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STUDY OF FNDC5 GENE POLYMORPHISM Rs3480 AND RS16835198 WITH OBESE PATIENTS AND SPORT INDIVIDUAL IN IRAQI POPULATIONS

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ABSTRACT : Weight of body maintenances is a result of the equilibrium among energy expenditure and intake. Inequality at either the level of consumption and energy expenditure given in underweight or overweight and obesity. The Irisin activation of browning operation, which is utilities in monitoring for diabetes, obesity and other energy-irregular related abnormal has been explored leading to great potentials surrounding "irisinemia" as novel concept in the guess disorders of metabolic. FNDC5 is a Fibronectin Type III Domain containing protein 5 a Molecular mass 23659 Da (23.659 KDa) with 212 amino acids an N-terminal 29-residue signal sequence, the FNDC5 is one of the target proteins of PPARγ coactivator-1 α (PGC1α) where discovered during a genome search for fibronectin type III domains FNDC5 is composed of a signal peptide, a fibronectin III domain and a hydrophobic C-terminal domain. From total number of participants of present study chosen one hundred (100) for Genetic study and correlation, it with clinical parameter. Where divided into (50) obese Group individual and (50) control group, which divided to the sub groups, (25) Control Sport apparently healthy individuals. The phenotypic clinical parameters analysis by spectrophotometric methods, while ELISA determined Insulin and Irisin concentrations. Genotyping of FNDC5 gene rs3480A/G and rs16835198G/T SNPs in obese persons by real time PCR technique. The analysis of results by using various descriptive statistical analyses and Hardy-Weinberg equilibrium (HWE) were used to evaluate data. The genotyping results of the studied SNPs were analysis within Hardy-Weinberg equilibrium the distributions genotype and allele frequencies for the observed gene polymorphisms among the obese and sport control for the FNDC5 gene for rs3480A/G SNP is shown the high significance in the sport control compared with obese group they were the frequency of homozygous (AA) and heterozygous (AG) and homomutant (GG) were 56.0%, 36.0% and 8.0% respectively while in obese patient group were 14.0%, 50.0% and 36.0% respectively and the result revealed frequencies of the A allele of 58.0% and 39.0% and G allele 32.0% and 61.0% in sport control and obese group respectively with OR 4.4 ((2.1-9.4) p =0.0001). But the result of FNDCF gene rs16835198G/T SNP shown significant differences of genotypes (GG), (GT) and (TT) where the distribution frequency in comparison of sport with obese patient result the significance different of genotypes of homozygote(TT) the distribution frequency in both groups as follows: sport control 16.0% and the obese patient 34.0% where OR 5.6, 95%CI (1.4-21.5), P = 0.011 for TT genotypes.

Biochemical characteristics were observed the result of comparison of the genotypes of FNDC5 gene SNPs with the clinical parameters reveals to the significance of rs3480A/G SNP with LDL-c level (P= 0.043) and the rs16835198 G/T SNP with gender (p = 0.031). The results revealed of with the clinical parameters reveals GG genotype and G allele of FNDC5 gene rs3480 (A/G) SNPs polymorphism and TT genotype and T allele of FNDC5 geners16835198 (G/T) SNPs might be genetic risk factor for obesity.

Key words : Irisin, exercise, obesity, FNDC5, SNPs.

INTRODUCTION

Weight of Body maintenances is a result of the equilibrium between energy expenditure and intake. Inequality either at the level of consumption and/or energy expenditure given in underweight on the other side or overweight and obesity (Stengel *et al*, 2013; Al-Daghri *et al*, 2014). Skeletal muscle can secretion substances as a cytokines or other specific protein collectively named myokines, through which it interacts with other tissues such as the adipose tissue (Lightfoot and Cooper, 2016; Ouchi *et al*, 2016; Anastasilakis *et al*, 2017) able of modulating metabolic pathway function as hormones. A novel peptide myokine named 'irisin'. This led the researchers to name the secreted polypeptide irisin, the Greek messenger goddess (Atherton and Phillips, 2013; Sanchis and Perez, 2013a). Irisin, the secretedas a product of fibronectin type III domain containing 5 protein (FNDC5), is a newly identified peptidicmyokine, where

the peroxisome proliferator-activated receptor coactivator-1-α (PGC1)-α activation response to regulate of Irisin (Bostrom *et al*, 2012). Its stimulates a brown process converting in white adipose tissue, acts on cells of white adipose tissue (WAT) which rises expenditure of energy (Alis *et al,* 2014) and has been offered to positive beneficial effects of exercise training on metabolism, inducing the 'browning' of adipocytes subcutaneous and thermogenesis by elevated uncoupling protein 1 (UCP1) levels, enhanced of expenditure energy (Moreno-Navarrete *et al*, 2013; Al-Daghri *et al*, 2014).

