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Vit D3 versus L Carnitine Supplement in Alleviating Age-Related Insulin Resistance in A Naturally Aging Rat Model

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Abstract

Background: Insulin Resistance (IR) has long been recognized as a characteristic of aging. Many studies recorded beneficial roles of Vitamin D3 (VD3) and L-Carnitine (LC) in obesity and type 2 diabetes.

Aim of Study: To evaluate the role of VD3 versus LC in attenuating the age-related IR in a naturally aging rat model, clarifying their possible underlying mechanisms.

Material and methods: Rats were divided into 4 groups; Young Control (YC), aged group, VD3-treated aged group (VD3-aged) and LC-treated aged group (LC-aged). Experimental procedures included measurement of body weight, Body Mass Index (BMI) and Oral Glucose Tolerance Tests (OGTT). The Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) index was also calculated. Assays of fasting serum glucose and insulin levels as well as serum tumor necrosis factor-a (TNF-a), interleukin-6 (IL-6) and adiponectin levels were performed. Moreover, skeletal muscle Sirtuin-1 (SIRT-1), Triglycerides (TGs) and TNF-a levels alongside with the glucose transporter-4 (GLUt4) mRNA expression were assessed.

Results: Compared to YC rats, aged rats exhibited significant increase in fasting serum insulin and HOMA-IR as well as an abnormal OGTT. Serum TNF-a and IL-6 levels were significantly increased, whereas serum adiponectin level was significantly decreased in old rats. Furthermore, the SIRT1 levels and GLUT 4 mRNA expression were significantly lower, whereas the TGs and TNF-a were significantly higher in skeletal muscle of aged rats compared to YC ones. On the other hand, these parameters, were equally and significantly attenuated in VD3 and LC supplemented rats compared to non-treated age matched group, except for the skeletal muscle SIRT-1 level which could be elevated only by VD3, while the anti-inflammatory and TGs lowering effects of LC were more prominent than VD3. Nevertheless, the overall effect of VD3 and LC on IR-related parameters were nearly equal.

Conclusion: Both VD3 or LC administrations were equally effective in alleviating the age-related IR, thus may provide a possible therapeutic intervention in the aging population.

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Key Words: *Vitamin D3 – L-carnitine – Insulin resistance – Aging – Proinflammatory cytokines – Adiponectin – SIRT-1 – GLUT 4.*

Introduction

OVER the few last decades, there has been a robust increase in the number of aging populations with metabolic disorders [1]. Glucose tolerance increasingly deteriorates with advancing age leading to high prevalence of impaired glucose tolerance and type 2 diabetes in elderly [2]. Such impairment is primarily as a result of decreased insulin sensitivity coincide with diminished glucose utilization by peripheral tissues, a condition of what so called, Insulin Resistance (IR) [3]. Insulin resistance has been long recognized as a characteristic of aging in human and rodents [4]. Thereby, aging has been considered as a main risk factor for development of metabolic syndrome [5]. Insulin resistance could be increased with age in relation to several well-known age-related changes, including hormonal changes, increased oxidative stress and inflammation [6]. Chronic, low-grade, systemic inflammation is widely accepted as a significant risk factor underlying aging and age-related type 2 diabetes [7]. Additionally, ageing-related IR usually presents low circulating levels of adiponectin [1,8]. Adiponectin, an adipocytokine secreted from adipose tissue, is involved in diverse biological processes, including sensitization of the insulin receptor signaling pathway, suppressing inflammation, and triggering the mitochondria biogenesis [9].

The skeletal muscle is regarded as the major site of IR in obesity and type 2 diabetes in elderly individuals [10]. During the ageing process, there is a gradual loss of the capability to adapt changing environments, alongside with excessive flux in fatty acids and impairment of fatty acids oxidation that are usually associated with development of

ageing-related IR [11,12]. Silent information regulator-1 (sirtuin SIRT-1), a NAD^+-dependent protein deacetylase, is found in various tissues and is considered to be a key metabolic regulator [13]. Recently, it had been concluded that, during aging, the decreased SIRT-1 in skeletal muscle fundamentally mediates IR [14], whereas, increased SIRT1 expression restricts age associated-diabetes and promotes insulin sensitivity [15,16]. Moreover, a decline in the skeletal muscle Glucose Transporter-4 (GLUT-4) with aging had been recorded in both human [17], and animal studies [18]. GLUT-4 is a glucose-transport protein which promotes the cellular uptake of glucose [19]. Several line of evidences indicate that the levels of GLUT4 mRNA expression in skeletal muscle are crucial for the regulation of total body glucose homeostasis [20].

