesity is one of the largest and fastest growing public health problems in the world. It is defined as a medical condition characterized by accumulation of excess body fat with negative impact on a person health. It is considered one of the major causes of death and disability. The worldwide prevalence of obesity nearly doubled during the last 30 years [1]. Obesity is associated with increased prevalence of multiple comorbidities like diabetes, hypertension, dyslipidemia and other features of the metabolic syndrome that leading to cardiovascular diseases. It is a known risk factor for certain types of cancer and other life-threatening conditions. It is also associated with increases in premature mortality, impaired quality of life and large health care cost. It presents a real burden for the modern human society in terms of the health care system and economy [2]. This is a potential health crisis that must be addressed sooner rather than later especially obesity is preventable and weight loss can largely improve & resolve most comorbidities associated with it [3-4]. We have to monitor science and promote research to fulfill the treatment gab between behavioral therapy, bariatric surgery and the quite limited pharmacotherapy [5]. Recently, major advances in our understanding of the basic energy homeostasis and appetite control have identified numerous targets for potential anti-obesity drug development such as innovative therapeutic molecular vaccines and immunoglobulins targeting hormones to reduce body weight [6]. In this scope, ghrelin hormone has attracted particular attention with the development of different neutralization approaches in order to develop alternative obesity treatments. Ghrelin administration potently stimulates feeding, lowers energy expenditure, shifts food preference toward diets rich in fat and shifts fuel preference away from metabolic utilization of fat as an energy source. Chronic ghrelin administration eventually results in increased body weight gain and adiposity. Collectively, these data have led to the hypothesis that inhibition of ghrelin action may be a feasible strategy to reduce body weight and food intake [7]. Unique acylation step is required for binding of ghrelin to its receptor (GHSR). The only enzyme known to catalyze acyl modification of ghrelin is Ghrelin O-acyltransferase (GOAT). The discovery of ghrelin O-acyltransferase (GOAT) opens the way to design of drugs that block the attachment of an octanoyl group to the appetite-stimulating peptide hormone ghrelin that potentially preventing obesity [8]. The usage of immunoglobulin Y as an immuno-intervention agent has superior advantages over mammalian IgG. Numerous studies have shown that IgY exhibits up to five times more affinity

to a specific antigen and reacts more rapidly to the same antigen when tested in competition assays [9]. Also, IgY preparation is more economic than that IgG. IgY does not bind human complement and Fc receptors. Therefore, IgY does not elicit non-specific inflammatory reaction. The manner of IgY extracting from the host is through a bloodless physiological process by egg laying whereas extracting of IgG involves bleeding and pain issues [10]. Here we developed novel Immunoglobulins against obesity containing anti-ghrelin O-acyltransferase (GOAT) IgY and demonstrated that oral immunization with this biologics successfully induced beneficial responses that attenuated body weight gain.

II. METHODOLOGY

1. Experimental Animals: Ten Single Comb White Leghorns SPF hens (6 months old) were purchased from a local poultry farm. The hens were kept in a standard poultry house with a 16/8 h light/dark cycle. The room temperature was approximately 25 °C. The hens were fed a laying hen diet, crushed egg shells and water ad libitum.

Sixty male mice were obtained from VACSERA, Egypt. All mice were housed with ad libitum food and water under standard 12-h light/12-h dark conditions. The mice were fed either standard pellet chow or a high-fat diet with metabolizable energy contents of 13% kcal from fat and 45% kcal from fat, respectively.

2. Synthesis of the immunogen: The immunogen used was synthetic peptide of Ghrelin O-acyltransferase that had the following sequence: CHLGLHYTEYYLGEP. It was constructed in multiple antigen peptide (MAP), tetra branched by:
Hangzhou Dangang Biological Technology Co., Ltd., China.

3. ANTI-GOAT IGY PREPARATION

3.1. Chicken Immunization: After keeping for three days, 6 months old Single Comb White Leghorns SPF laying hens were immunized with the prepared immunogen (synthetic peptide of Ghrelin O-acyltransferase) mixed with Freund's adjuvant, 4 successive doses with 2 week intervals according to the method described by Yokoyama et al., 1997[11] with some modifications.