Irisin, the novel myokine secreted as a product of fibronectin type III domain containing 5 protein (FNDC5), is a newly recognized peptidicmyokine, Irisin is regulated by response to(PGC1)-α peroxisome proliferator-activated receptor coactivator-1-α activation. It promotes a browning process (converted the with adipose tissue to the brown) and the useful effects of exercise on metabolism, encouraging the 'browning' of subcutaneous thermogenesis and adipocytes by elevated UCP1 levels and, hence, improved energy expenditure. Therefore, the current study was designed to elucidate to the association (Fibronectin Type III Domain-Containing Protein 5) FNDC5/irisin gene of the SNPs rs3480A/G and rs16835198G/T and rs726344A/G polymorphism and circulation of irisin level with obesity in Iraqi population.

The Irisin activation of browning operation which is utilities in monitoring for diabetes, obesity and other energy-irregular related abnormal has been explored (Spiegelman, 2013) leading to great potentials surrounding "irisinemia" as novel concept in the guess disorders of metabolic (Sanchis and Perez, 2013b).

A peptidic myokine is a novel product named 'irisin' recently identified by Boström *et al* (2012). A bodily activity life plays an pivotal role in protection and preventable metabolic syndrome, Coronary heart disease (CHD), cardiovascular disease (CVD) and T2DM. Thus, induction of myokine by training has suggested preventable and/or improve numerous illnesses(Anon 2012). Irisin concentrations level increase significantly after taken exercise in humans (Sanchis and Perez, 2013a). Exercise is the first mark of therapy for numerous metabolic diseases such as diabetic and obesity, but also progresses outcomes in diseases involving other peripheral tissues, such as the heart, liver and brain (Jedrychowski *et al*, 2015). Exercise is identified to induce weight loss which is more than probable due to energy expenditure during exercise (Anon n.d.) and physical exercise aids a variety of organ systems in living thing including insulin sensitivity and obesity. However. Many revisions have highlighted the role of exercise in several body part systems as a liver, adipose tissue, brain, and heart. The exercise affects the skeletal muscle directly between all other organs 3 (Pedersen and Febbraio, 2012; Panati *et al*, 2016).

There are more than one thousand genes are stimulated in skeletal muscle response to exercise and donate to improved health (Pedersen and Febbraio, 2012; Pinto *et al*, 2012; Thompson *et al*, 2012). Indeed, deficiency of exercise is a major reason of chronic diseases like muscle aging, obesity, metabolic syndrome, insulin resistance.

Genetic involvement obesity

The study of epidemiological and heritability proved the genetic factor contribution in obesity. Human genetic research pointed at identifying genetic basics associated with obesity started to appear in the late 19 century (Rojas *et al*, 2013). Rapid growths in the prevalence of overweight and obesity due to of lifestyle factors that good example to the etiology of obesity (Gull, 2016).

Together genetic issues and lifestyle factors have clear factor of the risk to the overweight, the Body Mass Index as a characteristic is determined not only by environmental reasons, but also basically by interaction through genetic factors (Rojas *et al*, 2013). Gene environment interaction (GEI) can be distinct as "a variety of genetic factors on illness risk in the persons with different environmental contacts" (Ottman, 1996). Some of studies about the twin and family have submitted that an person with a personal past of obesity has 1.5 to 5 times higher risk of obesity associated to the risk in population at extra-large (Lee and Price, 1997; Ziegler *et al*, 1997; Katzmarzyk *et al*, 1999).

The first study about gene relationship with obesity derive from study of the single gene complaints. Named Monogenic overweight that is caused by the single mutation. There are numerous different monogenic formulas of obesity have been labeled, where the first monogenic mutation caused by alterations in the leptin gene that leading to severe obesity, that which identified in 1997 (Montague *et al*, 1997; Gull Rukh, 2016).