Vitamin D is a lipid soluble vitamin synthesized from two main forms; Vitamin D2 (ergocalciferol) and Vitamin D3 (cholecalciferol). Both forms undergo two hydroxylation steps in liver and kidneys, generating the active Vitamin D3, 1, 25-dihydroxyvitamin D (1, 25[OH]2D) (calcitriol) [21]. Aging affects the formation of 1,25[OH]2D as a result of an age-related decline in renal function, which was further accentuated by decreased cutaneous synthesis and dietary intake of Vitamin D in elderly populations [22]. Beside the well-known function of Vitamin D on bone and calcium homeostasis, a large body of evidences suggested a potential influence of Vitamin D on glucose homeostasis, insulin secretion and IR, among these are the presence of specific Vitamin D receptors on pancreatic β-cells [23] and other tissues, such as vascular endothelial cells, neurons, osteoblasts and myocytes [24]. Furthermore, Vitamin D deficiency was shown to be related to IR, glucose intolerance and Type 2 diabetes [24,25], whereas increased circulating 25(OH) D concentration was shown to be associated with decreased prevalence of IR [26].

Many researchers suggested the positive impact of Vit. D supplementation on glucose homeostasis and IR, nevertheless , the results are still disputable. Vitamin D3 (VD3) administration was found to improve glucose and IR parameters in diabetic [27,28] and non-diabetic insulin resistant models [25,29,30]. On the contrary, neither the glucose homeostasis nor the insulin sensitivity were changed by Vitamin D supplementation in human experimental studies [31,32]. Therefore, it is unclear whether Vitamin D supplementation is beneficial or not in improving the glycemic control and IR in conditions of impaired glucose tolerance [33].

L-Carnitine (LC), a non-protein amino acid, is a ubiquitous constituent of mammalian plasma and tissues, mainly distributed among skeletal and cardiac muscles and found in plasma in the free or acyl-carnitine form [34]. It is supplied to the body through dietary sources (e.g., meat, dairy products), and by biosynthesis from essential amino acids, lysine and methionine [35]. The total LC levels in the serum, heart, and skeletal muscle were shown to be decreased in aged rats [36]. Carnitine functions to transport long-chain fatty acids across the inner mitochondrial membrane into the matrix for B-oxidation [37] and could promote oxidative glucose utilization and to improve insulin sensitivity [38]. Carnitine supplementation recorded beneficial effects in reducing the IR in fat rich diet and high fructose fed mice [34,39] and could improve glucose tolerance during insulin-resistant states, such as diabetes and obesity [40,41]. Nevertheless, a recent study showed that increasing carnitine availability did not modulate glucose tolerance or insulin sensitivity in diet-induced obese mice [42]. Additionally and up to our knowledge, the effect of LC on age-related IR in rats had not been yet investigated.

From the overmentioned data, we might hypothesize that VD3 or LC supplement could be beneficial in mitigating the aging associated IR. In this context, the present study was designed to evaluate the plausible modulating effect of vitamin D3 versus LC on alleviating the age-related IR in a naturally aging rat model, highlighting their possible underlying mechanisms.

Material and Methods

Chemicals and reagents:

1, 25(OH)2D3 was purchased from Sigma. Co, while LC was obtained from (Arab Company for Pharmaceuticals and Medicinal Plants, Cairo, Egypt). All other chemical reagents were of high purity and analytical grade.

Animals and study design:

This current work was conducted at Tanta Faculty of Medicine, from December 2018 to March 2019, and all experiments were conducted according to guidelines of the Ethical Committee of Medical Research, Faculty of Medicine, Tanta University, Egypt.

