Original article

Investigation of the relationship between DNA methylation of PTH, ESRRA, FSHR and obesity: A single center study

Huseyin Cagsin[1], Eda Becer[2], Rasime Kalkan[1,3]*

Abstract:

Background: Obesity is a multifactorial disorder that is an important predisposing factor for a number of disorders. Genetic and epigenetic variations play important roles during the development of obesity. ***Method:*** DNA methylation is the most studied as one of the epigenetic modifications and is directly related to gene expression. Here, the methylation status of *FSHR*, *PTH* and *ESRRA* genes were investigated in obese patients to discover novel associations between DNA methylation and obesity phenotype. A total of 69 patients with obesity and 76 patients without obesity were enrolled in this study. DNA methylation in *FSHR*, *PTH* and *ESRRA* genes was analyzed. ***Result:*** There is a statistically significant association between the methylation status of *ESRRA* and fasting glucose (P=0.04) and BMI and methylation of the *PTH* gene (P=0.036) in obese subjects. In non-obese subjects, a statistically significant association was detected between adiponectin, resistin levels and *FSHR* methylation status. It is concluded that the methylation status of *FSHR*, *PTH* and *ESRRA* genes play an important role in obesity phenotype, adiponectin and resistin levels. These genes had previously been associated with obesity-related variables, but their methylation status was not highlighted. ***Conclusion***: Our study will shed light on resolving the epigenetic contributions toward the development of obesity. Studies in the field of epigenetics will help discover predisposition markers for obesity. Based on the literature this was the first study that shows the interactions between *FSHR*, *PTH* and *ESRRA* methylation and obesity.

Keywords: Obesity; Epigenetics; Methylation; *FSHR; PTH; ESRRA*

Bangladesh Journal of Medical Science Vol. 22 No. 03 July'23 Page : 623-631
DOI: https://doi.org/10.3329/bjms.v22i3.65336

Introduction:

Obesity is a multi-factorial disorder that environmental, genetic and epigenetic factors play key roles during the disease development[1]. Obesity is a significant risk factor for metabolic, type II diabetes, and cardiovascular diseases. The genetic variations among individuals causes phenotypic differences in a population[2,3]. Additionally, ethnicity and ethnic-specific genetic variables influence how human body weight is regulated[4]. Genetic variations play a crucial role for percentage of body fat, waist circumference, eating behavior, degree of physical activity, energy expenditure, regulating metabolism, and hunger in obesity[5].

Recently, obesity and their interaction with vitamin D, type 2 diabetes and cardiovascular disease (CVD)

1. Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Near East University, Nicosia, Cyprus
2. Department of Biochemistry, Faculty of Pharmacy, Near East University, Near East Boulevard, ZIP. 99138 Nicosia, Cyprus
3. Department of Medical Genetics, Faculty of Medicine, Cyprus Health and Social Sciences University, Morphou, Cyprus. email: kalkanr@yahoo.com

Correspondence: Rasime Kalkan, Cyprus Health and Social Sciences University, Guzelyurt, Cyprus, email: kalkanr@yahoo.com

has been demonstrated. Potential biomarkers for obesity have been identified by genome-wide linkage studies and genome-wide association studies[6]. Most recently, studies are being concentrated on evaluation of SNPs (Single Nucleotide Polymorphisms) to assess the level of effect the genetic variations have in obesity development. The pathophysiology of body fat accumulation can be determined by looking at genetic and epigenetic changes in the hormones that control hunger and satiety, body glucose levels, basal metabolic rate, fat cell distribution and disposition, progenitor cell differentiation regulators, and adipocyte cell lineage-determining factors[6].

Several studies have found interaction between epigenetic modifications and the onset of obesity and environmental factors and their interaction with obesity[7].

Parathyroid hormone (PTH), one of the hormones that controls calcium, is released in response to low serum calcium levels. Studies showed the interaction between PTH level, BMI [8]and human human adipose tissue metabolism[9]. Obesity has also been associated with higher parathyroid hormone (PTH) levels[10] and PTH might promote weight gain[11,12].

The anterior pituitary gland produces follicle stimulating hormone (FSH), which affects bone remodeling through its receptor (FSHR)[13,14] and animal studies demonstrated the interaction between FSH and decreased level body fat mass[15]. *FSH* plays a key role during in human growth, development, pubertal maturation, reproduction[16], lipogenesis, inflammation[15,17], insulin sensitivity[15] and thermogenesis[18]. The pleotropic role of *FSH* makes as an important factor for metabolic disorders.