Some eggs were collected before immunization and stored in the refrigerator at 4 °C. At the first immunization, 1 ml of Freund's Complete Adjuvant (Sigma, USA) containing 6 ug of immunogen was injected into the hens intramuscularly. Repeat immunizations were performed at 2, 4 and 6 weeks using a complex solution containing Freund's Incomplete Adjuvant (Sigma, USA) and the same volume of immunogen. Two weeks after the first immunization, immunized eggs were collected, marked and stored at 4 °C.

3.2. Extraction and Purification of anti-GOAT IgY:

3.2.1. Extraction of anti-GOAT IgY: IgY was extracted from collected eggs using the water solution method as described by Akita and Nakai, 1993[12] and Chalghoumi et al., 2009[13]. Two weeks after the final immunization, collected eggs were harvested and pooled. The egg yolk was collected separately and carefully from the albumin and yolk membrane. The yolk membrane was perforated for allowing the flow of yolk into a graduated cylinder singly without the membrane. The egg yolk was mixed with fresh distilled water in a ratio (1:9). The pH of the mixture was adjusted to 4.0 with hydrogen chloride (HCl) and stored overnight at 4 °C. The aggregate lipoproteins of the yolk were separated by centrifugation at 10,000 X*g* for 25 min at 4 °C. Colorless and translucent supernatants that contain IgY were transferred to a graduated cylinder. The volume was recorded.

3.2.2. Salt precipitation of anti-GOAT IgY[14]: It was performed using ammonium sulphate 40% in two steps. First, the diluted yolk was precipitated by 40% ammonium sulfate at 4 °C using magnetic stirrer for 2 hours. Then, centrifugation was done at a speed of 10,000 Xg for 15 min. The pellet was re-suspended in 0.01 *M* Tris-HCl (pH 8.0) to a volume equal to half of the supernatant. The sample was precipitated by 40% saturated ammonium sulfate again at 4 °C for 2 hours, and the pellet was dissolved in PBS, pH 7.4, and dialyzed against 10 m*M* phosphate buffer, pH 7.0, for 3 successive days.

3.3. Anti-GOAT IgY concentration analysis: The protein concentration of the purified IgY was determined from the absorbance at 280 nm of purified IgY diluted at 1:10 using 1.33 as the extinction coefficient. The formula for calculating IgY concentration was as follows:

$$\text{IgY concentration} = A_{(280)} \times 10/1.33 \text{ mg/ml.}$$

3.4. Anti-GOAT IgY antibody titer was evaluated using ELISA: A polystyrene plate (Costar, USA) was coated with 100 ul of the immunogen in carbonate–bicarbonate (0.05 M, pH 9.6) coating buffer overnight at 4 °C. The plate was washed with PBS-T (0.01 M PBS containing 0.05% Tween 20), and then appropriately diluted IgY was added. After incubating for 2 h at 37 °C, the wells were washed with PBS-T, and 100 ul of an HRP-conjugated goat anti-chicken IgG (Sigma, USA) was added. After incubating for an additional 2 h, the plate was washed with PBS-T. Then, 100 ul of the TMB substrate solution was added. After further incubation for 15 min, 2 M H_2SO_4 was added to stop the reaction. The absorbance was measured at 450 nm on a micro plate reader (Thermo, USA). When the OD sample/OD negative ratio

was greater than 2:1, the maximum dilution multiple of the sample was determined as the IgY titer.

4. Preliminary pre-clinical evaluation of anti-GOAT IgY efficacy:

4.1. Body weight, food intake, and body temperature of the mice: Sixty mice were divided into 3 main groups (20 mice per group). Group (A) was fed standard pellet chow and subdivided into 2 subgroups. Subgroup (A1) included ten mice were immunized with anti-GOAT IgY daily and subgroup (A2) includes ten mice as a control. Group (B) was fed a high-fat diet with metabolizable energy contents of 13% kcal from fat and subdivided into 2 subgroups. Subgroup (B1) included ten mice were immunized with anti-GOAT IgY daily and subgroup (B2) includes ten mice as a control also. Group (C) was fed a high-fat diet with metabolizable energy contents of 45% kcal from fat and subdivided into 2 subgroups. Subgroup (C1) included ten mice were immunized with anti-GOAT IgY daily and subgroup (C2) includes ten mice as a control as well. All mice were weighed once weekly using a balance (Sartorus, Germany). Individual daily food intake was measured on 2 consecutive days at 2-3 weeks after the final vaccination and the mean was defined as the daily food intake. The body temperature of the mice was also measured rectally each week using a digital thermometer with a rectal Probe.