MATERIALS AND METHODS

One hundred (100) for genetic study and correlation it with clinical parameter, where divided to the sub Group, (50) obese individual and sub group (25) lean Sport Control apparently healthy individuals. Subjects with, coronary artery disease, diabetes mellitus, thyroid diseases and renal disease, nephropathy or liver disease were excluded from the study. However, after 12 hours overnight fasting, (6 ml) of venous blood were withdrawn from every subject by sterile searing and divided into three tubes;2

ml of blood were transferred into two EDTA tubes: one of them was used for quantitative colorimetric determination of glycated hemoglobin using kits supplied by StanBio USA, 2ml of blood to other EDTA tube for genotyping of irisin SNP to the extraction DNA by using FavorPrep™, Korea, 2 ml of blood was placed in plain tubes and took serum after centrifugation. Sera were used for colorimetric determination of LDL, TG, TC, HDL and irisin concentration.

The *FNDC5* gene was estimated by RT-PCR. To confirm the distribution of genotypes by qualitative real time RT-PCR TaqMan assay used. Primers sequences for *FNDC5* gene was design according synthesized by Alpha DNA Ltd (Canada) and stored lyophilized at (-23°C) until use.primers and probes were purchased from Alpha DNA/Canada: 1) rs3480 (F-primer) 5'CCAGCCTTGAGAGCTCTTGT3', (R-primer)5'CAGATGCCTGCATGGGAAGA 3', FAM 5'ATTAGGTGATGGCTTCTGG 3' and VIC-5' TGGGTGATGGCTTCGG3'; 2)-rs16835198 (F-primer)5' AGAGAATTAAGCCTGAGG3', (R-primer)5' GTGGGAGAAATGCTGGTCTGA3', FAM-5' AGAGAATTAACTTGCCTGAGG3' and VIC- 5' GATAATTAACTTGCCTGAGGT3'. Genotyping for (rs3480A/G) SNPs and (rs16835198G/T). SNPs was achieved by using TaqMan SNP Genotyping Assay (cepheid) *SmartCycler* Real-Time thermo cycler, according to manufacturer's recommendation.

The amplified product detected with the use of fluorescent dyes. These dyes are linked to oligonucleotide probes, which bind specifically to the amplified product during thermo cycling. The genotyping reaction of mixture for amplification of SNPs was prepared by mixing 10l rt-PCR Master (TaqMan), 3.2ì1 of the genotyping assay mix (probe and primers revers and forward). For each unknown reaction 2ì l DNA template and 4.8ì l of PCR, grade water added.

The cycling parameters were set as follows: initial denaturation step at 95°C for 5 min, 40 cycles of denaturation at 95°C for 20s, annealing at 60°C for 30s and extension at 72°C for 20s and a final extension step at 72°C for min.

Statistical analysis

Results collected and statistically analyzed by personal computer and statisticalprogram SPSS version 23 (IBM Corporation, Armonk, NY, USA). T-test was used for comparison between two groups having quantitative variables. Analysis of variance (ANOVA) was used for comparison between three or more groups having quantitative variables. Multiple regression analysis was performed to calculate the effects of risk factors. The Hardy-Weinberg equilibrium (HWE) was applied to study the frequencies of allele and genotype. The odds ratios (ORs) and 95% confidence intervals (95% CIs) was used to evaluate the potential associations between genetic variants of (rs3480A/G) and (rs16835198G/T) genes and the risk of obesity in this study compered with sport group. P value for all tests was considered significant if <0.05.

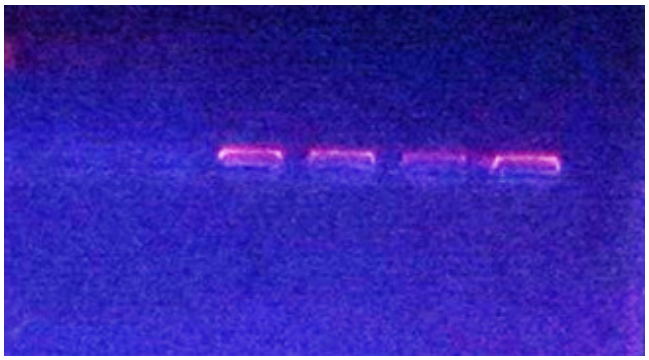

Fig. 1 : Gel electrophoresis for DNA extraction on agarose gel 1%, after electrophoresis at 70 volt for 30 minute.