The gene encoding ERRa (*ESRRA*) is located on chromosome 11q13, and this chromosomal region related with body mass index (BMI) and fat percentage[19]. This gene produces a protein that is essential as a transcription cofactor in controlling the expression of the majority of genes involved in cellular energy production[20], mitochondrial function, biogenesis, and lipid catabolism[21,22,23]. Studies showed a connection between ESRRA-null mice and a reduction in fat mass and resistance to obesity-causing high-fat diets[24]. Several studies highlighted a direct interaction between ESRRA and adipogenesis and lipogenesis in vivo[25,26]. Estrogen-related receptor alpha (ESRRA) was another receptor which mutations of *ESRRA* plays a key role development of eating disorders[27].

Our study's goal was to examine DNA methylation in *FSHR, PTH* and *ESRRA* genes which encodes hormones and receptors in obesity and to make a epigenetic explanation in patients with obesity.

Materials and Methods

Patients

The study included 69 patients with obesity and another 76 patients without obesity were enrolled as a control group. One patient without obesity did not complete the study. Each patient was assessed prior to inclusion for the presence of malignancy, diabetes mellitus, hypertension, dyslipidemias, liver cirrhosis, thyroid, cardiovascular, or any active inflammatory condition. In this study, professional athletes were not included. All subjects completed written informed consent forms and provided information about their medical histories. The Near East University's Research Ethics Committee gave its approval to the study protocol, which was carried out in conformity with the Helsinki Declaration (Project No: SAG-2018-1-013).

Biochemical analyses

BMI was calculated by dividing body weight (kg) by the square of height (m^2). BMI ≥30 kg/ m^2 was accepted as an an indicator of obesity. In addition to this, waist to hip circumference values as ≥ 0.85 in women and ≥0.9 in man was considered to be included with the patients with obesity cohort as it is a strong indicator of abdominal obesity. Blood samples were collected after an overnight fast. Circulating levels of serum glucose, triglycerides (TG), total cholesterol, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C), insulin concentrations were measured. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated according to the previously described formula[28].

Epigenetic analyses

Blood samples were collected from patients with obesity and normal control. DNA was extracted from all subjects using Qiagan AllPrep DNA/RNA/Protein isolation kit (Qiagen, Manchester, UK) according to manufacturer's protocol and NanoDrop ND-1000 Spec-trophotometer (Thermo Fisher Scientific, Waltham, MA USA) was used to measured its

quantity. Sodium bisulfite treatment was performed by using a Epitect Bisulfite Modification kit (Qiagen, Manchester, UK). The sequences of primers were designed according to the EpiTect® HRM™ PCR Handbook (Qiagen, Manchester, UK)). *ESRRA, PTH* and *FSHR* promotor methylation were analyzed according to EpiTect® HRM™ PCR Handbook protocol (Rotor Gene Q, Qiagen).

Statistical analysis

GraphPad Prism 7 software was used for data analysis. Data in tables are presented as mean ± standard deviation (SD) for continuous variables and absolute (percentage) values for discrete variables. $p<0.05$ was considered to indicate a statistically significant difference.

Results:

Patients with obesity had a mean age of 43.17 9.62 years and a BMI of 34.92 5.83 kg/m2. The average age and BMI of the control group were 42.92 13.14 years and 23.78 2.5 kg/m2, respectively. Table 1 displays the anthropometric and metabolic features of the patients.

DNA promoter methylation status of *ESRRA, PTH, FSHR* in obese and non-obese subjects

Methylation status of *ESRRA, PTH, FSHR* in obese and non-obese subjects has been shown in Table 2.

ESRRA gene methylated in 10 out of 69 patients with obesity (14.49%) and 14 out of the 75 non-obese subjects (18.67%). There was no statistically significant difference between methylation status and obesity identified ($p > 0.001$).

FSHR gene methylated in 18 out of the 69 obese subjects (26.09%) and 35 out of the 75 non-obese subjects (46.67%). There was significant difference between methylation status and non-obese subjects ($P <0.001$) (Table 2).

PTH gene methylated in 40 out of the 69 obese subjects (57.97%) and 22 out of the75 non-obese subjects (29.33%). There was significant difference between methylation status and obesity ($P <0.001$) (Table 2).

