Journal of Applied Pharmaceutical Science Vol. 7 (09), pp. 213-218, September, 2017
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2017.70929
ISSN 2231-3354

Effect of Ergonomic Exercises in Modifying Sirtuin1 Gene Expression in Obese Down Syndrome Patients

Hala M. Zeidan[1]*, Mohamed Maher Ahmed[1], Adel F. Hashish[1], Hend Rashad[2], Ehab R. Abdol Raouf[1]

[1]Research on Children with Special Needs Department, Medical Research Division, National Research Centre, Cairo, Egypt.
[2]Occupational and Environmental Medicine Department, Environmental Research Division, National Research Centre, Cairo, Egypt.

ARTICLE INFO

Article history:
Received on: 16/05/2017
Accepted on: 14/07/2017
Available online: 30/09/2017

Key words:
Obesity, Down syndrome, Sirtuin 1, gene expression.

ABSTRACT

The current study was conducted to investigate the effect of ergonomic exercises on modifying SIRT1 gene expression in obese Down syndrome patients. Forty obese Down syndrome patients (IQ level > 60) were included in current study. Their age were ranged from 14 to 18 years old, their body mass index (BMI) was ranged from 30kg/m^2 to 39.9 kg/ m^2, These patients were divided randomly and equally into two groups GI composed of twenty obese patients with down syndrome. They were received indoor ergonomic exercises two times / week for 3 months plus balanced diet and GII composed of twenty obese patients with Down syndrome. They were received balanced diet only. Data were obtained from each patient for BMI and SIRT1 level. Measurements were performed before study and after three months. Statistical analysis revealed that there was reduction of BMI within both groups after treatment in comparison to pre-treatment ($p<0.05$). Ergonomic exercises with balanced diet has a significant effect in reduction BMI and waist circumference (p; 0.0001* & 0.019*, respectively). Fold change as indicator for SIRT1 gene expression was changed in GII and GI respectively (40%, 27.27%). These results indicated that BMI and waist circumference reduced with exercises which may be an effective tool in modifying gene expression in Down syndrome obese patients.

INTRODUCTION

Down syndrome (DS) children have typical characteristic facial features, physical growth delays and mild to moderate intellectual impairment. This genetic disorder results from the presence of a piece or even an extra copy of chromosome 21 which means those DS patients have 47 chromosomes. 95% of DS cases have meiotic non-disjunction that result in trisomy 21. Robertsonian translocation and mosaic type are the other types of DS. Children with DS are shorter than normal age matched individuals and may have health problems including; immune dysfunction, leukemia, gastrointestinal abnormalities, heart and vision diseases, Alzheimer's disease, hypothyroidism and diabetes mellitus (Aburawi *et al*., 2015). Furthermore, Down syndrome has a higher risk for developing obesity. Childhood obesity is accompanied with health problems throughout life that may slow or reverse other health gains for children with DS. In addition, Obesity is a stigmatizing condition and can be another characteristic that identifies those children as different (Koebnick *et al*., 2012). The primary care providers can help children and adolescents with DS those are at higher risk to be obese by establishing an early screening and management plan to avoid or treat weight gain (Murray and Ryan-Krause, 2010). Sirtuin 1(SIRT1) stands for sirtuin (silent mating type information regulation 2 homolog) 1. It is also known as NAD+-dependent deacetylase sirtuin 1. This protein encoded by the SIRT1 gene in human. SIRT1 enzyme deacetylates proteins that contribute to cellular regulation. It also affects the activity of both members of the PGC1-alpha/ERR-alpha complex, which are essential metabolic regulatory transcription factors (Zhao *et al*., 2014; Schug and Li, 2011).