Ten young adult (3-4 month old, body weight 155± 15g) and thirteen aged (22-26 month old, body weight 410±27g) male albino rats were obtained from the Experimental Animal House of Faculty of Science, Tanta University. The rats were

kept in plastic cages (5 rats per cage) at room temperature (23 ±2ºC). Rats were maintained under regular 12h:12h day/night cycle and allowed free access to food and water.

Rats were weighed and divided randomly into four equal groups of ten rats each:

1- Young control (YC) group: Received rat chow diet and normal tap water.

2- Aged group: Similar to young control group which received only rat chow diet and normal tap water.

3- Vitamin D3-treated aged group (VD3-aged): This rat group received 1 ,25(OH)2D3 dissolved in ethanol and injected i.p., 3 times weekly for 6 weeks in a dose of 0.5 µg/Kg B.W. **[43,44]**.

4- L-Carnitine-treated aged group (LC-aged): Rats of this group received oral LC at a dose of 300 mg/kg, once daily for 6 weeks **[45]**.

At the end of the experimental regimen (6 weeks), Oral Glucose Tolerance Tests (OGTT) were conducted on overnight fasted rats, as previously described **[46]**. Rats received 1g/kg glucose by gavage and rats' blood samples were taken intermittently from the tail veins and glucose levels were measured using a glucometer at 0, 30, 60, 90 and 120min. Then the glucose area under the curve was calculated by trapezoid method **[47]**.

Blood and tissue sampling:

The next day of performing the OGTT, overnight fasted animals were weighed and anaesthetized with urethane. Body weights were determined and the BMI were calculated based on the method of Novelli et al. **[48]**, where BMI (gm/cm^2)=Body weight/length^2. The rats then sacrificed by decapitation and blood samples were collected, allowed to coagulate, at room temperature, centrifuged at 3000rpm for 15 minutes and serum was stored at –20ºC, till used for estimation of fasting serum glucose and insulin levels, HOMA-IR index along with TNF-α, IL-6 and adiponectin levels. The gastrocnemius muscle was extracted divided into two parts. One part was homogenized as 10% (w/v) in cold 50mM phosphate buffer (pH 7.4) and centrifuged at 5000xg for 30min at 4ºC. The resultant supernatant was stored at –80ºC until used for determination of Silent Information Regulator-1 (SIRT1), TNF-α and Triglycerides (TGs) content. Total protein content was assessed according to the method of Lowry et al. **[49]**. The other part of muscle used for RNA extraction for assessment of Glucose Transporter-4 (GLUT-4) mRNA expression using quantitative real time RT-PCR.

Biochemical assessment:

Glucose, insulin and HOMA-IR assay:

Serum glucose levels were estimated using oxidase-peroxidase method **[50]**, using kit (BIOMED Diagnostics Company, Germany). Insulin was measured using a rat insulin ELISA kit (Sun-Red Biological Technology Co., Ltd, Shanghai. China). HOMA-IR was used as a marker for IR using the equation previously described by Matthews et al., **[51]**.

$$\text{HOMA-IR} = \frac{\text{Fasting insulin (µIU/ml) X Fasting glucose (mg/dl)}}{405}$$

Determination of proinflammatory cytokines and serum adiponectin levels:

The levels of TNF-α in sera and skeletal muscle homogenates as well as the serum IL-6 level were determined using their corresponding ELISA kits purchased from (SunRed Biological Technology Co., Ltd, Shanghai. China), while serum adiponectin level was assayed using kits obtained from (Assaypro, Saint Charles, Missouri, USA), following the manufacturer's instructions.

Measurement of skeletal muscle SIRT1 and TGs content:

Skeletal muscle SIRT1 levels were measured using SunRed Biological Technology Co., Ltd, Shanghai. China), while the muscle TGs content was determined using colorimetric Biodiagnostic kits (Giza, Egypt) following the manufacturers' instructions.