Relationship between anthropometric and metabolic characteristics and of *ESRRA, PTH, FSHR* methylation status

The association between the levels of *ESRRA, PTH, FSHR*, insulin concentration, HOMA-IR, leptin, adiponectin, and resistin, as well as the waist and hip circumferences, body mass index (BMI), age, and levels of serum glucose, triglycerides (TG), total cholesterol, HDL-C, and LDL-C, was examined.

There is a statistically significant association detected between methylation status of *ESRRA* and fasting glucose in obese subjects ($P=0.04$). The level of fasting glucose was significantly higher in the *ESRRA* unmethylated obese subjects ($P=0.04$) (Table 3). There was a significant association between *ESRRA* methylation and HDL ($P=0.04$) cholesterol and triglyceride levels ($P=0.02$) in non-obese subjects (Table 3).

In non-obese subjects, statistically significant association detected between adiponectin, resistin levels and *FSHR* methylation status (Table 4). Adiponectin level was higher in FSHR unmethylated subjects and resistin level was higher in FSHR methylated subjects.

In our study group, there was a statistically significant association detected between BMI and methylation of *PTH* gene in obese subjects ($P=0.036$). The level of BMI was higher in PTH methylated obese subjects (36.16±6.67)(Table 5).

Discussion:

The pathophysiology of obesity involves both hereditary and epigenetic factors. Various number of studies demonstrated the interaction between obesity and epigenetic alterations. Methylation status plays an integral role in multifactorial diseases as environmental factors affect the gene expression patterns. In the case of obesity, the methylation status of involved genes are affected by gene-diet interactions[29]. Epigenetic changes in the following genes have been linked to obesity: *CLOCK, BMAL1, PER2, UBASH3A, TRIM3, LEP, ADIPOQ, PGC1, IGF-2, IRS-1, LY86, MEST, PEG3, NNAT, PLAGL1, MEG3, NPY, IL6, TNF, TFAM, GLUT4, RANKL*, and c-*FOS*[28,30,31,32,33]. Here, we have showed that *FSHR* gene tends to be unmethylated in patients with obesity whereas *PTH* gene tends to be methylated. On the other hand, *ESRRA* gene methylation was not found to have an association with obesity. Studies demonstrated that the abnormal calcium metabolism was also related with weight gain[29]. Vishnu and colleagues demonstrated interaction between obesity

and higher levels of serum parathyroid hormone[34]. They found that the increase level of serum PTH level was related with the increased grade of the obesity grade[34]. Kamycheva and colleagues analyzed calcium and PTH levels and their interaction between BMI and gender. They conclude that the highest level of serum PTH was a biomarker of obesity in both gender[8]. Kamycheva and colleagues noted that the age was an important determinant of PTH level[8]. Thus, an association between high serum PTH and obesity was demonstrated previously. The results presented here are not in line with this previous evidence as *PTH* gene has been found to be significantly methylated in patients with obesity. In addition to this, it is found that patients with obesity who have methylated PTH have higher BMI. There might be number of reasons behind the discrepancy between our results and previous evidence. In our opinion one of the most influential reason might be the difference between the populations that the cohorts were a part of. In different populations, the frequency of SNPs can vary greatly and since a significant number of genes can contribute to obesity, variation among other genes may have influenced our results. It should also be noted that life style factors were not accounted in our study. Therefore, the difference in culture and way of life might have altered the methylation patterns in *PTH* gene.

The incidence of eating disorders was found to be raised by missense mutations in the genes for histone deacetylase 4 (HDAC4) and estrogen-related receptor alpha (ESRRA)[35]. Studies demonstrated that ESRRA regulates mitochondrial activity, biogenesis, and lipid catabolism[36,37]. Luo et al showed decreased level fat mass and resistance to high-fat diet-induced obesity were observed in ESRRA-null mice[38]. In vivo studies demonstrated the interaction between ESRRA and adipogenesis and lipogenesis[39,40]. In our study, the methylation status of *ESRRA* gene was found to be non-significant when compared between patients with and without obesity. Metabolic characteristics evaluations showed that patients with obesity who have unmethylated *ESRRA* gene have higher fasting glucose levels indicating that *ESRRA* methylation status might also have a role in obesity related diabetes mellitus type II development. Contrary to this, patients without obesity have higher HDL levels and lower TG levels when *ESRRA* is unmethylated. Taken together, our results indicate that *ESRRA* gene expression contributes to obesity through disruption of hormonal balance rather than net impact on total weight gain.