* *Corresponding Author*
Tel: +201208434272-, Fax: +202-33370931
E-mail: halazeidan @ yahoo.com

SIRT1 is activated during calorie restriction which may be related to energy balance through glucose or lipid metabolism and insulin signaling. Therefore, SIRT1 suggested playing a significant role in the pathophysiology of visceral obesity. In addition, SIRT1 expression levels were negatively correlated with body mass index, waist circumference, and abdominal visceral fat area. The significant association between abdominal visceral fat accumulation and SIRT1 gene expression in circulating peripheral blood mononuclear cells (PBMCs) suggests that SIRT1 may be a new therapeutic target for the prevention of disease related to obesity, especially visceral obesity (Lee *et al*., 2013; Nasrin *et al*., 2009).

Essential factors to be consider before taking the decision for operation including the patient's; mental status, BMI, eating habits, health conditions related to obesity, and previous stomach surgeries (Mechanick *et al*., 2013).

Aerobic exercises are exercises having an adequate oxygen supply to the working groups of muscles without exhaustion. A wide variety of physical activities considered as exercises including yard work and many recreational activities such as dancing, tennis, basketball, and golfing. Also the traditional aerobic exercises (e.g., walking, running, bicycling, and swimming), stretching, and resistance training are considered to improve flexibility and muscle strength (Green and Smith, 2017).

There are two important factors in formulating an aerobic training program. The training must be graded to provide a sufficient cardiovascular overload to stimulate increases in stroke volume and cardiac output. This central overload should be accomplished with appropriate late muscle groups to concurrently enhance the local circulation and metabolic machinery within the specific muscles. This essentially embodies the specificity principle as applied to aerobic training. Brief bouts of repeated exercise (interval training) as well as continuous, long-duration work (continuous training) enhance aerobic capacity. Interval training, continuous training and farther training are 3 common methods to improve aerobic (Trigiani and Hamel, 2017; Green and Smith, 2017).

Therefore this current study is an attempt to evaluate the effect of ergonomic exercises on modifying SIRT1 gene expression in obese Down syndrome**.**

SUBJECTS AND METHODS

Subjects

Forty obese Down syndrome patients were included in current study. Their IQ level was greater than 60 . their ages were ranged from 14 to 18 years. Their body mass index was between 30 Kg/m² And 39.9 Kg/m² (moderate obesity). They were free from any pathological disorders or previous surgery which may affect the study and application of treatment modalities. The subjects were divided equally and randomly into two groups: Group I; In this group, twenty obese patients with Down syndrome were received a balanced diet and indoor ergonomic exercises therapy (each session was 30 minutes, 2 times per week for three months) and Group II; In this group, twenty obese patients with Down syndrome were received balanced diet only**.** Weight was determined, height was measured, then the body mass index was calculated where: Body mass index (BMI) = body weight (Kg)/ height $(m)^2$ (Ellulu *et al*., 2014). Skin fold was measured by using Electronic skin caliper and abdominal circumference was recorded for each patient.

Procedures

Therapeutic equipment

Bicycle ergometer

Electronic bicycle ergometer (Universal, made in New York, USA) equipped with pedals, electronic break, adjustable seat, handle bar, display screen and foot straps also provided with programmable control unit.

Program of aerobic training

Each patient was sit on stationary bicycle ergometer with her/his back in relaxed position, before exercises the limit of subject tolerance would be assessed by exercises test which was comprised of 3 minutes control period of unloaded pedaling, followed by an incremental ramp on a cycle ergometer at a rate of 10 W per minute to the limit of subject tolerance. Then the subject cycle was sit at 30 W for warming up, and then the intensity was increased every 60 seconds by 15 W until exhaustion, then the subject cycle was 30 W for cooling down. Duration was for three months, 2 times /week.

Exercises were performed on the electronic bicycle ergometer (Barlow, 2007; Maiorana *et al*., 2002) as the following stages:

- First stage (warming up): Consisted of 5 minutes warming up in the form of pedaling at speed of 60 revolutions per min without load.
- Second stage (active stage): Consisted of: Duration: 30 minutes. Mode: pedaling at speed of 60 revolution per min with Load: adjusted load to achieve 60% of the predictive age maximal heart rate which was calculated by the following equation: Maximal heart rate = 220 - age in years. Moderate work load = 60% of maximal heart rate. The heart rate was measured through pulsometer attached to the patient's ear.
- Third stage (cooling down): Consisted of 5 minutes cooling down in the form of pedaling at speed of 60 revolutions per min without load Frequency.

