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Original Research

An investigation of NIS expression in gastric tissue of obese individuals

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Abstract: Obesity is seen as one of the top ten (10) illness's listed by World Health Organization (WHO). It is a global problem that can affect people of all ages. Obesity is identified one of the most important factors leading to diabetes, heart disease and hypertension. Individuals with a Body Mass Index (BMI) above 40 kg/m^2 are defined with morbid obesity. Sodium Iodide Symporter (NIS) gene is a plasma membrane glycoprotein that mediates iodide uptake in thyroid glands, stomach, salivary glands, lactating mammary glands and intestine. NIS gene transports iodide from the blood to the gastric epithelial cells. NIS gene expression and regulatory role of NIS gene in gastrointestinal tract, hasn't been studied yet in the individuals with obesity (i.e., BMI >40 kg/m^2). In this study, gastric tissues were obtained by laparoscopic sleeve gastrectomy from 33 individuals diagnosed with obesity. Control group consisted of gastric tissue of 21 subjects with normal BMI obtained by endoscopy. RNA isolation, cDNA synthesis and qRT-PCR analyses were performed on the samples to determine NIS gene expression. Expression levels of NIS gene were compared between obese and control individuals, although an increase was observed in obese patients this difference was not found to be statistically important ($p>0.05$).

Key words: Obesity; NIS; Gene Expression; Ion Channel; TSH.

Introduction

It is only the ages ago, the affair of obesity wasn't seen a public health problem, affect up children to adults. Nowadays obesity and related metabolic diseases are alarming problem which effects people in developed and developing countries. The obese group has approximately 10-20 years less life span than normal individuals (1)

Although obesity primarily results from disequilibrium among energy intake and consumption is also affected by multiple factors, such as age, sex, educational level, socio-cultural condition, level of income, malnutrition, insufficient physical activity, hormonal disorders (2). Obesity is determined by different markers just as body mass index (BMI), waist-to-hip ratio (WHR), waist circumference (WC), body surface area (BSA), and waist-to-height ratio (3). Body Mass Index (BMI) is used for determining adults weight scale who are underweight or overweight. It is calculated by dividing the weight in kilogram by the square of the height in meter (kg/m^2). The degree of weight is according to BMI, adults with BMI < 18.5 kg/m^2 are classified as underweight, BMI between 18.5-24.9 kg/m^2 as normal, BMI between 25-29.9 kg/m^2 overweight, adults with BMI > 30 kg/m^2 obese, BMI between 30-34.9 kg/m^2 as class I obesity, BMI between 35-39.9 kg/m^2 as class II, BMI > 40 kg/m^2 as severe or extreme obesity class III (4). The prevalence of the metabolic syndrome and cardiovascular disease is considered to rise dramatically in parallel to the global obesity epidemic (5). Metabolic syndrome is defined as a combination of the following features: central obesity, high serum triglyceride (TG) levels, low serum high-density lipoprotein (HDL), increased cholesterol levels, hypertension, and elevated fasting blood glucose levels. The existence of at least three of these features proofs the obesity (6) .

Adipocytes are the major form of adipose tissue and are estimated the crucial component of homeostasis of the body metabolism (7). Adipose tissue is currently known to secrete a large number of proteins termed adipokines that control various metabolic functions. Enlarged, molecularly and cellularly altered adipocytes that affect systemic metabolism are observed in obesity (8). Accumulation of the adipose tissue results obesity progression (9). This process depends on ion channels functionality. Ion channels have important functions, such as homeostasis, cell proliferation and signal transduction in a variety of cell types and different cell stages (10). Despite of their metabolic roles, more specific functions of ion channels are considered regarding the central regulation of food intake, energy expenditure, and glucose homeostasis (11). In this context, the investigation of ion channels has been emerging as a new approach in the study of the pathogenesis of obesity (10). A large variety of ion channels has been identified in the pathogenesis of obesity such as potassium, sodium, calcium and chloride channels.