RESULTS AND DISCUSSION

This study conceded the first study in Iraq shed light on the relationship between FNDC5 gene of obesity and approximately healthy sport as a control, to understanding the associated effects of genetic variants on weight changes at different ages may help to understand what the effect of FNDC5 gene on the obesity. This study achieved genetic analysis (SNPs) on a case control of 50 obese individuals, compared the results with those obtained in healthy controls 25 sport SNPs (rs3480A/G and rs16835198G/T) were analyzed by Hardy-Weinberg equilibrium for all of the variants investigated in the FNDC5 gene.

Genomic DNA was extracted from blood samples of subjects (obese and control groups). Results showed in Fig. 1 that there are a clear DNA bands obtained after DNA extraction and electrophoresis on agarose gel (1%). Depending on these, the purity (1.9) of DNA solutions were recommended and suitable for genetic analysis and the concentrations mean was 23 µg/ml measured by nanodropusing. the purity of DNA solutions were recommended and suitable for further genetic analysis by using real time PCR technique as mentioned by Boesenberg-Smith *et al* (2012).

Polymorphisms of Single Nucleotide at position rs3480 in the FNDC5 gene between obese patient and sport groups

The prevalence of FNDC5 rs3480 SNPs AA, AG, and GG genotypes obese group was shown in table 1 It

Table 1 : Comparison of the Genotype and Allele Frequencies of FNDC5 gene polymorphism rs3480 between Sport group and obese patient group.

rs3480	Frequencies (%)		P value	Odd ratio (95% CI)
	Sport(n=25)	Obese Patient (n=50)		
AA	56.0 (n=14)	14.0 (n=7)	—	1.00 (Reference)
AG	36.0 (n=9)	50.0 (n=25)	0.004**	5.5 (1.6-18.1)
GG	8.0 (n=2)	36.0 (n=18)	0.001**	18.0 (0.3-10.0)
Allele frequency				
A	58.0 (34)	39.0 (39)	—	1.00 (Reference)
G	32.0 (16)	61.0 (61)	0.0001**	4.4 (2.1-9.4)

Significance difference* p 0.05, ** p< 0.05.

Table 2 : Comparison of the Genotype and Allele Frequencies of FNDC5 gene polymorphism rs16835198 between Sport group and obese group.

rs16835198	Frequencies (%)		P value	Odd ratio (95% CI)
	Sport(n=25)	Obese Patient (n=50)		
GG	28.0 (n=13)	24.0 (n=11)	—	1.00 (Reference)
GT	32.0 (n=8)	42.0 (n=20)	0.06	2.9 (0.9-9.3)
TT	16.0 (n=4)	34.0 (n=19)	0.011	5.6 (1.4-21.5)
Allele frequency				
G	68.0(n=34)	42.0 (42)	—	1.00 (Reference)
T	32.0 (16)	58.0 (58)	0.0032**	2.9 (1.4-5.9)

Significance difference** p< 0.05

revelations 14% (n=7) persons with wild type AA and 50% (n = 25) individuals with heterozygous AG. The mutant homozygous GG was seen in 36% (n=18) subject.

While in control group (sport), the highest genotype was the A/A (56%) and A/G (36%) followed by GG (8%) genotype as showed the odds ratio for the rs3480 AG was 5.5 (1.6-18.1), p = 0.004. The difference from the control sport was statistically significant with obese persons, p<0.05. Besides, there is rs3480 GG revealed a statistically significant difference from the Healthy sport with an odd ratio of 18.0 (0.3-10.0), P-value = 0.001 compared with obese patients.

FNDC5 rs3480 G polymorphism was found for allele, the OR was 4.4 (2.1-9.4) (95%CI); p=0.0001 with significant difference from the control sport P< 0.05 while compared with obese patient.

Relationship of genotype distributions and allele frequencies of FNDC5 gene for SNP rs16835198 G/T SNPs polymorphisms with obese and sport control group

The genotype distributions and allele frequencies for the observed gene polymorphisms between the obese and control sport are presented in Table 2, Fig. 3 the allelic distributions where shown the distribution of genotype and allele frequencies between obese patients group compared with apparently healthy control sport for the FNDC5 gene to SNPrs16835198 is shown the genotypic frequencies of obese were 24.0% (n = 11) normal GG and 42.0% (n = 20) heterozygous GT. The mutant homozygous was set up in TT 34.0% (n=19). In the healthy sport controls, the results display 28.0% (n=13) wild type GG, 32.0% (n=8) heterozygous GT and the mutant homozygous TT 16.0% (n=4). The results of genotype frequencies of obese individual's study reveal that the wild type allele and wild type genotype occupied as reference.