4.2. Measurement of the plasma concentrations of ghrelin, leptin, adiponectin, and growth hormone: Concentrations of AG, UAG, leptin, adiponectin, and growth hormone in plasma obtained from control and immunized mice fasted for 16 h were measured by double-antibody sandwich technique (SPI-Bio, Montigny Le Bretonneux, France) or ELISA methods (EMD Millipore Corporation, Billerica, MA).

5. Statistical analysis: The data are expressed as mean ± s.e.m. Statistical comparisons were made by analysis of variance followed by an unpaired two-tailed Student's t-test between the two groups. P-values of < 0.05 were considered to be statistically significant.

III. RESULTS

Anti-GOAT Ig Y concentration: Two weeks after the final immunization with the prepared immunogen (synthetic peptide of Ghrelin O-acyltransferase), laying hens produced specific IgY. The concentration of IgY was nearly 8.66 mg/ml egg yolk, as determined by the absorbance at 280 nm.

Anti-GOAT Ig Y purity: The purified IgY samples showed antibody concentrations ranging from 8.18 mg/ml to 8.29 mg/ml as measured by Bradford assay.

Anti-GOAT IgY antibody titer in egg yolk: Anti-GOAT IgY produced by the immunized hens increased over time, as revealed by ELISA (*Fig. 1*). The specific IgY titer increased starting the second week after the first immunization, with the titer peaking at the eighth week. After twelve weeks, the antibody titer slowly declined.

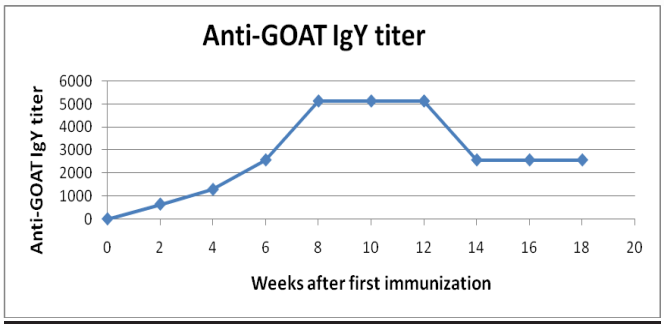
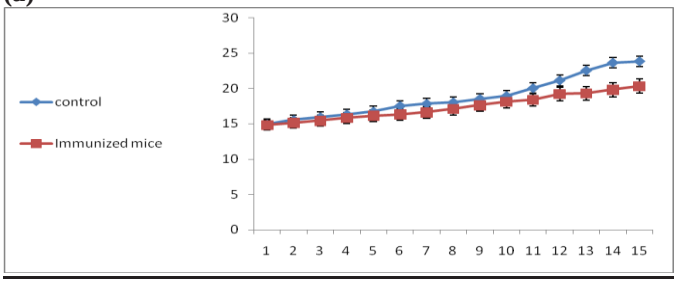


Fig. 1: The titer of anti-GOAT IgY antibody in egg yolks increases over time. Egg yolks were collected from immunized hens over a course of 18 weeks after the first immunization.

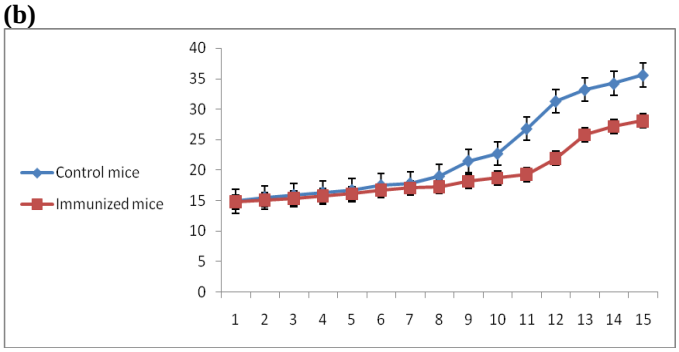
Body weight, food intake, and body temperature of the mice:

Body weight gain: To evaluate the effect of anti-GOAT IgY on body weight gain, control and immunized mice were weighed every week. The results revealed that anti-GOAT IgY attenuated body weight gain in the three main groups. Also, body weight gain with growth was accelerated with initiation of the high-fat diet in control mice and in immunized mice. However, immunization with anti-GOAT IgY attenuated the body weight gain induced by the high-fat diet. The response of Group (B) which fed a high-fat diet with metabolizable energy contents of 13% kcal from fat (control 35.61 ± 0.6 g vs. immunized mice 28.10 ± 0.2 g at 14 weeks after the final immunization, n = 10 per group, $P < 0.05$) was significantly more than group (C) which fed a high-fat diet with metabolizable energy contents of 45% kcal from fat (control 38.60 ± 0.1 g vs. immunized mice 32.10 ± 0.5 g at 14 weeks after the final immunization, n = 10 per group, $P = 0.55$). While the response in group (A) was (control 23.80 ± 0.7 g vs. immunized mice 20.30 ± 0. 4 g at 14 weeks after the final immunization, n = 10 per group, $P < 0.05$). To elucidate whether the attenuation in body weight gain in immunized mice of all groups was due to a decrease in food intake or due to an increase in energy expenditure, we measured food intake and rectal temperature.

(a)



(b)



(c)

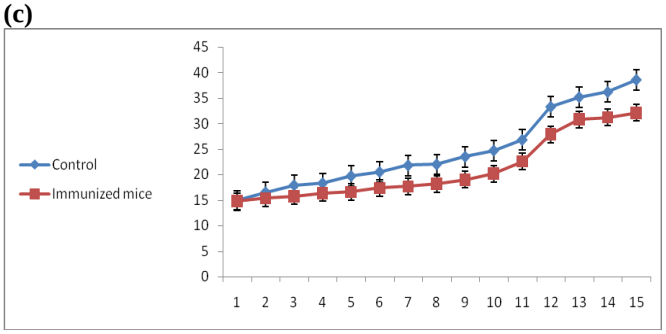


Fig. 2: *Anti-GOAT IgY attenuated body weight gain. Control and immunized mice were weighed every week. (a) Group (A) was fed standard pellet chow immunized with or without anti-GOAT IgY daily for 14 weeks. (b) Group (B) was fed a high-fat diet with metabolizable energy contents of 13% kcal from fat immunized with or without anti-GOAT IgY daily for 14 weeks. (c) Group (C) was fed a high-fat diet with metabolizable energy contents of 45% kcal from fat immunized with or without anti-GOAT IgY daily for 14 weeks. Body weight gain with growth was accelerated with initiation of the high-fat diet in control mice and in immunized mice. However, immunization with anti-GOAT IgY significantly attenuated the body weight gain induced by the high-fat diet. Data are shown as means ± s.e.m. with differences between mean values evaluated for significance by using Student's t-test (P value < 0.05).*

Daily food intake: The result revealed that anti-GOAT IgY immunization significantly decreased the daily food intake of immunized mice than control mice in the three main groups. The decrease in the daily food intake was more in group (C) which fed a high-fat diet with metabolizable energy contents of 45% kcal from fat (control 14.00 ±0.82 K cal per day vs. immunized mice 8.63 ± 0.75 K cal per day, n = 10 per group, $P < 0.05$), followed by group (B) which fed a high-fat diet with metabolizable energy contents of 13% kcal from fat (control 13.25 ± 0.96 K cal per day vs. immunized mice 9.00 ± 0.82 K cal per day, n = 10 per group, $P < 0.05$) and followed by group (A) which fed standard pellet chow (control 13.00 ± 0.82 K cal per day vs. immunized mice 9.88 ± 0.54 K cal per day, n = 10 per group, $P < 0.05$) respectively (*Fig. 3*).

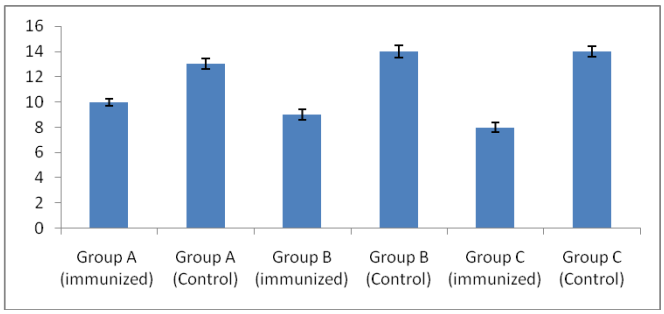


Fig. 3: Anti- GOAT IgY decreased food intake of immunized mice than control. Individual daily food intake was measured on 2 consecutive days and the mean intake over the 2 days was defined as the daily food intake. Data are shown as means ± s.e.m. with differences between mean values evaluated for significance by using Student's t-test ($P < 0.05$).