Low caloric diet protocol

Patients were beginning adding thicker liquids that are high in protein and low in fat and sugar. They were used high – protein, Low- calorie liquid supplement drinks during this period. Daily caloric intakes were not exceeding 1500 calories. They were drinking 1 to 1.5 liters of water or other non-caloric liquids per day.

Recommended thicker liquids

- Nonfat or 1% milk.
- Lactose-free or soy based low calorie drinks.
- Sugar free pudding
- Sugar free nonfat yogurt
- Low fat cottage cheese
- Blended broth based soup or other low fat soups.

Gene expression of SIRT1 gene

Samples of whole blood were collected from each patient in 5-ml sterile RNAase free vaccutainer tubes containing EDTA. Samples were collected two times throughout the study, before starting (pre-test) and after training sessions (post-test). Blood samples were collected on ice and processed within 30 minutes after collection. Total RNA was extracted from whole blood using PureLink RNA Mini Kit (Invitrogen; Thermo Fisher Scientific, USA) according to manufacturer's instructions. RNA extraction was carried out using cooling centrifuge (Labofuge 400R-Heraeus, Germany). RNA concentration and purity were identified using nanodrop spectrophotometer device (2000 C, Thermo Scientific, USA). RNA samples were kept immediately at -80 ˚C until assayed. cDNA synthesis was carried out for each RNA sample in a thermal cycler (*T* gradient, Biometra, Germany) using High Capacity cDNA Reverse Transcription Kit (Applied biosystem, USA, Foster) according to manufacturer's instructions. Gene expression of SIRT1gene in obese children of the two groups was studied to investigate the effect of ergonomic exercises in SIRT1 gene expression in obese Down syndrome children. Gene expression assay for SIRT1 gene was carried out using customized ready-to-use SIRT1 gene expression assay and TaqMan universal master mix II (Applied biosystem, USA, Foster). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as internal control. Quantitative real-time polymerase chain reaction (qRT-PCR) (DNA Technology, Moscow, Russia) was applied for quantitative SIRT1 gene mRNA expression (Lee *et al*., 2013).

Ethical Approval

All procedures performed in the current study involving human participants were in accordance with the ethical standards of the Medical Research Ethics Committee of the National Research Centre- Egypt (MREC, NRC, no. 14136), and with 1964 Helsinki declaration and its later amendments. Informed consent was obtained from the parents of each participant included in the study.

STATISTICAL PROCEDURES

Data collection

Data were collected two times as follow: before starting (pre-test) and after three months (post-test) including body weight BMI and SIRT 1 gene expression .

Data analysis

Statistical analysis was conducted using SPSS for windows, version 20 (SPSS, Inc., Chicago, IL). The current test involved two independent variables. The first independent variable was the (interventions); between subjects factor which had two levels (group I receiving ergonomic exercises and group II receiving balanced diet only). The second independent variable was the (measuring periods); within subject factor which had two levels (pre & post). In addition, this test involved three tested dependent variables (body weight, waist circumference, and skin fold). Accordingly, 2×2 mixed design MANOVA was used to compare the tested variables of interest at different tested groups and measuring periods. With the initial alpha level set at 0.05.

RESULTS

Body mass index

Table (2) revealed the results for the Body mass index pre- and post- treatment between two groups of the study. There was no significant difference in pre-treatment values. But there was a significant difference in the post-treatment values (P<0.05) .

Table 1: General and Clinical Characteristics (age and height) of the Study Groups.

Items	Group I Mean ±SD	Group II Mean ± SD	Comparison t-value	*p* value	Level of significance
Age (yrs)	16.11±1.36	15±1.95	-2.76	0.05	NS
Height (cm)	147.11±7.70	140.25±13.89	-1.33	0.199	NS

SD: standard deviation, *p*: probability, NS: non-significant

Table 2: Mean ±SD and *p* values of body mass index of the study groups.