Liu et al., demonstrated that the blocking of FSH action results with decreased level body fat mass in mice[15]. Cui and colleagues found an interaction between abdominal fat mass and *FSHR* mRNA expression in chickens[27]. They reported that FSH affects the expression of Dci, Lpl, RarB, Rdh10, Dgat2, and Acsl3 genes and alters lipid metabolism[27]. These studies demonstrated the importance of FSH receptors in bone loss and obesity. In our study, we found *FSHR* was significantly unmethylated in patients with obesity and significantly methylated in patients without obesity. The methylation status of *FSHR* was not able to distinguish any anthropometric and metabolic indicators in patients with obesity. However, patients without obesity have higher adiponectin if FSHR in unmethylated and have lower resistin if it is methylated. Both of these indicators and their associations with methylation status are in line with the previous evidence.

Overall, we showed a correlation between *ESRRA, PTH, FSHR* methylation status and waist, hip, and age as well as circulating levels of glucose, triglycerides (TG), total cholesterol, HDL-C, and LDL-C. We also showed a correlation between insulin concentration and HOMA-IR, as well as levels of leptin, adiponectin, and resistin in obese subjects. We found methylation status of *FSHR*, *PTH* and *ESRRA* genes were associated with obesity. Our study demonstrates the importance of epigenetic studies to be able to find out obesity associated biomarkers.

Conclusion:

Numerous obesity susceptibility genes and their significance in the disease's development have been shown by studies. There is a limited number of epigenetic studies was performed in this field. In this point of view, our study will shed light and gives critical information to further epigenetic studies.

Funding

This study was supported by Near East University Scientific Research Project Unit—Grant Number: SAG-2018-1-013. We would like to thank all the participants involved in this study.

Consent of Participate

The study protocol was approved by the Research Ethics Committee of the Near East University and performed in accordance with the Declaration of Helsinki (Project No: SAG-2018-1-013). Written informed consent form obtained from all the subjects.

Compliance with Ethical Standards

Written informed consent form obtained from all the subjects. The study protocol was approved by the Research Ethics Committee of the Near East University and performed in accordance with the Declaration of Helsinki (Project No: SAG-2018-1-013).

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' contributions:

Data gathering and idea owner of this study: RK

Study design: RK

Data gathering: EB,RK

Writing and submitting manuscript: RK,HC

Editing and approval of final draft: RK,EB,HC

Data Availability Statement

The genetics data that support the findings of this study are available on request from the corresponding author, (RK).

Table 1. The anthropometric and metabolic characteristics of studied patient population.

Parameter	Non-obese subjects (n=75)	Obese subjects (n=69)	*p*
Age	42.92 ± 13.14	43.17 ± 9.62	0.9
BMI (kg/m²)	23.78 ± 2.5	34.92 ± 5.83	<0.0001
Waist circumference (cm)	83.69 ± 8.8	113.1 ± 13.25	<0.0001
Hip circumference (cm)	100.3 ± 6.95	119.1 ± 9.56	<0.0001
Fasting glucose (mg/dL)	89.84 ± 7.45	102.1 ± 20.28	<0.0001
Total cholesterol (mg/dL)	203.1 ± 23.15	225.5 ± 35.48	<0.0001
LDL-cholesterol (mg/dL)	128.5 ± 25.23	141.7 ± 31.02	0.0056
HDL-cholesterol (mg/dL)	57.22 ± 9.01	46.77 ± 9.79	<0.0001
Triglycerides (mg/dL)	99.71 ± 43.57	166 ± 69.62	<0.0001
HOMA-IR	1.98 ± 0.67	4.62 ± 3.02	<0.0001
Leptin (ng/ml)	9.21 ± 4.98	23.89 ± 13.08	<0.0001
Adiponectin (µg/mL)	20.73 ± 9.23	10.43 ± 4.85	<0.0001
Resistin (ng/mL)	6.143 ± 2.5	8.612 ± 2.44	<0.0001

BMI body mass index, LDL low-density lipoprotein, HDL high-density lipoprotein, HOMA-IR homeostasis model assessment of insulin resistance

Table 2. Methylation status of the *ESRRA, PTH, FSHR* genes in obese and non-obese subjects

Subjects	*ERRA* gene		*FSHR* gene		*PTH* gene	
	Methylation	Unmethylation	Methylation	Unmethylation	Methylation	Unmethylation
Obese % (n/n)	14.49% (10/69)	85.51% (59/69)	26.09% (18/69)	73.91% (51/69)	57.97% (40/69)	42.67% (29/69)
Non-obese % (n/n)	18.67% (14/75)	81.33% (61/75)	46.67% (35/75)	53.33% (40/75)	29.33% (22/75)	70.67% (53/75)
P Value	*p*=0.52		*p*=0.015*		*p*=0.0004*	