The browning effects contributed of muscle created irisin, observed only in SAT (10), we have found that FNDC5 in muscle was linked to the UCP1 level (Jose´ *et al*, 2012).

This current study detected an exercise-induced increase of FNDC5/Irisin in human muscle and adipose tissue beneficial effects on metabolism, where inducing the browning of adipocytes and thermogenesis by elevated uncoupling protein 1 (UCP1) levels that increase the fat consumption.

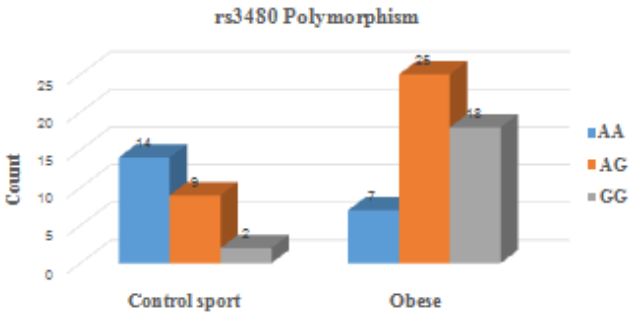

Fig. 2 : Genotype distribution of the FNDC5 rs3480 (A/G) polymorphism between two studied groups.

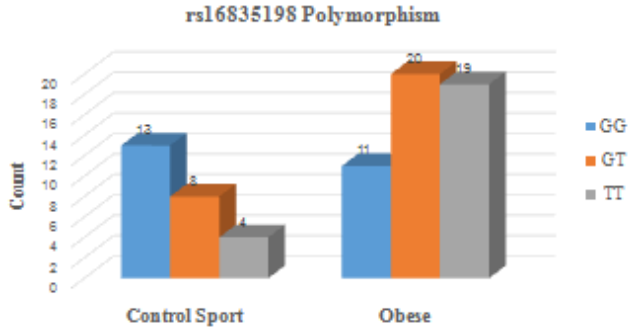

Fig. 3 : Genotype distribution of the FNDC5 rs16835198 (G/T) polymorphism between two studied groups.

Table 3 Clinical parameters of the obese patients with genotypes of FNDC5 rs16835198 (G/T).

rs16835198 G/T				
Clinical parameters	**GG**	**GT**	**TT**	**P value**
Gender M/F	1.09±0.30	1.35±0.48	1.05±0.22	0.031*
Age/ year	40.18±12.13	40.20±11.15	36.65±7.32	0.476
BMI (kg/m2)	31.44±3.36	33.40±4.55	31.94±3.54	0.340
WHR	0.96±0.07	1.03±0.38	0.95±0.05	0.551
Systolic/ mmHg	12.40±1.11	12.82±1.26	12.45±1.14	0.524
Diastolic/ mmHg	8.18±0.33	8.60±1.27	8.22±0.75	0.354
FBG mg/dl	101.52±16.53	99.76±26.49	94.64±14.42	0.605
TG mg/dl	184.88±48.95	194.60±46.42	193.50±61.55	0.879
TC mg/dl	247.27±64.66	270.69±82.98	236.00±58.15	0.295
HDL-C mg/dl	74.59±25.16	81.21±27.92	81.10±18.65	0.726
VLDL mg/dl	36.97±9.79	38.92±9.28	38.69±12.31	0.879
LDL mg/dl	135.70±73.29	150.56±88.87	116.19±60.74	0.362
HbA1c mg/dl	7.25±0.86	7.84±1.50	7.67±1.06	0.440
Insulin µUI/ml	11.83±6.52	11.27±7.05	8.84±5.80	0.365
HOMA-IR	3.08±1.97	2.84±2.18	2.12±1.56	0.335
Irisin ng/ml	8.67±0.89	7.26±2.72	8.87±0.82	0.018*

*Significance difference p 0.05

Association among the FNDC5 gene SNPs and the clinical parameters

The determination the effect of FNDC5 gene SNPs on clinical and biochemical parameters focus in this study which analyzed by the levels parameters and various genotypes.

The results of comparison three different genotypes of FNDC5 rs3480 polymorphism (AA, AG and GG) with serum clinical parameters of obese patients are presented in the Table 2. There was no significant between genotypes and most clinical parameters such as gender, age, BMI, and WHR and with lipid profile TC, TG and HDL-c except LDL-c was significance difference in subjects (obese patients) with AA genotype and also the study showed there is no significance with blood pressure systolic anddiastolic when compared with genotypes in the obese patient.