Skeletal muscle GLUT4 mRNA expression:

Total RNA was isolated from gastrocnemius skeletal muscle using Gene JET RNA Purification Kit (Thermo Scientific, USA). Total RNA was reverse transcribed to produce cDNA by means of Revert Aid H Minus Reverse Transcriptase (Thermo Scientific, USA). The cDNA then was used as a template to detect GLUT4 mRNA relative expression using Step One Plus real time PCR system (Applied Biosystem, USA). The sequences of GLUT4 primer was as follow: F (5'-TTCTGG-CTCTCACAGTACTC-3'; R (5'-CATTGATGC CT-GAGAGCTGT-3') the housekeeping gene β-actin F (5'-TGGAATCCTGTGGCATCCATGAAAC-3'); R (5'-TAAAACGCA GCTCAGTAACAGTCCG-3'). Finally, mRNA relative expression was assessed using $2^{-\Delta\Delta Ct}$ method of Livak and Schmittgen **[52]**.

Statistical analysis:

The statistical analysis was conducted using the SPSS software. The collected data were subjected to one-way ANOVA followed by Tukey's

post hoc test to determine the significance between more than two groups. Data are expressed as the mean ± SD. p-value <0.05 was considered significant.

Results

Effect of VD3 or LC on body weight, BMI, glucose regulation and IR-related parameters:

As exhibited in (Table 1), the body weights at the end of the experimental period in all studied groups were not significantly different from their corresponding initial values. The body weights and BMI of VD3 or LC treated-aged rats were not significantly different from that of aged group.

Additionally, significant elevation of fasting serum insulin level and HOMA-IR index were observed in aged rats versus YC ones. Meanwhile, there was insignificant change in the fasting glucose levels in aged versus YC group. VD3 or LC administration significantly decreased the levels of fasting insulin and HOMA-IR with no significant difference on fasting glucose level in treated VD3 and LC-aged groups versus aged group (Table 1).

Table (1): Changes in initial and final body weights (BW1, BW2), BMI, serum levels of fasting glucose, fasting insulin, and HOMA-IR in all studied groups.

Parameters	Young control	Aged	VD3-aged	LC-aged
BW1 (g)	155±14.5	415±35.7*	409±27.9*	417±30.4*
BW2 (g)	169±16.8	432±26.9*	417.8±24.3*	424.4±29.5*
BMI (g/cm^2)	0.521±0.074	0.738±0.089	0.728±0.064	0.742±0.076
Fasting glucose (mg/dl)	89.4±6.1	95.1±9.8	93.4±6.3	94.7±5.8
Fasting insulin (μIU/ml)	4.73±0.76	7.65±0.65*	4.84±0.59#	4.644±0.56#
HOMA-IR index	1.105±0.19	1.763±0.32*	1.119±0.24#	1.122±0.27#

Data are expressed as mean ± standard deviation of a group of 10 rats.
Significance of difference from young control group is illustrated as *: p<0.05.
Significance of difference from aged group is illustrated as #: p<0.05.

Concerning OGTT, the values of blood glucose at 30, 60, 90, and 120min. (during OGTT) and the AUC glucose were significantly elevated in aged rats compared to YC group. Meanwhile, VD3 or LC administration significantly attenuated the blood glucose levels throughout the OGTT along with the AUC glucose in VD3 and LC-aged rats compared to aged group (Table 2), Fig. (1).

In comparison to YC group, the values of fasting insulin, HOMA-IR along with blood glucose during OGTT and the AUC glucose were insignificant in the VD3-aged and LC-aged groups. Moreover, the values of these parameters in VD3-aged were insignificant versus LC-aged. These findings elucidated that both VD3and LC were equally effective in ameliorating the age-induced IR in this rat model.

Table (2): The fasting blood glucose levels (mg/dl) at different time intervals (0, 30, 60, and 120min) during OGTT in all studied groups.

Time (min)	Young control	Aging	VD-aging	LC-aging
0	89±5.6	95±6.7	93±5.9	95±4.4
30	140±7.9	167±10.6*	145±10.3#	143±8.9#
60	110±5.8	154±9.1*	115±7.8#	115±6.7#
90	105±6.2	140±8.9*	104±6.9#	103±7.1#
120	90±5.7	122±8.5*	94±5.2#	96±5.2#

- Data are expressed as mean ± standard deviation of a group of 10 rats.
- Significance of difference from young control group is illustrated as *: p<0.05.
- Significance of difference from aged group is illustrated as #: p<0.05.