Table 3. Methylation status of *ESRRA* gene and anthropometric and metabolic characteristics of obese and non-obese subjects

Parameter	Non-obese subjects			Obese subjects		
	Methylated (14)	Unmethylated (61)	p	Methylated (10)	Unmethylated (59)	p
Age	39.64±10.44	43.7±13.65	0.3	42.8±9.2	43.24±9.76	0.89
BMI (kg/m²)	23.56±1.8	23.83±2.6	0.72	33.65±10.62	35.13±5.82	0.46
Waist circumference (cm)	84.43±7.68	83.52±9.08	0.73	114.5±9.9	112.9±13.78	0.72
Hip circumference (cm)	100.7±7.08	100.2±6.98	0.82	117.5±8.82	119.4±9.72	0.57
Fasting glucose (mg/dL)	91.79±6.53	89.39±7.63	0.28	90.4±5.81	104.1±20.47	**0.04***
Total cholesterol (mg/dL)	203.1±30.74	203.1±21.35	0.99	238.7±12.21	223.3±37.66	0.2
LDL-cholesterol (mg/dL)	133.4±31.84	127.4±23.63	0.42	153.9±15.37	139.6±32.58	0.18
HDL-cholesterol (mg/dL)	53±9.56	58.25±8.67	**0.04***	49.9±15.61	46.24±8.5	0.27
Triglycerides (mg/dL)	122.7±57.67	94.43±38.32	**0.02***	164.6±37.7	166.3±73	0.94
HOMA-IR	2.052±0.62	1.964±0.69	0.66	2.82±1.6	4.48±2.89	0.03
Leptin (ng/ml)	9.766±5.19	8.972±4.97	0.59	24.86±10.88	23.72±13.49	0.8
Adiponectin (µg/mL)	17.47±9.6	21.47±9.05	0.14	9.558±3.48	10.58±5	0.54
Resistin (ng/mL)	6.027±1.98	6.039±2.43	0.45	8.367±2.93	8.654±2.38	0.73

Data are expressed as mean ± SD. For the comparison of subgroups, analysis of variance followed by Mann–Whitney U test was performed BMI body mass index, LDL low-density lipoprotein, HDL high-density lipoprotein, HOMA-IR homeostasis model assessment of insulin resistance

Table 4. Methylation status of *FSHR* gene and anthropometric and metabolic characteristics of obese and non-obese subjects

Parameter	Non-obese subjects			Obese subjects		
	Methylated (35)	Unmethylated (41)	p	Methylated (18)	Unmethylated (51)	p
Age	45.34±11.69	40.85±14.41	0.14	41.94±9.28	43.61±9.79	0.53
BMI (kg/m²)	24.24±2.3	23.38±2.55	0.13	33.81±4.61	35.31±6.19	0.35
Waist circumference (cm)	82.86±9.06	84.43±8.6	0.44	111.3±11	113.7±14	0.51
Hip circumference (cm)	100.2±7.02	100.4±6.61	0.90	117.6±6.94	119.6±10.34	0.45
Fasting glucose (mg/dL)	89.49±5.8	90.15±8.71	0.7	102.2±22.38	102.1±18.81	0.97
Total cholesterol (mg/dL)	202.5±24.26	203.7±22.42	0.82	229.4±32.55	224.2±36.67	0.59
LDL-cholesterol (mg/dL)	126.8±27.23	129.9±23.59	0.59	142.1±27.5	141.6±32.42	0.95
HDL-cholesterol (mg/dL)	58.34±8.42	56.33±9.50	0.33	47.78±10.71	46.41±9.53	0.61
Triglycerides (mg/dL)	98.06±39.63	101.2±47.2	0.76	175.5±72.75	162.7±68.91	0.5
HOMA-IR	1.871±0.59	2.077±0.73	0.18	4.49±2.63	4.477±2.5	0.98
Leptin (ng/ml)	9.219±5	9.034±5	0.87	24.01±12.33	23.85±13.45	0.96
Adiponectin (µg/mL)	18.51±7.43	22.67±10.26	**0.04***	9.393±4.95	10.79±4.81	0.29
Resistin (ng/mL)	6.809±2.82	5.382±1.83	**0.012***	8.196±2.02	8.759±2.58	0.4

