Original Article

Effect of Dehydroepiandrosterone and Muscular Exercise on Insulin Sensitivity and Tumour Necrosis Factor Alpha in High Fat Diet Fed Rats

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Abstract:

Background: Visceral obesity is considered as an inflammatory condition with production of pro-inflammatory mediators such as IL-6 and TNF-α that play a role in the induction of insulin resistance and type 2 diabetes in obese individuals. Both high fat diet and lack of physical activity are risk factors for obesity. DHEA is a precursor of steroid hormones that may play a role in improving insulin resistance in obese individuals. **Aim** of the present study was to investigate the possible effects of muscular exercise, DHEA and their combination on insulin sensitivity, TNF-α in high fat diet fed rats. **Materials and Methods:** The study was conducted on 40 adult male Albino rats with body weight of approximately 100-150g. All rats were fed on high fat diet (60% fat) for 14 weeks. Body weight was measured at the end of the 8th week and those who had 10% increase in their body weight or more had continued the last 6 weeks of the experiment. During the last 6 weeks of experiment, rats were distributed randomly as follows: **Group I (Control, n=10):** This group received oral sesame oil every day for 6 weeks. **Group II (DHEA, n=10):** This group received DHEA orally dissolved in sesame oil every day for 6 weeks. **Group III (Exercise, n=10):** This group had an exercise protocol of swimming for 5 days/week for 6 weeks. **Group IV (Combined, n=10):** They received DHEA as group II and had the exercise program as group III for 6 weeks. At the end of the study, all rats were sacrificed and visceral fat was weighed. Fasting serum glucose, insulin and TNF-α were measured. Insulin sensitivity was calculated by QUICKI (quantitative insulin sensitivity check index). **Results:** rats of the combined group showed significant decrease in bodyweight and visceral fat than all the other groups. They also showed significant improvement in fasting serum glucose level, insulin and TNF-α levels than the other 3 groups. The combined group was significantly more insulin sensitive than the other 3 groups. There was no significant difference between the DHEA group and the Exercise group regarding all the parameters measured though both were significantly improved than the Control group. **Conclusion:** our results support that the combination of DHEA and exercise showed better results for body weight, visceral fat and insulin sensitivity than DHEA or exercise alone. Both modalities showed better results for serum TNF-α than either alone.

Keywords: obesity, DHEA, exercise, insulin sensitivity, TNF-α, visceral fat.

Introduction:

Obesity is a growing epidemic worldwide; its prevalence has been rising tremendously over the last 30 years (WHO, 2013).[1] It results from the imbalance between energy intake and energy expenditure. [2] It is a pro-inflammatory condition in which hypertrophied adipocytes and adipose tissue-resident immune cells (primarily lymphocytes and macrophages), both contribute to increased circulating levels of pro-inflammatory cytokines. [1,3]

Abbreviations:

T2DM, type 2 diabetes mellitus; **HFD**, high fat diet; **TNF-α**, tumour necrosis factor alpha; **IL-6**, interleukin 6; **IRS**, insulin receptor substrate; **DHEA**, dehydroepiandrosterone; **QUICKI**, quantitative insulin sensitivity check index; **RMR**, resting metabolic rate; **PPAR**, peroxisome proliferator-activated receptor; **GLUT4**, glucose transporter 4; **PI3K-PKB**, phosphatidylinositol 3-kinase-protein kinase B; **AMPK**, AMP-activated protein kinase; **PGC-1α**, peroxisome proliferator-activated receptor-γ coactivator-1α .