Body mass index (Kg)	Pre treatment Mean ±SD	Post treatment Mean ± SD	MD	% of change	*p* value
Group I	34.18±3.31	29.29±3.89	2.371	6.93	0.0001*
Group II	33.23±8	29.86±7.89			
MD	0.94	-0.57	3.887	11.69	0.0001*
p- value	0.744	0.844			

*Significant level is set at alpha level <0.05, SD: standard deviation, MD: mean difference, *p*: probability value.

SIRT 1 gene expression

Table (3) shows median score, U, Z and P-values of the fold change (a fold change is basically a ratio (post-treatment and pre-treatment). It indicates the number of times gene expression has changed in comparison to an original amount) at both groups. In group I, the median score of fold change for gene expression was 0.07205. While in group II, the median score of fold change for gene expression was 0.26375. "Mann-Whitney tests ″ revealed there was no significant difference between the both groups in fold change (U = 39, Z = -1.385, and p = 0.166).

Table 3: Median, U, Z, and *p* values of fold change at both groups.

Fold change	Group I	Group II	U-value	Z-value	p-value
Median	0.07205	0.26375	39	-1.385	0.166

*Significant level is set at alpha level <0.05

Correlation among fold change and BMI of group I at post-treatment

As illustrated at figure (1) the correlations among fold change at post-treatment and BMI. Waist circumference, skin fold and BMI at group I were studied through the Pearson product moment correlation coefficient. It revealed that there was no significant correlation between fold change and BMI (r= 0.261, p= 0.466).

While, there was positive strong significant correlation between fold change and waist circumference (r= 0.69, p= 0.019*). But, there were no significant correlation between fold change and skin fold (r=0.347, p= 0.295).

Correlation among fold change and BMI of group II at post treatment

As at figure (2) the correlations among fold change and BMI at post-treatment. Waist circumference, skin fold and BMI at group II were studied through the Pearson product moment correlation coefficient. It revealed that there was no significant correlation between fold change and BMI (r= -0.099, p= 0.801). As well as, there was no significant correlation between fold change and waist circumference (r= 0.001, p= 0.998). Additionally, there were no significant correlation between fold change and skin fold (r=0.042, p= 0.915).

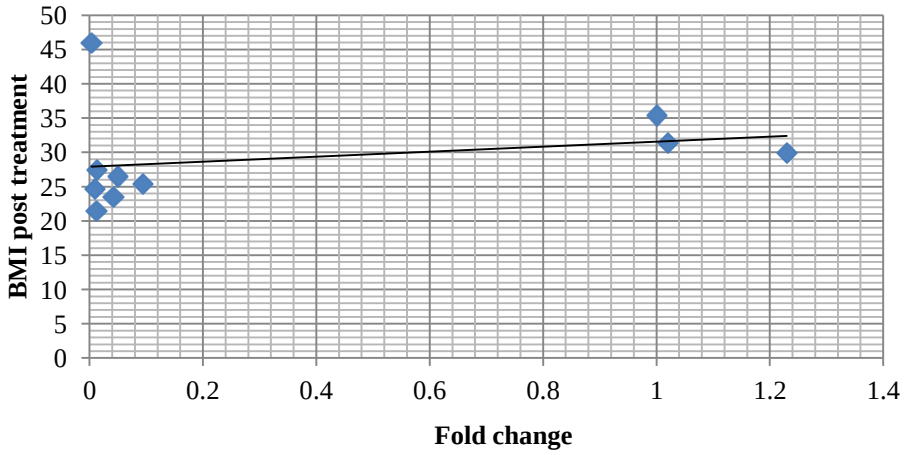

Fig. 1: Scatter plot for the bivariate correlation between fold change and BMI post treatment at group I.