Data are expressed as mean ± SD. For the comparison of subgroups, analysis of variance followed by Mann–Whitney U test was performed BMI body mass index, LDL low-density lipoprotein, HDL high-density lipoprotein, HOMA-IR homeostasis model assessment of insulin resistance

Table 5. Methylation status of *PTH* gene and anthropometric and metabolic characteristics of obese and non-obese subjects

Parameter	Non-obese subjects			Obese subjects		
	Methylated (22)	Unmethylated (53)	p	Methylated (40)	Unmethylated (29)	p
Age	36.95±10.21	45.43±13.5	**0.01***	42±9.15	44.79±10.18	0.23
BMI (kg/m²)	23.14±2.81	24.04±2.33	0.15	36.16±6.67	33.2±3.91	**0.036***
Waist circumference (cm)	81.86±9.42	84.45±8.5	0.24	114.9±14.78	110.7±10.56	0.2
Hip circumference (cm)	99.05±7.18	100.9±6.85	0.3	120.4±10.2	117.3±8.45	0.2
Fasting glucose (mg/dL)	90.82±6.75	89.43±7.75	0.46	100.5±17.51	104.3±22.36	0.42
Total cholesterol (mg/dL)	210.6±27.28	200±20.69	0.069	225.2±32.83	226±39.45	0.93
LDL-cholesterol (mg/dL)	132.2±28.86	126.9±23.68	0.41	140.4±26.78	143.5±36.5	0.68
HDL-cholesterol (mg/dL)	58.95±9.61	56.57±8.75	0.29	47.85±9.56	45.28±10.07	0.28
Triglycerides (mg/dL)	93.09±40	99.85±40.73	0.27	156.5±67.74	179.2±71.19	0.18
HOMA-IR	2.068±0.74	1.944±0.65	0.47	4.768±3.17	4.43±2.79	0.62
Leptin (ng/ml)	10.49±6.39	8.551±4.21	0.12	26.19±14.23	20.71±10.75	0.08
Adiponectin (µg/mL)	21.73±12.97	20.31±7.25	0.54	9.637±3.75	11.52±5.95	0.11
Resistin (ng/mL)	6.876±2.92	5.839±2.26	0.10	8.619±2.22	8.603±2.76	0.97

Data are expressed as mean ± SD. For the comparison of subgroups, analysis of variance followed by Mann–Whitney U test was performed BMI body mass index, LDL low-density lipoprotein, HDL high-density lipoprotein, HOMA-IR homeostasis model assessment of insulin resistance

Reference:

1. Soufi M, Sattler AM, Herzum M, Maisch B, Schaefer JR. Molecular basis of obesity and the risk for cardiovascular disease. *Herz*. 2006;**31**(3):200-6.
2. Wardle J, Carnell S, Haworth CM, Plomin R. Evidence for a strong genetic influence on childhood adiposity despite the force of the obesogenic environment. *Am. J. Clin. Nutr.* 2008; **87**(2):398–404.
3. Karita, D., Sadewa, A. H., & Hastuti, P. (2018). Association between polymorphism of lys198asn endothelin-1 gene and endothelin-1 plasma level in javanese obesity population. *Bangladesh Journal of Medical Science*, **18**(1), 46–49. https://doi.org/10.3329/bjms.v18i1.39546
4. Beardsall K, Ong KK, Murphy N, Ahmed ML, Zhao JH, Peeters MW, Dunger DB. Heritability of childhood weight gain from birth and risk markers for adult metabolic disease in prepubertal twins. *J. Clin. Endocrinol*. Metab. 2009;**94**: 3708–3713.
5. Mustelin L, Latvala A, Pietilainen KH, Piirila P, Sovijarvi AR, Kujala UM, Rissanen A, Kaprio J. Associations between sports participation, cardiorespiratory fitness and adiposity in youngadult twins. *J. Appl. Physiol*. 2011;**110**: 681–686.
6. Lyon HN, Hirschhorn JN. Genetics of common forms of obesity: a brief overview, Am. *J. Clin. Nutr*. 2005; **82**: 215S–217S.
7. Samblas, M., Milagro, F. I., & Martínez, A. (2019). DNA methylation markers in obesity, metabolic syndrome, and weight loss. *Epigenetics*, **14**(5), 421–444. https://doi.org/10.1080/15592294.2019.1595297
8. Kamycheva E, Sundsfjord J, Jorde R. Serum parathyroid hormone level is associated with body mass index. The 5th Tromsø study. *Eur J Endocrinology*. 2004; **151**(2):167-72
9. Breining, P., Pedersen, S. B., Kjolby, M., Hansen, J. B., Jessen, N., & Richelsen, B. (2021). Parathyroid hormone receptor stimulation induces human adipocyte lipolysis and browning. *European journal of endocrinology*, **184**(5), 687–697. https://doi.org/10.1530/EJE-20-0713
10. Turer CB, Lin H, Flores G (2013) Prevalence of vitamin D deficiency among overweight and obese US children. Pediatrics. 131: e152–e161
11. McCarty MF, Thomas CA () PTH excess may promote weight gain by impeding catecholamine-induced lipolysis-implications for the impact of calcium, vitamin D, and alcohol on body weight. Med Hypotheses2003; **61**:535–542
12. Ishrat, S., & Hossain, M. (). Obesity in relation to clinical, endocrine and metabolic parameters infertile women with polycystic ovary syndrome: the South Asian perspective. *Bangladesh Journal of Medical Science*, 2021; ***20***(4): 864–870. https://doi.org/10.3329/bjms.v20i4.54146
13. Sun L, Peng Y, Sharrow AC, Iqbal J, Zhang Z, Papachristou DJ, Zaidi S, Zhu LL, Yaroslavskiy BB, Zhou H, Zallone A, Sairam MR, Kumar TR, Bo W, Braun J, Cardoso-Landa L, Schaffler MB, Moonga BS, Blair HC & Zaidi M. FSH directly regulates bone mass. Cell 2006 125 247260.
14. Zaidi M, Blair HC, Iqbal J, Zhu LL, Kumar TR, Zallone A & Sun L. Proresorptive actions of FSH and bone loss. Annals of the New York Academy of Sciences 2007 1116 376–382.
15. Liu P, Ji Y, Yuen T, Rendina-Ruedy E, DeMambro VE, Dhawan S, Abu-Amer W, Izadmehr S, Zhou B, Shin AC, Latif R, Thangeswaran P, Gupta A, Li J, Shnayder V, Robinson ST, Yu YE, Zhang X, Yang F, Lu P, Zhou Y, Zhu LL, Oberlin DJ, Davies TF, Reagan MR, Brown A, Kumar TR, Epstein S, Iqbal J, Avadhani NG, New MI, Molina H, van Klinken JB, Guo EX, Buettner C, Haider S, Bian Z, Sun L, Rosen CJ, Zaidi M. Blocking FSH induces thermogenic adipose tissue and reduces body fat Nature. 2017 Jun 1; **546**(7656):107-112.
16. Papadimitriou K, Kountourakis P, Kottorou AE, Antonacopoulou AG, Rolfo C, Peeters M, Kalofonos HP. Follicle-stimulating hormone receptor (FSHR): a promising tool in oncology? *Mol Diagn Ther*. 2016;**20**:523–530.
17. Blüher M, Klöting N, Wueest S, Schoenle EJ, Schön MR, Dietrich A, Fasshauer M, Stumvoll M, Konrad D Fas and FasL expression in human adipose tissue is related to obesity, insulin resistance, and type 2 diabetes. *J Clin Endocrinol Metab*. 2014 Jan; **99**(1):E36-44.
18. Wueest S, Mueller R, Blüher M, Item F, Chin AS, Wiedemann MS, Takizawa H, Kovtonyuk L, Chervonsky AV, Schoenle EJ, Manz MG, Konrad D. Fas (CD95) expression in myeloid cells promotes obesity-induced muscle insulin resistance. *EMBO Mol Med*. 2014 Jan; **6**(1):43-56.
19. Pe´russe L, Rankinen T, Zuberi A, Chagnon YC, Weisnagel SJ, Argyropoulos G et al., The human obesity gene map: the 2004 update. *Obes Res* 2005; **13**: 381–490
20. RefSeq, Jun 2019
21. Vega, R.B.; Kelly, D.P. A role for estrogen-related receptor alpha in the control of mitochondrial fatty acid beta-oxidation during brown adipocyte differentiation. *J. Biol. Chem.* 1997;**272**: 31693–31699. [CrossRef]
22. Alaynick, W.A. Nuclear receptors, mitochondria and lipid metabolism. Mitochondrion 2008; **8**:329–337
23. Singh, B.K.; Sinha, R.A.; Ohba, K.; Yen, P.M. Role of thyroid hormone in hepatic gene regulation, chromatin remodeling, and autophagy. Mol. Cell. Endocrinol. 2017; **458**: 160–168. [CrossRef
24. Luo, J.; Sladek, R.; Carrier, J.; Bader, J.A.; Richard, D.; Giguere, V. Reduced fat mass in mice lacking orphan nuclear receptor estrogen-related receptor alpha. Mol. Cell. Biol. 2003; **23**: 7947–7956
25. Ju, D.; He, J.; Zhao, L.; Zheng, X.; Yang, G. Estrogen

related receptor alpha-induced adipogenesis is PGC-1beta-dependent. Mol. Biol. Rep. 2012, 39, 3343–3354. [CrossRef]