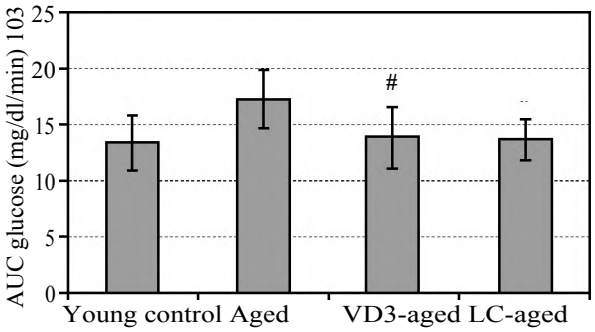

Fig. (1): The area under the curve of glucose (AUC glucose) in all studied groups.

Data are expressed as mean ± standard deviation of a group of 10 rats. Significance of difference from young control group is illustrated as *: p<0.05. Significance of difference from aged group is illustrated as #: p<0.05.

Effect of VD3 or LC on serum TNF-α and IL-6 and adiponectin levels: As exhibited in (Table 3), aged rats presented a significant elevation in serum levels of TNF-α and IL-6 by 2.11 and 1.51 fold, respectively, in comparison to YC rats. Meanwhile, a significantly lower serum adiponectin level by 38.5% was observed in aged rats versus YC ones.

On the contrary, VD3 supplement in aged rats significantly reduced serum levels of TNF- α and IL-6 by 38.1% and 20.47% respectively, while LC administration in aged rats reduced serum levels of TNF-α and IL-6 by 54.64% and 32.28%, respectively, when compared with the untreated aged group, denoting a better effect of LC than VD3 in this regard (Table 3).

Moreover, serum adiponectin level was significantly increased by 1.36 and 1.41 fold, respectively in VD3-aged and LC-aged rat, as compared to aged rats. However, the observed values of adiponectin in VD3 and LC-aged rats were still significantly different from those recorded in the YC group, which revealed that VD3 and LC partially restored the serum adiponectin levels (Table 3).

Table (3): Serum TNF-α, IL6 and adiponectin levels in all studied groups.

Parameters	Young control	Aged	VD3-aged	LC-aged
Serum TNF-α (ng/ml)	7.98±1.14	16.8±2.78±2.5*	10.4±1.42#	7.62±1.42#
Serum IL6 (pg/ml)	83.98±7.99	127±11.45*	101±8.91 *#	86±7.73#$
Serum Adiponectin level (ng/ml)	70.6±6.78	43.4±3.76*	58.9±4.53*#	ы .3±5.23*#

Data are expressed as mean ± standard deviation of a group of 10 rats.
Significance of difference from young control group is illustrated as *: $p<0.05$.
Significance of difference from aged group is illustrated as #: $p<0.05$.
Significance of difference from VD3-aged group is illustrated as $: $p<0.05$.

Effect of VD3 or LC on skeletal muscle SIR T-1 and GLUt4 mRNA expression:

As presented in (Table 4) and Fig. (2), levels of SIRT-1 and GLUT4 mRNA expression were significantly decreased by 29.8% and 50.4%, respectively, in skeletal muscle of aged group versus YC group. On the other hand, Vit D3 significantly increased SIRT1 levels by (1.07 fold increase) in VD3-aged versus aged group, while, no noticeable changes in SIRT-1 levels were recorded in LC-aged versus aged group. Meanwhile, treatment of aged group with VD3 or LC significantly increased GLUT-4 mRNA expression by 1.80 and 1.84 fold, respectively, as compared to aged group, denoting their comparable effect on skeletal muscle GLUT4 mRNA expression.

Table (4): Skeletal muscle SIRT1, TGs and TNF-α levels in all studied groups.