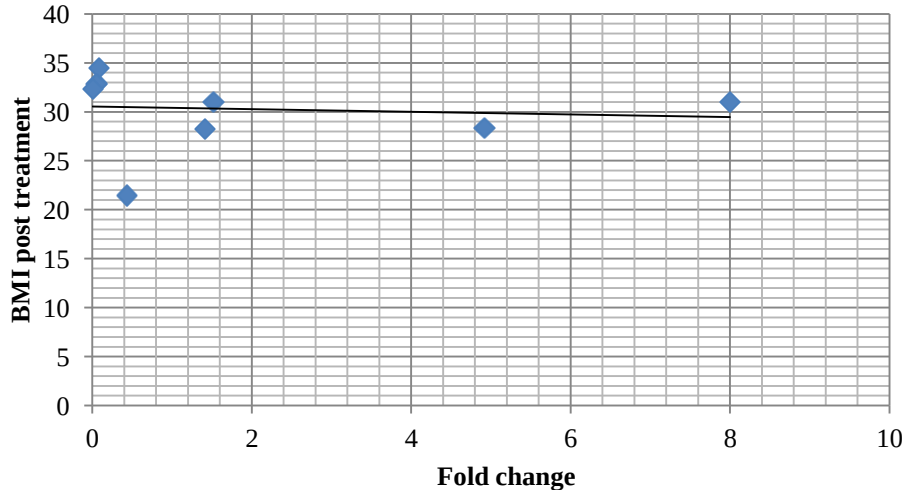

Fig. 2: Scatter plot for the bivariate correlation between fold change and weight post treatment at group II.

DISCUSSION

Role of exercises therapy in controlling obesity

The results of the present study showed that there is a significant difference in BMI in both studied groups pre- and post-treatment. According to (Petridou *et al*., 2017) low density lipoprotein (LDL) was decreased by increasing of lipoprotein lipase activities. These increased activities cause clearance of residual chylomicron and cholesterol-rich LDL. Cholestryl ester transfer protein control the exchange of triglycerides for cholesterol ester in LDL and HDL. Moreover, weight loss and exercises decrease the level of cholestryl ester transfer protein, may be due to part of this protein is synthesized in adipose tissue.

On the other hand, regular training by using cycle ergometer showed a decrease in abdominal fat and body mass index as a result of decrease in total amount of stored calories (Driss and Vanderwalle, 2013). This decrease in energy stores was attributed to a negative energy balance produced by exercises (Fearnbach *et al*., 2016).

(Fernström *et al*., 2017) Studies have demonstrated that aerobic fitness is associated with low risk for cardiovascular diseases in Swedish young adults .

Moreover, (Nelson *et al*., 2014) found that physical activity causes a long term weight loss and decrease body mass index. They revealed that this loss, due to regular physical activity that enhance fat oxidation (Arquer *et al*., 2010) and partially prevent the age related increase in central body fatness. The present study revealed that ergonomic exercises with balanced diet has a significant effect in BMI reduction.

Modifying SIRT1 gene expression

The results of the current study found that the median score of fold change for SIRT1 gene expression in the study groups I and II (0.07205 and 0.26375, respectively). On the other hand, there was no significant difference between both groups concerning fold change. However, a significant positive correlation was found between waist circumference and fold change of SIRT1 in group I but was not found in group II. According to (Ogorodnikov *et al*., 2016; Covington *et al*., 2015) Mechanisms that control gene expression are relatively well understood. The dynamic regulation in the cell control each step in this process. This regulation includes structural changes in the chromatin to make a particular gene accessible for transcription into RNA, splicing of RNA into mRNA, editing and other covalent modifications of the mRNA, translation of mRNA into protein, and, finally, post-translational modification of the protein into its mature, functional form.

However, it is not clear that visceral adiposity affects SIRT1 expression or SIRT1 regulates fat distribution. This relationship could be explained on the basis that visceral fat increases free fatty acid flux and inhibit insulin effect, but SIRT1 decreases lipogenesis, increase fatty acid oxidation, affect insulin signaling and protect against oxidative stress. In addition, the relationship between SIRT1 and visceral adiposity may also be explained on the basis of mitochondrial function. Visceral adiposity is associated with mitochondrial dysfunction and diminished glucose tolerance in elderly, but SIRT1 induce mitochondrial activity and biogenesis in several tissues (Lee *et al*., 2013).