Insulin resistance is a common feature of obesity and is strongly associated with the etiology of type 2 diabetes mellitus (T2DM), hypertension and coronary heart disease.[4,5] It is defined as the decreased peripheral tissue response to insulin-mediated cellular actions and the term "insulin resistance" refers to reduced whole-body glucose uptake in response to physiological levels of insulin.[6] It correlates strongly with central-visceral obesity but not with lower body (i.e., hip, lower extremity) obesity.[7]

Among all environmental influences, both high-fat diet (HFD) and lack of or decline in daily physical activity are the most important factors for obesity development.[8] Excessive intake of dietary fat promotes adipocyte hypertrophy, altering their normal endocrine function to an inflammatory pathologic condition that increases the secretion of tumour necrosis factor alpha (TNF-α) and interleukin-6 (IL-6), and concomitantly reducing adiponectin secretion.[9] TNF-α is a potent pro-inflammatory cytokine, secreted mainly by adipose tissue-resident macrophages in obese individuals. TNF-α induces insulin resistance mainly by defective phosphorylation of insulin receptor substrate 1 (IRS-1).[1] This reduces the expression of glucose transporters and adiponectin in adipocytes, which contributes to the development of insulin resistance.[10] TNF-α also indirectly induces insulin resistance by altering adipocyte differentiation and adipocyte lipid metabolism.[11] Moreover, it inhibits the conversion of preadipocytes to mature adipocytes allowing further recruitment of uncommitted cells and thus possible expansion of adipose tissue mass.[1] TNF-α also has an enhancing effect on the production of other inflammatory cytokines, such as IL-6. [12]

Dehydroepiandrosterone (DHEA) is a natural steroid that serves as a precursor of male and female sex hormones. It is produced from the adrenal gland, and together with its sulfated form (DHEA-S), are the most abundant steroid hormones in humans.[13] Patients with metabolic syndrome have lower DHEA and DHEA-S levels.[14,15] The obesity and type 2 diabetes patients show lower concentrations of DHEA and other sex steroid hormones.[16] While some studies indicated that DHEA administration leads to improving insulin sensitivity, reducing fat mass and normalizing glucose metabolism,[17,18] others demonstrated no improvement in insulin sensitivity or body composition. [19]

Exercise training is considered as an important environmental factor associated with body weight regulation and has shown to control obesity and weight gain and to improve insulin sensitivity and help in reducing the risk of diabetes.[20,21] Research groups have already demonstrated that intermittent swimming exercise is more efficient than continuous swimming exercise in decreasing adiposity in rats fed a high fat diet.[22]

The aim of the present study was to investigate the possible effects of muscular exercise, DHEA and their combination on insulin sensitivity, TNF-α in high fat diet fed rats.

Materials and Methods:

The study was conducted on 40 adult male Albino rats (10-12 weeks old), with bodyweight of approximately 100-150g. Rats were housed at room temperature with a 12-h light–dark cycle; 5 rats per cage; with free access to food and water. The duration of the study was 14 weeks. All rats were fed on HFD [23] (60% fat, 20% carbohydrate and 20% protein) with vitamin and mineral mix for the 14 weeks. The HFD was prepared every 3 days to ensure its freshness.[24] All rats were subjected to body weight measurement at the end of the eighth week of the experiment and those who had 10% increase in their body weight or more had continued the last 6 weeks of the experiment.[25]

During the last 6 weeks of experiment, rats were distributed randomly into 4 groups as follows: **Group I (Control, n=10):** This group received sesame oil (1mg/Kg body

weight) administered orally by gastric tube in the morning at 9 am every day for 6 weeks and served as a control. **Group II (DHEA, n=10):** This group received DHEA (1mg/kg body weight) (bought from LKT Laboratories, Inc) dissolved in sesame oil administered orally by gastric tube in the morning at 9 am every day for 6 weeks.[26] **Group III (Exercise, n=10):** The animals of this group had an exercise program consisted of swimming in individual tanks filled with water and maintained at 28-32°C.[27] They swam for 10, 20, and 30 minutes twice daily on the first, second, and third days respectively to adapt. The swimming period was maintained at 30 minutes twice, [28] separated by 45-min rest period.[29] The exercise protocols were performed for 5 days/week for 6 weeks.[27] Rats were continuously monitored during swimming to prevent them from drowning.[30] **Group IV (Combined, n=10):** This group received DHEA in the same dose as group II and had the exercise program as group III for 6 weeks.