The Na/I Symporter gene belongs to Solute Carrier Gene super family (SLC). This gene encodes a member

of the sodium glucose cotransporter family (12). Solute carrier family 5 (SLC5A5), sodium iodide symporter, member 5 mediates iodide uptake in the thyroid gland. NIS gene located in chromosome 19p13.2-p12 and contains 15 exons, 14 introns (13). NIS is consisted of 13 transmembrane helices and 643 amino acids in human (14). The Na/I Symporter (NIS) is an integral membrane bound glycoprotein which located at the basolateral side of thyroid follicular cells and a key molecule for thyroid hormone biosynthesis (12).

NIS is expressed also in salivary glands, endometrium, placenta, intestine, lactating breast, gastric mucosa, lacrimal ducts (13).

Gastric epithelial cells secretes I^- into the gastric juice. I^- intake into the gastric epithelial cells from serum modulated by NIS independent from I^- absorption. NIS may be a new biomarker for the diagnosis of gastric cancer and intestinal metaplasia due to downregulated expression in gastric cancer (15).

The Na/I Symporter (NIS) is an integral plasma membrane protein that assists in the thyroid, lactating, breast, and other tissues uptake of I^- (16). No study has been carried out to date to elucidate the expression of sodium iodide symporter in obesity. The aim of this study was to determine the level of NIS gene expression in obese and non-obese individuals.

Materials and Methods

Study group

33 morbid obese (BMI>40 kg/m^2) individuals (i.e., patient group) who got sleeve gastrectomy surgery were selected as the patient group. These subjects were between 24 and 58 years of age. Female to male ratio was 26:7, mean of ages was 33.45 ± 9.18. For the control group, 21 non-obese (BMI in the range of 18.5 to 24.9 kg/m^2) individuals who got endoscopy operation. The subjects which belong to the control group were between 23 and 75 years of age. Female to male ratio was 16:5, mean of ages was 46.62 ± 14.12. From both groups, tissue samples were collected. Upon collection, tissue samples were placed in "RNA later" solution and stored at -86°C until testing.

Total RNA extraction

RNA was extracted from 10 mg frozen tissue using high pure RNA tissue kit (12183018A PureLink® RNA Mini Kit, Thermo Fisher Scientific) following manufacturer's instructions. The quality of extracted RNAs were confirmed by nano-spectrophotometer based on absorbance density in 260 nm/280 nm. Extracted RNAs were stored at −86°C prior to use.

cDNA synthesis

cDNA synthesis were performed from 300 ng/µl of extracted of RNA, using 4374966 High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific) based on manufacturer's instructions. Reverse transcriptase random primers were used to amplify all RNA. RNA samples (10 µl) were performed in 20 µl reaction mixtures containing 10 X RT Buffer 2.0 µl , 25 X dNTP Mix (100 mM) 0.8 µl, 10 X RT Random Primers 2.0 µl, MultiScribe™ Reverse Transcriptase 1.0 µl, Nuclease-free H_2O 4.2 µl, using a thermal cycler. Reaction conditions were as follows; 25°C for 10 min., 37°C for 120 min. and 85 °C for 5 min.

Table 1. Comparing of demographic factors between obese and control groups.

Quantitative Variable	Obese Group (n=33)	Control Group (n=21)	p
Age† (years)	33,45 ± 9,18	46,62 ± 14,12	0,001*
Height† (cm)	166,3 ± 9,4	167,14 ± 7,19	0,606
Weight† (kg)	127,76 ± 20,96	64,24 ± 6,04	0,001*
BMI† (kg/m^2)	46,07 ± 5,47	23 ± 1,66	0,001*
Qualitative Variable	**Obese Group (n=33)**	**Control Group (n=21)**	**p**
Sex‡ (No. of Female/No. of Male)	26/7	16/5	0,823

†: Avarage±Standard deviation; Mann Whitney u test. ‡: Chi-square test. *p<0,05.