The mechanism by which SIRT1 gene affect obesity could be attributed to its effect on the nuclear peroxisome proliferator-activated receptor (PPARγ). This receptor plays an important role in glucose metabolism and maturation of preadipocytes into mature fat cells. PPARγ in mature fat cells induce the genes that accumulate free fatty acid and synthesis triglycerides. SIRT 1 was found to suppress PPARγ by anchoring to a negative cofactors of this receptor. This process down regulate genes such as adipocyte protein 2 (aP2) in mice. Therefore, mice starvation induced SIRT1 to bind the aP2 promoter in white adipose tissue (WAT), thereby down regulate gene expression, and accelerate mobilization of fat into the blood. This explain the association between decreasing fat storage and increasing lipolysis with up regulation of SIRT1 in differentiated adipose cells (Li, 2013). Moreover, treatment of mice on a high fat diet with resveratrol that is an activator of SIRT1 was shown to reduce weight gain. These findings suggest that SIRT1 affect lipid regulating transcription factors which adapt gene transcription with changes in nutrients (Zhou *et al*., 2016; Kauppinen *et al*., 2013).

CONCLUSION

In summary, fold change as indicator for SIRT1 gene expression was changed in the study groups GII and GI (40%, 27.27%, respectively). This could be explained by the effect of ergonomic exercise with balanced diet on the reduction in BMI and waist circumference of cases which may affect gene expression in the studied Down syndrome obese patients.

Financial support and sponsorship: Nil.

Conflict of interest: The authors declare no conflict of interest.

REFERENCES

Aburawi EH, Nagelkerke N, Deeb A, Abdulla S, Abdulrazzaq YM. National growth charts for United Arab Emirates children with Down syndrome from birth to 15 years of Age. J Epidemiol, 2015; 25(1):20-9.

Arquer A, Elosua R, Marrugat J. Physical activity and lipid oxidation. Apunts Med Esport. 2010; 45(165): 30-39.

Barlow SE. Expert committee recommendations regarding the prevention, assessment, and treatment of child and adolescent overweight and obesity: summary report. Pediatrics. 2007; 120 (Suppl 4): S164-92.

Covington JD, Bajpeyi S, Moro C, Tchoukalova YD, Ebenezer PJ, David H Burk DH, *et al*. Potential effects of aerobic exercise on the expression of perilipin 3 in the adipose tissue of women with polycystic ovary syndrome: a pilot study. European Journal of Endocrinology. 2015; 172: 47–58.

Ellulu M, Abed Y, Rahmat A, Ranneh Y, Ali F. Epidemiology of obesity in developing countries: challenges and prevention. Global Epidemic Obesity, 2014; doi: 10.7243/2052-5966-2-2

Fearnbach S, Masterson T, Schlechter H, Ross A, Rykaczewski M,

Loken E *et al*. Impact of imposed exercise on energy intake in children at risk for overweight. Nutrition Journal, 2016; 15: 92; doi: 10.1186/s12937-016-0206-5

Fernström M, Fernberg U, Eliason G, Hurtig-Wennlöf A. Aerobic fitness is associated with low cardiovascular disease risk: the impact of lifestyle on early risk factors for atherosclerosis in young healthy Swedish individuals – the Lifestyle, Biomarker, and Atherosclerosis study. Vascular Health and Risk Management, 2017; 13: 91–99.

Green DJ, Smith KJ. Effects of Exercise on Vascular Function, Structure, and Health in Humans. Cold Spring Harb Perspect Med, 2017; doi: 10.1101/cshperspect.a029819

Koebnick C, Smith N, Black MH, Porter AH, Richie BA, Hudson S, *et al*. Pediatric obesity and gallstone disease: results from a cross-sectional study of over 510,000 youth. Journal of Pediatric Gastroenterology and Nutrition, 2012; 55(3):328–33.

Kauppinen A, Suuronen T, Ojala J, Kaarniranta K, Salminen A. Antagonistic crosstalk between NF-κB and SIRT1 in the regulation of inflammation and metabolic disorders. , 2013; 25(10): 1939-48.