This study agree with Al-Daghri (2016), which found no significance with most clinical parameter when compared with genotype exception with AA genotype was associated with higher HDL-c levels and BMI (Al-Daghri *et al*, 2016) which related to Peroxisome proliferator-activated receptor alpha (PPAR-α), a main transcriptional regulator of lipid metabolism, is up regulated in adipose cells treated with FNDC5 as mentioned by Wu *et al* (2012).

In contrast, the result of this study agree with Staiger *et al* (2013) did not detect any association between rs3480 polymorphism and parameters of body mass index and WHR, also found did not detect any association or significance with insulin level and HDL-c. In addition, this result disagree with Abdu Allah *et al* (2017) were detected the rs3480 associates with body mass index (BMI).

Previous researches reported that irisin might be beneficial not only for the preventable of obesity and T2DM, but also for a wide-ranging of pathological conditions characterized by an imbalance between energy

intake and energy expenditure which convention with the results in this study.

The result of comparison three different genotypes of FNDC5 rs16835198 SNPs G/T polymorphism (GG, GT and TT) with serum clinical parameters of obese patients were presented in Table 3 showed the no significant effect with most clinical parameters such as gender, age, BMI and WHR and with lipid profile TC, TG, and HDL-c except irisin was elevated significance difference with homozygote TT genotype p = 0.018 and also the study showed the no significance effect with blood pressure systolic anddiastolic when compared with genotypes in the obese patient like the results of genotype SNP rs3480 (A/G).

In our opinion of this study, the high levels of insulin and insulin resistance considered risk factor of obese where to limited this risk should be increase regular exercise to increase irisin level secretion that make reduced insulin resistance in obese person and contributed by 'browning' (process converted white adipose tissue to the brown adipose tissue), and elevated energy expenditure and fat consumption that due to decreased weight gain.

CONCLUSION

The results indicate that GG genotype and G allele of FNDC5 rs3480 A/G polymorphism and TT genotype, T allele of FNDC5 rs16835198 G/T SNPs might be genetic risk factor for obesity and the irisin level significance with the metabolic parameters and clinical significance deference circulation of the irisin levels between sport and obese patient and between non-sport with obese individuals that preventable of obesity, T2D and decrease insulin resistance. Also, increase energy expenditure by activation the browning and thermogenesis process.

REFERENCES

Al-Daghri Nasser M (2014) Irisin as a Predictor of Glucose Metabolism in Children: Sexually Dimorphic Effects. *European Journal of Clinical Investigation* **44**(2), 119–124.

Al-DaghriNasser M (2016) SNPs in FNDC5 (Irisin) are Associated with Obesity and Modulation of Glucose and Lipid Metabolism in Saudi Subjects. *Lipids in Health and Disease* **15**(1), 54.

AlisRafael (2014) Association between Irisin and Homocysteine in Euglycemic and Diabetic Subjects. *Clinical Biochemistry* **47**(18), 333–335.

Anastasilakis Athanasios D (2017) Circulating Irisin Levels Are Lower in Patients with Either Stable Coronary Artery Disease (CAD) or Myocardial Infarction (MI) versus Healthy Controls, Whereas Follistatin and Activin A Levels Are Higher and Can Discriminate MI from CAD with Similar to CK. *Metabolism : Clinical and Experimental* **73**(Mi), 1–8.

Anonymous (2012) The Skeletal Muscle–Metabolism Axis in Prostate-Cancer Therapy. *New England Journal of Medicine* **367**(23), 2257–2258.

Anon. n.d. Irisin, a Novel Myokine Responsible for Exercise Induced.Pdf.

Atherton P J and Phillips B E (2013) Greek Goddess or Greek Myth: The Effects of Exercise on Irisin/FNDC5 in Humans. *Journal of Physiology* **591**(21), 5267–5268.

Bostrom P (2012) A PGC1-Alpha-Dependent Myokine That Drives Brown-Fat-like Development of White Fat and Thermogenesis. *Nature* **481**(7382), 463–468.

Gull Rukh (2016) Genetic Determinants of Obesity in Relation to Diet, Weight Gain and Mortality. Pdf. Lund University.