Body temperature: The results show significant difference between control and immunized mice rectal temperature, which is the representative of systemic thermogenesis. It was 37.0 ± 0.2 °C in control mice versus 37.9 ± 0.5 °C in immunized mice ($P < 0.05$).

All The previous data indicate that immunization by anti-GOAT IgY attenuated body weight gain by decreasing food intake and increasing energy expenditure.

Plasma concentrations of ghrelin, leptin, adiponectin, and growth hormone: Ghrelin, leptin and adiponectin are important hormones that control energy homeostasis. The plasma level of acylated ghrelin was significantly altered by immunization (Control mice 676.68 ± 84.9 pg/ml vs. immunized mice 134.40 ± 16.4 pg/ml, n = 9, $P < 0.01$). While the plasma leptin and adiponectin levels of immunized mice were comparable to those of control mice (leptin: control mice 2.62 ± 0.39 ng/ml vs. immunized mice 2.36 ± 0.83 ng/ml, n = 9 per group, $P < 0.05$; adiponectin: control mice 1569±181 ng/ml vs. immunized mice 1944 ± 832 ng/ml, n = 9 per group, $P < 0.01$). The plasma level of growth hormone also did not significantly alter by immunization (control mice 5.33±0.97 ng/ml vs. immunized mice 9.32 ± 3.66 ng/ml, n = 9 per group, $P = 0.399$).

IV. DISCUSSION

Obesity is one of the important biggest and speediest growing public health problems worldwide. The prevalence of obesity nearly doubled during the last thirty years all over the world [1-2]. It is a medical condition caused by excessive accumulation of body fat with negative impact on an individual's health, including reduced life expectancy. It is associated with numerous comorbidities such as type 2 diabetes mellitus, hypertension, metabolic syndrome, cardiovascular diseases, respiratory, musculoskeletal, infectious, psychiatric disorders and cancer and represents a huge economic issue on the health-care system. However,

weight loss can largely improve or resolve most of these comorbidities. Recently, it has been considered the leading cause of preventable death. It has been reported that sixty-five percent of the world's population lives in countries where overweight and obesity kills more people than underweight. So, we can conclude it presents a real burden for the modern human society in terms of the health care system and economy [2].

The cause of obesity is mainly resulted from a positive energy balance due to excessive food intake in concomitant with lack of physical activity in genetically predisposed persons. In a limited number of cases are secondary to mono genetic causes such as endocrine disorders or administration of drugs that lead to weight gain [15]. Therefore, it is a potential health crisis that must be addressed sooner rather than later.

The cornerstone of overweight and obesity treatment is diet and physical exercise. However, many patients find lifestyle modifications difficult to comply and prone to failure in the long-term. Weight loss surgery (WLS) such as adjustable gastric banding, gastric bypass surgery and sleeve gastrectomy provides sustainable weight loss but this method has significant peri-operative risks such as peri-operative mortality, surgical complications and the frequent need for reoperation. Also, it presents an economic burden for the patient. In addition to the mechanisms of action that lead to weight loss after operation are not completely understood.

Therefore, many patients consider anti-obesity drugs an important adjuvant if not a better alternative to behavioral therapy or weight loss surgery [5]. Since the pharmacological options for obesity treatment remain quite limited, this is an exciting research area, with new treatment targets and strategies on the horizon.

One of the key segments of anti-obesity drugs pipeline is Appetite Suppressants/Satiety Inducers. In this context, major recent advances in our understanding of the basic energy homeostasis and appetite control have identified numerous targets for potential anti-obesity drug development and pathophysiological mechanisms involved in appetite control have opened new possibilities [16-18].

One of the recent advances in obesity pharmacotherapy is the development of innovative therapeutic agents as the use of molecular vaccines and immunoglobulins that target hormones to reduce body weight. These kinds of therapeutic agents could be a new approach in developing anti-obesity medications. Therapeutic vaccines and immunoglobulins could alternatively treat obesity by suppressing appetite-stimulating hormones and/or by blocking nutrient absorption[6].

Regulatory peptides such as adipose tissue antigens, somatostatin, glucose-dependent insulinotropic polypeptide (formerly known as gastric inhibitory polypeptide "GIP") and ghrelin that originated from the stomach, gut, pancreas, and adipose tissue were studied as potential targets for anti-obesity drugs [19].