Sampling: At the end of the study, rats of all groups were fasted overnight, weighed, anaesthetized using ether then euthanized by decapitation.[30] Visceral fat mass was assessed by weighing the epididymal, mesenteric, and retroperitoneal fat pads.[31] Blood was collected from trunk after decapitation into a clean dry non-heparinized Wasserman tubes for separation of serum. [30] Samples were allowed to clot for 10-20 mins at room temperature before centrifugation for 20-mins at the speed of 2000-3000 r.p.m. A fraction of the sera was taken and used for estimation of fasting glucose levels immediately.[32] The rest of the sera were aliquoted and stored at-20°C until assayed. Samples were centrifuged again after thawing before the assay.

The following parameters were assessed: 1.Serum insulin levels were assayed with enzyme-linked immunosorbent assay (ELISA) kits (supplied by WKEA MED supplies, USA).[32] 2.Insulin sensitivity was assessed using the QUICKI method (quantitative insulin sensitivity check index). This method was calculated using fasting glucose and insulin values.[33,34] QUICKI = 1 / [log (I_0) + log (G_0)], Where I_0 is fasting insulin (μU/ml), and G_0 is fasting glucose (mg/dl). 3. TNF-α was assessed with ELISA kits. (supplied by WKEA MED supplies, USA) [35]

Statistical analysis:

The values of the measured parameters were expressed as mean ± SD. The difference between the studied groups was determined using ANOVA test (F-test) and least significant difference (LSD). Correlations between two quantitative variables were assessed using Pearson correlation coefficient (r). $P < 0.05$ values were considered significant. All statistical analyses were processed using SPSS for windows Version 20.0.

Results:

Comparison between body weights (gm) in the studied groups.

At the beginning of the study, the body weight was 132 ± 2.34 gm.

At the end of the eighth week, all rats exhibited increase in body weight which ranged between 190 ± 19.7 gm with a mean % of increase 43.7% ± 14.9%. Then rats were distributed randomly into 4 groups and there was no significant difference between these groups.

At the end of the 14th week, the body weight was measured. There was a significant difference between the four groups where F=7.908 and P= <0.001. The DHEA group had a significant decreased body weight than the Control group where P= 0.007, while it had a significant increased body weight than the Combined group where P=0.050. The Exercise group had a significant decreased body weight than the Control group where P= 0.022, while it had a significant increased body weight than the Combined group where P=0.020. The Combined group had a significant decreased body weight than the Control group where P=<0.001. There was no significant difference between the DHEA group and the Exercise group.

Comparison between visceral fat (gm) in the studied groups at the end of the study:

At the end of the study, visceral fat (epididymal, mesenteric, and retroperitoneal) was weighed. The mean values of the Control group, DHEA group, Exercise group and Combined group were 5.15 ± 0.92 gm, 4.11±0.63 gm, 3.91 ± 0.63 gm and 3.14 ± 0.52 gm respectively. There was a significant difference between the four groups where F= 14.366 and P= <0.001.

The DHEA group had a significant decreased visceral fat than the Control group where P= 0.002, while it had a significant increased visceral fat than the Combined group where P=0.003. The Exercise group had a significant decreased visceral fat than the Control group where P= <0.001, while it had a significant increased visceral fat than the Combined group where P=0.018. The Combined group had a significant decreased visceral fat than the control group where P=<0.001. There was no significant difference between the DHEA group and the exercise group.

Comparison between insulin sensitivity in the studied groups at the end of the study:

At the end of the study, the fasting serum glucose and insulin levels were measured. The mean values of serum glucose for the Control group, DHEA group, Exercise group and Combined group were 133 ± 8.61 mg/dl, 113 ± 6.62 mg/dl, 113 ± 8.37 mg/dl and 98.7 ± 8.04 mg/dl respectively. There was a significant difference between the four groups where F= 31.626 and P= <0.001.

The mean values of serum insulin for the Control group, DHEA group, Exercise group and Combined group were 22.7 ± 2.37 μU/ml, 16.9 ± 0.90 μU/ml, 16.6 ± 1.13 μU/ml and 14.4 ± 2.22 μU/ml respectively. There was a significant difference between the four groups where F= 39.770 and P= <0.001.