Parameters	Young control	Aged	VD3-aged	LC-aged
Skeletal muscle SIRT1 (ng/mg tissue protein)	139.4±11.67	97.8±8.91*	133.8±10.67#	104.8±9.34*$
Muscle triglycerides (mg/g tissue)	6.19±1.13	11.71±1.78*	8.09±1.26#	6.42±0.93#
Skeletal muscle TNF-α (ng/mg protein)	0.644±0.12	1.62±0.65 *	1.18±0.53*#	0.68±0.23#$

Data are expressed as mean ± standard deviation of a group of 10 rats.
Significance of difference from young control group is illustrated as *: $p<0.05$.
Significance of difference from aged group is illustrated as #: $p<0.05$.
Significance of difference from VD3-aged group is illustrated as $: $p<0.05$.

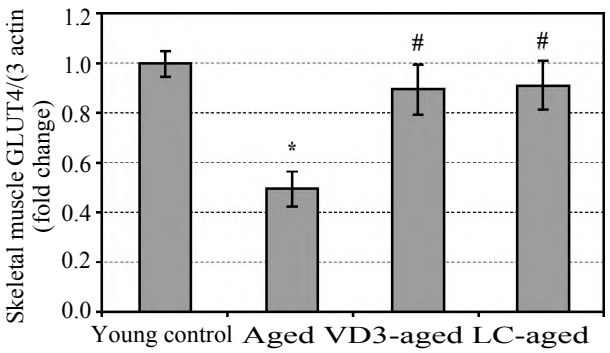

Fig. (2): Skeletal muscle GLUT 4 mRNA expression in all studied groups.

Data are expressed as mean ± standard deviation of a group of 10 rats. Significance of difference from young control group is illustrated as *: $p<0.05$. Significance of difference from aged group is illustrated as #: $p<0.05$.

Effect of VD3 or LC on skeletal muscle TG and TNF-α: The results of (Table 4) revealed that the levels of TNF-α and TG levels were significantly increased by 2.52 and 1.89 fold, respectively in skeletal muscle homogenates of aged group versus YC group.

In comparison with the aged group, the levels of TNF-α and TG were significantly decreased by 27.16%, 30.9%, respectively in VD3-aged group, and by 58.02%, 45.18%, respectively, in LC-aged group. Moreover, a significant difference was observed in skeletal muscle levels of TNF-α and TG levels between the VD3-aged and LC-aged groups, referring the more prominent effect of LC than VD3 in this respect (Table 4).

Discussion

The interaction of multiple factors associated with aging contributes to age-related changes in IR noticed in elderly population [53]. The current study examined the development of IR in a naturally aging rat model. It was found that aged rats showed an increase in IR revealed by, significant elevation of HOMA-IR index which was supported by the significant fasting hyper insulinemia that seems to be a compensatory mechanism to maintain glucose homeostasis in light of high IR [53]. Our results were in line with previous observations [54,55]. Additionally, the glucose clearance was slower in aged versus YC rats, so that the AUC glucose was significantly higher in aged group, which agree with earlier studies [56,57], and confirming the aging associated high IR and glucose intolerance.

Noteworthy, treatment with either VD3 or LC for 6 weeks were shown to counteract age- related IR, which was in accord with findings of previous studies [28,35,58,59]. Moreover, their effect on IR was nearly comparable to each other's.

The effect of VD3 or LC on glucose tolerance and IR were previously attributed to their anti-obesity and body weight lowering effects [59,60]. Surprisingly, we did not find any significant differences in the body weight or BMI when VD3 or LC treated aged rats were compared with age matched non-treated counterparts. Thus, we could attribute their beneficial effect on IR to anti-obesity-independent mechanisms in this rat model. In support to this hypothesis, many studies elucidated that IR occurs in non-obese elderly humans [61,62]. Moreover, both VD3 and LC were shown to mediate an improvement in IR regardless of their effect on obesity [25,41,45,63].

One of the mechanisms by which aging contributes to IR is chronic inflammation, in which mild persistent accumulation of pro-inflammatory cytokines, a phenomenon called; inflammaging, was observed in elderly and had negative impact on insulin signaling [64,65]. In consistence, our results showed an increased level of serum TNF-α and IL-6 alongside with increased TNF α in skeletal muscle of aged rats compared to YC ones.