Ghrelin has attracted particular attention with the development of different neutralization approaches in order to develop alternative obesity treatments. Ghrelin is a hormone that increases hunger feeling. It is made and released by cells located in the upper part of the stomach. Multiple studies have approved that ghrelin administration potently stimulates feeding, decreases energy expenditure, shifts food preference toward diets rich in fat and shifts fuel preference away from metabolic utilization of fat as a source of energy. Therefore, chronic administration of ghrelin eventually leads to the increase in body weight gain and adiposity [20].

However, the results presented by Zorrilla *et al.* (2006) [21] supporting the use of methods to neutralize acylated ghrelin by therapeutic vaccines, many important questions still remain such as who should be treated. Also, how long the immune-neutralization would be efficacious. For example, it is well-known that antibody titers could wane over time resulting in less inhibition of ghrelin action. On the other end of the spectrum, the vaccination's efficacy might be long-lasting, which could be problematic if its effects were irreversible leading to Cachexia. In addition to that the main limitation of these anti-ghrelin vaccines is the need to use adjuvants in order to achieve adequate antibody titers. Adjuvants may be associated with inflammatory reactions[22]. On the other hand, a different study showed that IgG anti-ghrelin auto-antibodies are able to protect ghrelin from degradation [23].

Passive immunization using a polyclonal anti-ghrelin and monoclonal anti-ghrelin antibody also studied to treat obesity. Lu et al., 2009 [24] prepared anti-ghrelin antibodies which bind with high affinity to acylated ghrelin but not to des-acyl ghrelin. Recently, monoclonal antibodies targeting different ghrelin epitopes resulted in maintain increased energy expenditure and reduce overall food intake [25].

For binding of ghrelin to its receptor (GHSR), unique acylation step is required. Till now the only enzyme known to catalyze acyl modification of ghrelin is Ghrelin O-acyltransferase (GOAT). The inactivation of GOAT has been also suggested as an alternative strategy to block ghrelin activity since antibodies targeted to hydrolyze the octanoyl moiety of ghrelin to form des-acyl ghrelin, which has no biological activity [4]. Therefore, the discovery of ghrelin O-acyltransferase (GOAT) also opens the way to the design of drugs that block the attachment of an octanoyl group to the

appetite-stimulating peptide hormone ghrelin that is potentially preventing obesity [26].

Recently, there has been a growing appreciation of using immunoglobulin Y (IgY) by oral administration against host pathogens to establish protective immunity especially against gastrointestinal pathogens both in the human and animals. Eggs are normal dietary components and there is practically no risk of toxic side effects. Besides, IgY does not activate mammalian complement system nor interact with mammalian Fc-receptors that could mediate inflammatory response in the gastrointestinal tract. This IgY provides a safer, more efficient pharmacotherapy and needs less expensive method for preparation than conventional mammalian antibodies [27]. Here we developed novel Immunoglobulins against obesity containing anti-ghrelin O-acyltransferase (GOAT) IgY and demonstrated that oral immunization with this biologics successfully induced beneficial responses that attenuated body weight gain.

The immunization of hens with the prepared immunogen (synthetic peptide of Ghrelin O-acyltransferase) resulted in the production of specific IgY in egg yolk. The concentration of IgY was nearly 8.66 mg/ml egg yolk, as determined by the absorbance at 280 nm. The purified IgY samples showed antibody concentrations ranging from 8.18 mg/ml to 8.29 mg/ml as measured by Bradford assay.

Anti-GOAT IgY produced by the immunized hens increased over time, as revealed by ELISA. The specific IgY titer increased starting the second week after the first immunization, with the titer peaking at the eighth week. After twelve weeks, the antibody titer slowly declined.