26. Ijichi, N.; Ikeda, K.; Horie-Inoue, K.; Yagi, K.; Okazaki, Y.; Inoue, S. Estrogen-related receptor alpha modulates the expression of adipogenesis-related genes during adipocyte differentiation. Biochem. Biophys. *Res. Commun*. 2007; **358**: 813–818. [CrossRef]
27. Cui, H., Lu, Y., Khan, M. Z., Anderson, R. M., McDaniel, L., Wilson, H. E., Yin, T. C., Radley, J. J., Pieper, A. A., & Lutter, M. (2015). Behavioral disturbances in estrogen-related receptor alpha-null mice. *Cell reports*, **11**(3), 344–350. https://doi.org/10.1016/j.celrep.2015.03.032
28. Kalkan, R., & Becer, E. (2019). RANK/RANKL/OPG pathway is an important for the epigenetic regulation of obesity. *Molecular biology reports*, **46**(5), 5425–5432.
29. Huang T, Zheng Y, QI Q et al., DNA Methylation Variants at HIF3A Locus, B-Vitamin Intake, and Long-term Weight Change: Gene-Diet Interactions in Two U.S. Cohorts. *Diabetes*, 2015; **64**(9):3146-54. doi: 10.2337/db15-0264.
30. Van Dijk SJ, Molloy PL, Varinli H, Morrison JL, Muhlhausler BS, Members of EpiSCOPE (2015) Epigenetics and human obesity. *Int J Obes* **39**(1):85–97
31. Carless MA, Kulkarni H, Kos MZ, Charlesworth J, Peralta JM et al (2013) Genetic effects on DNA methylation and its potential relevance for obesity in Mexican Americans. *PLoS ONE* **8**(9):e73950
32. Xu X et al (2013) A genome-wide methylation study on obesity: differential variability and differential methylation. Epigenetics **8**(5):522–533
33. Bouchard L, Rabasa-Lhoret R, Faraj M, Lavoie ME, Mill J et al (2010) Differential epigenomic and transcriptomic responses in subcutaneous adipose tissue between low and high responders to caloric restriction. *Am J Clin Nutr* **91**(2):309–320
34. Vishnu K, Indumathi S and Harishkiran N.E., The study of association of serum parathyroid hormone level with obesity in subjects admitted to a tertiary care centre. *International Journal of Biomedical Research* 2018; **09**(01): 40-44. Journal DOI: https://doi.org/10.7439/ijbr
35. Cui H, Zhao G, Liu R, Zheng M, Chen J, Wen J. FSH stimulates lipid biosynthesis in chicken adipose tissue by upregulating the expression of its receptor FSHR. *J Lipid Res*. (2012) **53**:909–17. doi: 10.1194/jlr.M0 25403
36. Alaynick, W.A. Nuclear receptors, mitochondria and lipid metabolism. Mitochondrion 2008; **8**: 329–337.
37. Villena, J.A.; Kralli, A. ERRalpha: A metabolic function for the oldest orphan. Trends Endocrinol. Metab. 2008;**19**,:269–276.
38. Luo, J.; Sladek, R.; Carrier, J.; Bader, J.A.; Richard, D.; Giguere, V. Reduced fat mass in mice lacking orphan nuclear receptor estrogen-related receptor alpha. *Mol. Cell. Biol*. 2003, 23, 7947–7956
39. Ju, D.; He, J.; Zhao, L.; Zheng, X.; Yang, G. Estrogen related receptor alpha-induced adipogenesis is PGC-1beta-dependent. *Mol. Biol. Rep*. 2012, 39, 3343–3354.
40. Ijichi, N.; Ikeda, K.; Horie-Inoue, K.; Yagi, K.; Okazaki, Y.; Inoue, S. Estrogen-related receptor alpha modulates the expression of adipogenesis-related genes during adipocyte dierentiation. Biochem. Biophys. *Res. Commun*. 2007, 358, 813–818.