The fasting serum glucose and insulin levels were significantly higher in the Control group than other studied groups, while they are significantly lower in the Combined group than the other groups. There was no significant difference between the DHEA group and the Exercise group.

Insulin sensitivity was calculated using the QUICKI method. There was a significant difference between the four groups where F= 32.714 and P= <0.001.

The DHEA group had a significant increased insulin sensitivity than the Control group where P= <0.001, while it had a significant decreased insulin sensitivity than the Combined group where P=<0.001. The Exercise group had a significant increased insulin sensitivity than the Control group where P= <0.001, while it had a significant decreased insulin sensitivity than the Combined group where P=<0.001. The Combined group had a significant increased insulin sensitivity than the control group where P=<0.001. There was no significant difference between the DHEA group and the Exercise group.

Comparison between serum tumour necrosis factor alpha (TNF-α) (ng/L) in the studied groups at the end of the study:

At the end of the study, the serum TNF-α level was measured. There was a significant difference between the four groups where F= 37.599 and P= <0.001.

The DHEA group had a significant lower serum TNF-α level than the Control group where P=< 0.001, while it had a significant higher serum TNF-α level than the Combined group where P=<0.001. The Exercise group had a significant lower serum TNF-α level than the Control group where P= <0.001, while it had a significant higher serum TNF-α level than the Combined group where P=<0.001. The Combined group had a significant lower serum TNF-α level than the Control group where P=<0.001. There was no significant difference between the DHEA group and the Exercise group.

Correlation study:

I- Correlation study between visceral fat and serum TNF-α:

A significant positive correlation was detected between visceral fat and fasting serum TNF-α in total cases where r=0.934 and p= <0.001.

II- Correlation between visceral fat and insulin sensitivity measured by QUICKI:

A significant negative correlation was detected between visceral fat and insulin sensitivity measured by QUICKI in total cases where r= -0.919 and p= <0.001.

Table I : Comparison between body weights (gm) in the studied groups.

	Control (n=10)	DHEA (n=10)	Exercise (n=10)	Combined (n=10)	F	p
Baseline						
Mean ± SD.	132 ±2.5	132 ± 2.5	132 ± 2.2	131 ± 2.4	0.434	0.730
At end of 8 weeks						
Mean ± SD.	196 ± 20.6	181 ± 17.1	194 ± 19.2	187 ± 21.3	1.159	0.339
Mean % of change	48.5	37.3	46.7	42.4		
At end of 14 weeks						
Mean ± SD.	244 ± 27.5	217 ± 16.6	221 ± 16.4	198 ± 22.4		
p_1		0.007*	0.022*	<0.001*	7.908*	<0.001*
p_2			0.654	0.050*		
p_3			0.020*			

F: F test (ANOVA), SD= standard deviation.
p1: p value for Post Hoc test (LSD) for comparing between Control and each other group.
p2: p value for Post Hoc test (LSD) for comparing between DHEA group with each other group.
p3: p value for Post Hoc test (LSD) for comparing between Exercise group with Combined group.
*: Statistically significant at p ≤ 0.05

Table II: Comparison between insulin sensitivity in the studied groups at the end of the study:

	Control (n=10)	DHEA (n=10)	Exercise (n=10)	Combined (n=10)	F	p
Insulin sensitivity measured by QUICKI						
Mean ± SD.	0.288 ± 0.006	0.305 ± 0.004	0.306 ± 0.006	0.318 ± 0.010		
p_1		<0.001*	<0.001*	<0.001*	32.714*	<0.001*
p_2			0.761	<0.001*		
p_3			<0.001*			

F: F test (ANOVA), SD= standard deviation.
p1: p value for Post Hoc test (LSD) for comparing between Control and each other group.
p2: p value for Post Hoc test (LSD) for comparing between DHEA group with each other group.
p3: p value for Post Hoc test (LSD) for comparing between Exercise group with Combined group.
*: Statistically significant at p ≤ 0.05

Table III: Comparison between serum tumour necrosis factor alpha (TNF-α)(ng/L) in the studied groups at the end of the study:

	GroupI (Control) (n=10)	GroupII (DHEA) (n=10)	GroupIII (Exercise) (n=10)	Group IV (DHEA+Exercise) (n=10)	F	p
TNF -α						
Mean ± SD.	518± 43.8	407 ± 49.3	389 ± 53.4	293 ± 42.0		
p_1		<0.001*	<0.001*	<0.001*	37.599*	<0.001*
p_2			0.396	<0.001*		
p_3			<0.001*			

F: F test (ANOVA), SD= standard deviation.
p1: p value for Post Hoc test (LSD) for comparing between Control and each other group.
p2: p value for Post Hoc test (LSD) for comparing between DHEA group with each other group.
p3: p value for Post Hoc test (LSD) for comparing between Exercise group with Combined group.
*: Statistically significant at p ≤ 0.05

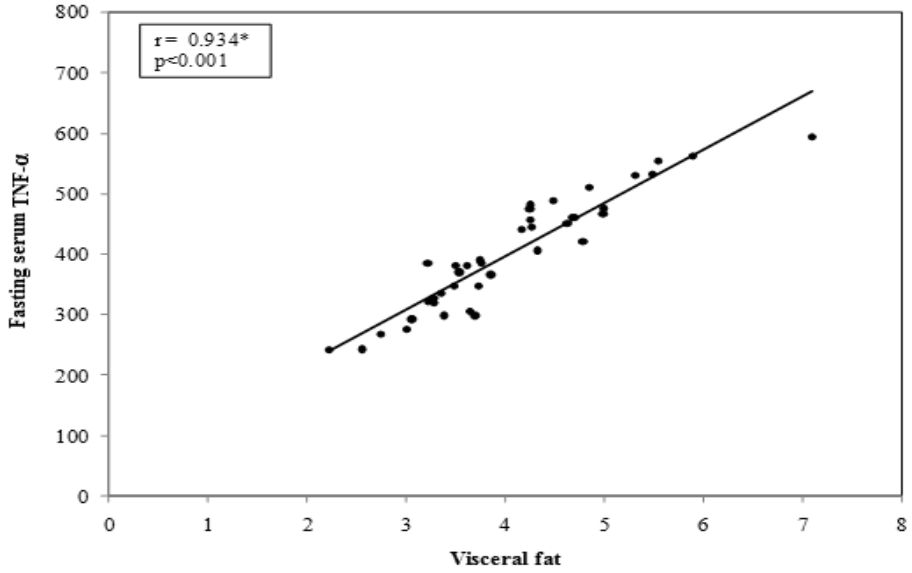

Fig.1: Correlation between visceral fat and fasting serum TNF-α in total cases.

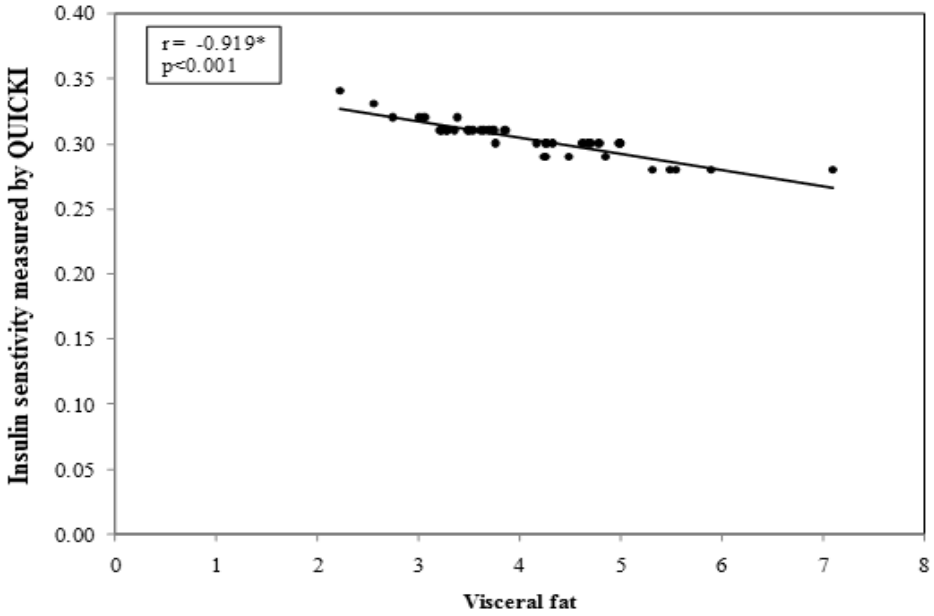

Fig.2: Correlation between visceral fat and insulin sensitivity measured by QUICKI in total cases.