Real-Time Polymerase Reaction

Real-Time PCR performed with NIS and GAPDH (as housekeeping) genes using cDNA template. cDNA samples (2 µl) were performed 20 µl reaction mixtures containing 10 µl 20X Taqman Gene Expression master mix, 1 µl 2X Taqman Gene Expression Assay for NIS and GAPDH genes (4331182 TaqMan® Gene Expression Assay, Thermo Fisher SLC5A5, Hs00166567, 4331182 TaqMan® Gene Expression Assay, Thermo Fisher GAPDH, Hs03929097) 7 µl RNase-free water, using StepOnePlus Real-Time PCR. Reaction conditions were as follows; 50°C for 2 min., 95 °C for 10 min., 40 cycles of 95 °C for 15 seconds, 60°C for 1 min.

Results

Demographic data of obese and control groups are presented in Table 1. Obese and control groups had significant differences (p=0,001) in age, weight and BMI. However, there were no significant differences between the groups about the subjects' height or female/male ratios.

Thyroid Stimulating Hormone (TSH) values were obtained from medical health records of individuals. Preoperation and postoperation (after 12 months from operation) TSH levels of obese patients were compared to non-obese group. Statistically significant difference was found between preoperation of morbid obese and control groups (p=0,015). TSH levels were significantly different between preoperation and postoperation of obese group (p=0,001). There was no significant changes in TSH value in between control and postoperation obese groups (p=0,663).

No significant difference (p>0.05) was observed between the obese and control groups in NIS gene expres-

Table 2. Comparison of TSH values for obese and control groups.

Variables†	Obese Group (n=33)	Control Group (n=21)	$P^{‡}_{intergroups}$
Preoperation TSH	1.48 ± 0.82	1.99 ± 0.74	0.015*
Postoperation TSH	2.02 ± 0.89	1.99 ± 0.74	0.663
$P^{§}_{in\text{-}group}$	0.001*		

†: Avarage±Standard deviation. ‡: Mann Whitney U test. §: Willcoxen test. *p<0,05.

Table 3. Comparison of NIS gene expression for obese and control groups.

Gene	Descriptive Statistics	Obese Group (n=33)	Control Group (n=21)	p†
2^(–ΔΔCT)	Average ± Standard Deviation	3.54±8.61	11.04±25.58	0.965
	Median [%25-%75]	1.10[0.46-1.85]	0.92[0.15-7.57]	

†: Mann Whitney U test.

sion levels. NIS gene expression results are shown in Table 3.

Discussion

Obesity is a global health problem affecting not only adults, but also children. It is mainly caused by the high intake and lack of use of calories (17). Several diseases such as hypertension, cardiovascular diseases, metabolic syndromes have been related with obesity (3). New studies intend to find out about relationship between ion channels and obesity. In this study, our approach was to investigate whether NIS is a contributing factor to obesity. NIS gene expression hasn't been studied yet in gastrointestinal tract in obese individuals. The Sodium Iodide Symporter (NIS) is a basal membrane glycoprotein mainly related with the thyroid gland, responsible for transportation of iodide and important factor of thyroid hormone biosynthesis (12). Besides the thyroid gland, NIS expression can be found in other tissues such as, salivary glands, stomach, small intestine, lactating breast, kidney, placenta (15). Characteristic hallmark of NIS is its dependence on Na^+. While Na^+ is critical for NIS, presence of perchlorate (ClO_4^-) can inhibit Na^+ somethesia of NIS (18). Furthermore, hNIS has been shown to relate to autoimmune diseases such as autoimmune gastritis and Sjögren's syndrome, because of linked with autoantibodies and affect as a target antigen for T cells (19).

Using the realtime PCR technique, we studied NIS gene expression in 33 subjects identified as obese and 21 control subjects' stomach tissue samples. NIS gene expression levels of obese and control groups are shown in Figure 1. There was no statistically significant difference in NIS gene expression between obese and control groups (P = 0,965) selected in this study.