Jedrychowski Mark P (2015) Detection and Quantitation of Circulating Human Irisin by Tandem Mass Spectrometry.

Jose´ M M, Francisco O, Marta S, Ester G, Gerard P, Francisco T, Wifredo R and Jose´ Ma´ndez-Real and Department (2012) Irisin is Expressed and Produced by Human Muscle and Adipose Tissue in Association with Obesity and Insulin Resistance. 1–10.

Katzmarzyk P T, Perusse L, Rao D C and Bouchard C (1999) Familial Risk of Obesity and Central Adipose Tissue Distribution in the General Canadian Population. *American Journal of Epidemiology* **149**(10), 933–942.

Lee J H, Reed D R and Price R A (1997) Familial Risk Ratios for Extreme Obesity: Implications for Mapping Human Obesity Genes. *International Journal of Obesity and Related Metabolic Disorders/ : Journal of the International Association for the Study of Obesity* **21**(10), 935–940.

Lightfoot Adam P and Robert G Cooper (2016) The Role of Myokines in Muscle Health and Disease.*Current Opinion in Rheumatology* **28**(6), 661–666.

Montague C T (1997) Congenital Leptin Deficiency is Associated with Severe Early-Onset Obesity in Humans. *Nature* **387**(6636), 903–908.

Moreno-Navarrete Jose Maria (2013) Irisin is Expressed and Produced by Human Muscle and Adipose Tissue in Association with Obesity and Insulin Resistance. *The Journal of Clinical Endocrinology and Metabolism* **98**(4), E769-E778.

Ottman R (1996) Gene-Environment Interaction: Definitions and Study Designs. *Preventive Medicine* **25**(6), 764–770.

Ouchi N, Koji O, Rei S and Toyoaki M (2016) Protective Roles of Adipocytokines and Myokines in Cardiovascular Disease. *Circulation Journal/ : Official Journal of the Japanese Circulation Society* **80**(10), 2073–2080.

Panati K, Suneetha Y and Narala V R (2016) Irisin/FNDC5—An Updated Review. *European Review for Medical and Pharmacological Sciences* **20**(4), 689–697.

Pedersen Bente K and Mark A Febbraio (2012) Muscles, Exercise and Obesity: Skeletal Muscle as a Secretory Organ. *Nature Reviews Endocrinology* **8**(8), 457–465.

Pinto A (2012) Effects of Physical Exercise on Inflammatory Markers of Atherosclerosis. *Current Pharmaceutical Design* **18**(28), 4326–4349.

Rojas J, Miguel A, Manuel V and Valmore B (2013) Obesity Genetics: A Monopoly Game of Genes. *American Journal of Therapeutics* **20**(4), 399–413.

Sanchis-Gomar F and Carme P (2013a) Irisinemia: A Novel Concept

to Coin in Clinical Medicine? *Annals of Nutrition and Metabolism* **63**(1–2), 60–61.

Schumacher M A, Nagababu C, Tomoo O, Riddhi S S and Harold P E (2013) The Structure of Irisin Reveals a Novel Intersubunit â-Sheet Fibronectin Type III (FNIII) Dimer. *Journal of Biological Chemistry* **288**(47), 33738–33744.

Spiegelman Bruce M (2013) Banting Lecture 2012: Regulation of Adipogenesis: Toward New Therapeutics for Metabolic Disease. *Diabetes* **62**(6), 1774–1782.

Staiger Harald (2013) Common Genetic Variation in the Human FNDC5 Locus, Encoding the Novel Muscle-Derived 'Browning' Factor Irisin, Determines Insulin Sensitivity. *PLoS ONE* **8**(4).

Stengel, Andreas (2013) Circulating Levels of Irisin in Patients with Anorexia Nervosa and Different Stages of Obesity—Correlation with Body Mass Index. *Peptides* **39**, 125–130.

Thompson D, Fredrik K, Max L and Keith F (2012) Physical Activity and Exercise in the Regulation of Human Adipose Tissue Physiology. *Physiological Reviews* **92**(1), 157–191.

Wu Jun (2012) A PGC1- a -Dependent Myokine That Drives Brown-Fat-like Development of White Fat and Thermogenesis.

Ziegler A, Schafer H and Hebebrand (1997) Risch's Lambda Values for Human Obesity Estimated from Segregation Analysis. *International Journal of Obesity and Related Metabolic Disorders/ : Journal of the International Association for the Study of Obesity* **21**(10), 952–953.