Discussion:

Obesity is a complex metabolic disorder that is one of the most prevalent public health problems.[36] Excess adiposity is an established risk factor for metabolic diseases including insulin resistance, T2DM, hypertension, nonalcoholic fatty liver disease, polycystic ovarian diseases, and several types of cancer.[37] HFDs are known to lead to a positive fat balance and consequently to adipose mass accumulation.[38] Adult mice fed a HFD (60% fat) developed glucose intolerance, insulin resistance, increased expression of TNF-α, decreased expression of adiponectin and hyperlipidemia.[39] Our results showed that after 8 weeks of HFD (60% fat), all rats exhibited increase in body weight which ranged between 190 ± 19.7 gm with a mean % of increase 43.7% ± 14.9%. Chen et al[40] demonstrated that mice fed on HFD (45% fat) for 12 weeks had achieved a higher body

weight, visceral adiposity, fasting plasma glucose and insulin levels than rats fed on a low fat diet (10% fat) for the same period.

Six weeks of combined DHEA treatment and swimming exercise induced significant lower body weight and visceral fat than results observed from DHEA administration alone or intermittent swimming alone for the same period of time. This may be due to DHEA administration alone had shown to reduce adiposity by increasing resting metabolic rate (RMR) which is the largest component of the daily energy budget in most human societies. DHEA also down-regulates adiposity through the reduction of peroxisome proliferator-activated receptor gamma (PPARγ) in adipocytes.[41,42,43] Exercise also enhances glucose/lipid metabolism in skeletal muscle which is involved in white adipose tissue reduction. Also, both aerobic and resistance training in human subjects had also shown to increase the RMR so increasing energy expenditure during rest as well as during exercise.[10,44] Therefore, 6-weeks of combination treatment may have promoted additive reductions in body weight and visceral fat. According to Sato et al[26], body weight and abdominal fat were reduced by 6-weeks DHEA administration combined with treadmill training in rats fed on high sucrose diet for 20 weeks. They hypothesized that DHEA administration and exercise training, both can up regulate fatty acid metabolism which can help in adipose tissue reduction.[45,46]

Our results showed that 6-weeks of combined DHEA treatment with exercise training induced significant decrease in serum TNF-α level than results observed from DHEA administration alone or exercise training alone for the same period of time. This may be due to the synergistic effect of DHEA and exercise together in decreasing the body weight and visceral fat as our results showed that there is a positive correlation between visceral fat and TNF-α levels.[47] Similarly, Hostamilgil et al[48] reported that the adipose-tissue expression of TNF-α was positively related to BMI and decreased in proportion to the loss in body weight.

Several studies showed that DHEA reduced TNF-α serum concentrations and its induced inflammatory response.[32,49] DHEA may have lowered the circulating levels of inflammatory cytokines such as TNF-α and IL-6 as demonstrated by Weiss et al through activation of PPARα in aging humans causing a decrease in release of these inflammatory cytokines by various cell type.[50] Brignardello et al[51] showed that DHEA treatment reduced mRNA expression of TNF-α, TNF-receptor I (TNF-RI) and TNF-receptor II (TNF-RII) in peripheral blood mononuclear cells. A reduction in mRNA expression of TNF-α indicated a down regulation of this system.