The driving force of iodide uptake by NIS is mediated by sodium gradient (20). $Na(^+)/K(^+)$-ATPase enzyme/pump has a major role for the Na^+ gradient. Based on the electrogenic stoichiometry, carriage of I^- is performed by two Na^+ per I^- (12). Na^+ comes from $Na(^+)/K(^+)$-ATPase to the NIS. In recent years, significance of $Na(^+)/K(^+)$-ATPase pump has been demonstrated in several diseases including obesity, diabetes, and atherosclerosis (21). Altered $Na(^+)/K(^+)$-ATPase levels are related to obesity and associated with hyperinsulinemia, thermogenesis, energy equilibrium and negatively related with body mass index, oral glucose tolerance test (22). Researchers showed that stimulation of $Na(^+)/K(^+)$-ATPase activity accelerate Na^+ -couple glucose absorption (2). Voltage-activated K^+ channel mRNA levels in different carriers were significantly increased in adipose tissue and adipose tissue–derived cells (23). Leptin is well known for its anti-obesity effects, which are mediated by $Na(^+)/K(^+)$-ATPase channels. Increased $Na(^+)/K(^+)$-ATPase channel activity in neurons have been shown to lead to hyperphagia and diet-induced obesity (11). In the literature, it has been shown that obesity can lead to low-dose chronic inflammation (24). NIS gene is target for immune system cells. Activation of immune system causes obesity related insulin resistance (25). It is not clear yet if gastric NIS expression is controlled by gastric hormones such as gastrin, glucagon, and secretin (26).

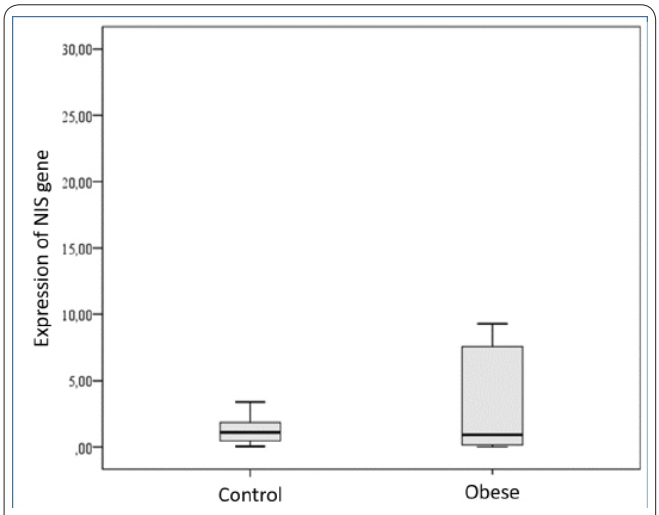

Figure 1. Comparation of NIS gene expression levels between obese and control groups.

Weight of an individual is dependent on the energy equilibrium (energy intake vs. energy use). Energy consumption is linked with exercise and basal metabolism energy expenditure. Many metabolic pathways are moderated by thyroid hormones which are related in basal metabolic rate (27). TSH levels of obese individuals were examined before Sleeve Gastrectomy Surgery. TSH level of obese individuals before Sleeve Gastrectomy Surgery was significantly lower than control group (p=0,015). We also determined pre and post-operation TSH levels in obese group. Compared to pre-operation, higher levels of TSH were detected after the surgery (p=0,001) in the obese group. Many studies on adult obese individuals, thyroid hormone and TSH concentrations have been shown as normal, increased or reduced, compared with a control group (28). Our data about TSH values were in the normal range in obese and control group.

To the best of our knowledge, this is the first NIS gene expression study in gastric tissue of individuals with obesity. Further researches are needed for better understanding the ion channel mechanisms and relevant signal pathways in obesity. The results of our study can inspire for the research to clarify the effect of ion channels on obesity progression and can be used to design more effective studies for individuals with obesity. New studies explaining the role of NIS could be a new aspect of therapeutic strategy for the treatment of obesity. Further research is warranted to investigate using different markers such as medicines, hormones, immune system cells and other ion pumps to identify NIS gene's medical significance.

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Interest conflict
The authors declare no conflict of interests.

Author's contribution
H.S.N. devised the project, G.O.F. worked out almost all of the technical details, A.B. and Y.N. collected the subjects, M.D. performed the experiments and analysed the data. All authors discussed the results and contributed to the final manuscript.

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