Preliminary pre-clinical evaluation of anti-GOAT IgY efficacy was applied using sixty mice divided into 3 main groups (20 mice per group). Group (A) was fed standard pellet chow, group (B) was fed a high-fat diet with metabolizable energy contents of 13% kcal from fat and group (C) was fed a high-fat diet with metabolizable energy contents of 45% kcal from fat. All mice were weighed once weekly. Also, individual daily food intake and the body temperature of the mice were measured. The results revealed that anti-GOAT IgY attenuated body weight gain in the three main groups. Also, body weight gain with growth was accelerated with initiation of the high-fat diet in control mice and in immunized mice. However, immunization with anti-GOAT IgY significantly attenuated the body weight gain induced by the high-fat diet. The response of Group (B) which fed a high-fat diet with metabolizable energy contents of 13% kcal from fat was significantly more than group (C) which fed a high-fat diet with metabolizable energy contents of 45% kcal from. This finding confirms the theory of life style as diet and physical exercise is still the cornerstone for obesity management. Anti-obesity drugs achieve weight loss when used in concomitant with weight regain [28]. In addition to, Azegami, et al. (2017) [29] who prepared nanogel-based ghrelin vaccine to prevent obesity, found that vaccination may be more effective when used in combination with dietary restriction. Polytherapy such as combination agents that are planned to simultaneously target more than one biological mechanism might be more effective in inducing sustained weight loss and improving the related comorbidities. Beside, polytherapy provide a lot of advantages such as using lower drug doses, possible synergistic or at least additive weight loss, less serious side effects and reducing potential for counter-regulation [30].

To elucidate whether the attenuation in body weight gain in immunized mice of all groups was due to a decrease in food intake or due to an increase in energy expenditure, we measured food intake and rectal temperature. The result revealed that anti-GOAT IgY immunization decreased the daily food intake of immunized mice than control mice in the three main groups. The decrease in the daily food intake was significantly more in group (C) which fed a high-fat diet with metabolizable energy contents of 45% kcal from fat, followed by group (B) which fed a high-fat diet with metabolizable energy contents of 13% kcal from fat and followed by group (A) which fed standard pellet chow, respectively. This finding is matched with Tschop et al. (2000) [6] who concluded that ghrelin shifts food preference toward diets rich in fat. Moreover, ghrelin shifts fuel preference away from metabolic utilization of fat as a source of energy.

Also, the results show difference between control and immunized mice rectal temperature, which is the representative of systemic thermogenesis. It was 37.0±0.2 ^{o}C in control mice versus 37.9±0.5 ^{o}C in immunized mice. This result agrees with Wren et al. (2001) [31] who concluded that ghrelin administration potently stimulates feeding and lowers energy expenditure.

Ghrelin, leptin, adiponectin and growth hormone are important hormones that control energy homeostasis. In this study we measured their plasma level. We found that acylated ghrelin was significantly altered by immunization (Control mice 676.68 ± 84.9 pg/ml vs. immunized mice 134.40 ± 16.4 pg/ml). While the plasma leptin and adiponectin levels of immunized mice were comparable to those of control mice (leptin: control mice 2.62 ± 0.39 ng/ml vs. immunized mice 2.36 ± 0.83 ng/ml; adiponectin: control mice 1569 ± 181 ng/ml vs. immunized mice 1944 ± 832 ng/ml per group). Although, growth hormone is likely to be directly altered by modification of ghrelin–GHSR signaling as ghrelin is the endogenous ligand of GHSR [7]; the plasma level of growth hormone also did not significantly alter by immunization (control mice 5.33±0.97 ng/ml vs. immunized mice 9.32± 3.66 ng/ml per group).

The previous data indicate that immunization by anti-GOAT IgY attenuated body weight gain by both mechanisms, decreasing food intake and increasing energy expenditure. From all of the above, the novel anti-GOAT IgY developed here has the potential to prevent and treat obesity. The concept for using immunoglobulin Y (IgY) by oral administration against host pathogens to establish protective immunity especially against gastrointestinal pathogens both in human and animals is a novel and creative challenge for the development of future pharmacotherapy and nutraceutical. Anti-GOAT IgY is a promising approach for the treatment of obesity. Further studies are still required before entry into clinical trials as its effect on physical activity and visceral adipose tissue.

V. CONCLUSION AND FUTURE SCOPE

In this study, we prepared a novel anti-GOAT IgY for inactivation of GOAT as a new approach to block ghrelin activity. The preclinical trial revealed that immunization by anti-GOAT IgY attenuated body weight gain by both mechanisms, decreasing food intake and increasing energy expenditure. Therefore, the novel anti-GOAT IgY developed here has the potential to prevent and treat obesity.

Furthermore, the concept for using immunoglobulin Y (IgY) by oral administration is also a novel and creative challenge for the development of future pharmacotherapy and nutraceutical. So it can be concluded that the prepared anti-GOAT IgY is a promising approach for the treatment of obesity while further studies are still required before entry into clinical trials such as its effect on physical activity and visceral adipose tissue.

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