Exercise also showed reductions in the expression of TNF-α in white adipose tissue.[52] Studies suggested that long-term exercise reduces adipose inflammation via suppression of macrophage infiltration and a switch from the inflammatory M1 macrophages to the anti-inflammatory M2 macrophages.[53,54] Weight reduction through exercise decreased the volume and number of adipocytes and also reduced the number of endothelial and macrophage cells that are lodged inside adipose tissue that produce pro-inflammatory mediators. Increased production of anti-inflammatory mediators by adipocytes and decreased hepatic production of fibrinogen and other pro-inflammatory mediators were other consequences of exercise-induced weight reduction. Weight loss also influenced the immune system by reducing the number of mononuclear cells in the circulation; these are important sources of pro-inflammatory cytokines.[55,56]

We also demonstrated that 6-weeks of DHEA treatment combined with swimming exercise induced significant decrease in fasting serum glucose and insulin levels than results observed from DHEA administration alone or exercise training alone for the same period of time. The combined interventions were more beneficial for insulin sensitivity than either DHEA administration or exercise training alone. This may be due to the significant decrease in body weight and

visceral fat than the DHEA group or the exercise group. As evidenced by the present study, visceral fat was negatively correlated with insulin sensitivity.[57,58] The improvement in insulin sensitivity may be also correlated to the improvement in the fasting serum TNF-α level as demonstrated by Plomgaard et al.[59] Similarly, Sato et al[26] had demonstrated that the combination of DHEA administration and treadmill running had additively or synergistically improved blood glucose levels and activated the glucose uptake in skeletal muscle because both modalities significantly increased basal muscular DHEA and dihydrotestosterone levels which were positively correlated with insulin sensitivity.[60]

In patients with obesity and T2DM, skeletal muscles have reduced glucose metabolic capacity due to insulin resistance and the ability of insulin to stimulate glucose transporter 4 (GLUT4) translocation decreases, resulting in a reduced GLUT4 content at the plasma membrane.[61,62] DHEA treatment enhanced glucose transport rates through GLUT4 transporter translocation to the cell surface. This effect seems to involve stimulation of insulin receptor substrate tyrosine phosphorylation and the associated phosphatidylinositol 3-kinase-protein kinase B (PI3K-PKB) activity.[63,64] Veras et al[65] had demonstrated that DHEA supplementation in ovariectomized female rats fed a HFD had maintained glucose-induced insulin secretion and pancreatic islet function. This effect was mediated by an increase in the phosphorylated PKB.

Exercise also had an “insulin-like effect” to facilitate glucose transport from the circulation into the working muscles.[66] Many studies had shown that exercise training can increase mitochondrial proliferation and boost the expression of GLUT4, and can in turn enhance lipid and glucose metabolic capacities.[67,68] Some demonstrated that exercise-induced, contraction-mediated GLUT4 translocation from an intracellular pool to the muscle membrane where glucose uptake takesplace, is independent of insulin and occurs through calcium/calmodulin-dependent protein kinase IV and, secondarily, through AMP-activated protein kinase (AMPK), which induces expression of peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α), a transcriptional coactivator that is essential for mitochondrial biogenesis.[68,69] Exercise improved HFD-induced metabolic dysfunction, including insulin resistance, fat accumulation, and mitochondrial dysfunction may also be due to the decreased expression of protein kinase Cβ in both skeletal muscle and liver as demonstrated by Rao et al.[70]

Despite positive findings in most rodent models and some of human studies, indicating that DHEA treatment improved glucose tolerance and insulin sensitivity, other DHEA studies in humans had yielded inconclusive results.[71,72] One possibility to explain these discrepancies was that one or many metabolites of DHEA, rather than DHEA itself, may be necessary for its full action in human physiology. DHEA undergoes extensive conversion to multiple products by phase 1 reactions involving the cytochrome P450 system. Studies showed that these phase 1 products, which frequently decline in elderly who had been the major participants in human DHEA treatment studies, can be more potent than parental DHEA.[73,74] It is also possible that qualitative changes in DHEA metabolism between rodents and humans may account for these differences.[75,76]

In conclusion, on the basis of this study, we support other results showing that combined treatment of DHEA and exercise showed better results for body weight, visceral fat and insulin sensitivity than DHEA or exercise alone. We report that combination of both modalities may have a better effect in reducing serum TNF-α than either alone.

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