Lee H, Chu SH, Park JY, Park HK, Im JA, Lee JW. Visceral adiposity is associated with SIRT1 expression in peripheral blood mononuclear cells: A pilot study. Endocrine Journal, 2013; 60(11):1269-1273.

Li X. SIRT1 and energy metabolism. Acta Biochim Biophys Sin, 2013; 45:51–60.

Maiorana A, O'Driscoll G, Goodman C, Taylor R, Green D. Combined aerobic and resistance exercise improves glycemic control and fitness in type 2 diabetes. Diabetes Res Clin Pract. 2002; 56(2):115-23.

Mechanick JI, Youdim A, Jones DB, Garvey WT, Hurley DL, McMahon MM, *et al*. Clinical practice guidelines for the perioperative nutritional, metabolic, and nonsurgical support of the bariatric surgery patient--2013 update: cosponsored by American Association of Clinical Endocrinologists, the Obesity Society, and American Society for Metabolic & Bariatric Surgery. Endocr Pract. 2013; 19(2):337-72.

Murray J, Ryan-Krause P. Obesity in children with Down syndrome: Background and recommendations for management. Pediatric Nursing, 2010; 36(6):314-319.

Nasrin N, Kaushik VK, Fortier E, Wall D, Pearson KJ, de Cabo R, *et al*. JNK1 phosphorylates SIRT1 and promotes its enzymatic activity. PLOS ONE, 2009; 4(12):e8414.

Nelson C, Wagner G, Caban-Martinez A, Buxton O, Kenwood C, Sabbath E, *et al*. Physical Activity and Body Mass Index: The Contribution of Age and Workplace Characteristics. Am J Prev Med, 2014; 46(3 0 1): S42–S51.

Ogorodnikov A, Kargapolva Y, Danckwardt S. Processing and transcriptome expansion at the mRNA 3′ end in health and disease: finding the right end. Eur J Physiol. 2016; 468: 993–1012.

Petridou A, Chatzinikolaou A, Avloniti A, Jamurtas A, Loules G, Papassotiriou I, *et al*. Increased Triacylglycerol Lipase Activity in Adipose Tissue of Lean and Obese Men During Endurance Exercise. J Clin Endocrinol Metab. 2017; doi: 10.1210/jc.2017-00168.

Schug T and Li X. Sirtuin 1 in lipid metabolism and obesity. Ann Med, 2011; 43(3): 198–211.

Trigiani LJ, Hamel E. An endothelial link between the benefits of physical exercise in dementia. J Cerebr Blood Flow Metab, 2017; doi: 10.1177/0271678X17714655

Driss T, Vandewalle H. The measurement of maximal (anaerobic) power output on a cycle ergometer: a critical review. BioMed Research International, 2013, Article ID 589361; doi.org/10.1155/2013/589361

Zhao Y, Ling F, Griffin TM, He T, Towner R, Ruan H, *et al.* Up-regulation of the Sirtuin 1 (Sirt1) and Peroxisome Proliferator-activated Receptor γ Coactivator-1 α (PGC-1α) Genes in White Adipose Tissue of Id1 Protein-deficient Mice IMPLICATIONS IN THE PROTECTION AGAINST DIET AND AGE-INDUCED GLUCOSE INTOLERANCE.

The Journal of Biological Chemistry, 2014; 289(42) 29112–29122.

Zhou W, Ni TK, Wronski A, Glass B,Skibinski A, Beck A, *et al*. The SIRT2 Deacetylase Stabilizes Slug to Control Malignancy of Basal-like Breast Cancer. Cell Reports, 2016; 17:1302–1317.

How to cite this article:

Zeidan HM, Ahmed MM, Hashish AF, Rashad H, Abdol Raouf ER. Effect of Ergonomic Exercises in Modifying Sirtuin1 Gene Expression in Obese Down Syndrome Patients. J App Pharm Sci, 2017; 7 (09): 213-218.