In line with these findings, Al-Ghamdi and Aboodi [57] recorded a significant positive correlation between IR and pro-inflammatory cytokines in the sera and insulin sensitive tissues of aged rats, and stated that these cytokines can act in an autocrine and paracrine fashion to induce IR in peripheral tissues. These cytokines were also linked to reduction of the GLUT4 mRNA expression [66], which consequently decreased glucose uptake by the muscle, as recorded later in this current study and supported previously [67]. Additionally, Plomgaard et al., [68] stated that increasing the serum level of TNF-α down regulated the insulin signaling and whole body glucose uptake; mostly via reducing the insulin-mediated glucose uptake by the skeletal muscle. TNF-α could stimulate the inhibitory phosphorylation of serine residues of Insulin Receptor Substrate 1 (IRS-1) [69]. Specifically, phosphorylation of these residues impedes the normal association of IRS-1 with insulin receptors, thereby impairing the downstream of insulin signaling [70,71]. TNF-α could also induce skeletal muscle IR via suppressing AMPK signaling [72]. AMPK is a vital energy sensor that positively modulates insulin-dependent glucose uptake [73] and it is well known for its insulin sensitizing effects [74,75].

On the other hand, our investigations showed that treatment with VD3 or LC for 6 weeks significantly suppressed the notable increase in the inflammatory cytokines in the sera and skeletal muscle of aged rats, with superiority of LC above VD3 in this respect.

In line with this proposal, both VD3 and LC were shown to modulate the expression and release of TNF-α and IL-6 in different experimental models [76-79]. Vitamin D3 could suppress NF-κB activation and consequently the transcription of its downstream targets, TNF α, in different cell types [80-82] including the muscle cells [59]. Meanwhile, LC could counteract the TNF-α-induced IR [83,84], possibly via its activation to AMPK signaling bathway and upregulating IRS-1 with a sequel of improvement of insulin signaling [75].

Another important observation in this current study was the significant decrease in the serum level of adiponectin in aged group versus YC group, which came in line with previous reports [1,8,85]. This observed diminution in the circulating adiponectin levels may be as a consequence of age associated redistribution of adipose tissue and visceral adiposity which could influence the adiponectin level and insulin sensitivity [86].

Adiponectin has been identified as a beneficial marker for metabolic disorders with its well documented insulin sensitizing effects [85]. It could maintain glucose homeostasis by increasing hepatic and muscle insulin sensitivity. Its insulin sensitizing effects could be attributed to its suppression to TNF-α expression [87], its stimulation to AMPK

signalling pathways in liver, adipose tissue and skeletal muscle as well as its promotion to fatty acid oxidation **[88,89]**.

Notably, treatment with either vitamin D3 or LC increased serum adiponectin levels in VD3 and LC-aged rats compared to untreated aged ones. In accordance, it had been concluded that Vitamin D is involved in the regulation of adipocyte gene expression as well as cell signaling **[90]**. Vitamin D administration in type 2 diabetic patients can elevate serum levels of adiponectin, suggesting a role of Vitamin D in adiponectin secretion **[91]**. Furthermore, Minto et al., **[92]** suggested a potential association between low Vit. D levels and adiponectin and elucidated a significant dose-response relationship between Vitamin D3 supplementation and adiponectin levels. On the other side, LC was shown to enhance adiponectin secretion in non-diabetic subjects and diabetic rats **[93]**.Consistently, the beneficial effects of LC in a diabetic model was suggested to be related to its increment to adiponectin levels **[35]**. From the above overmentioned data, we may speculate that the effect of Vit. D3 or LC in mitigating the aging related IR could be mediated partially in part, via their induced increment of serum adiponectin.

Additionally and depending on the hypothesis of that during aging the skeletal muscle is the principle site where the early metabolic disturbances leading to IR take place **[94]**. The skeletal muscle SIRT1, and TGs levels alongside with GLUT4 mRNA expression were also assayed herein, for further dissecting the underlying mechanisms of age-induced IR with the possible modulating role of VD3 and LC in this aspect.

The findings of our research elucidated that, the IR observed in aging rats was in concomitance with a significant decline in SIRT-1 levels in skeletal muscle of aged rats versus YC ones. Similar results were recorded previously **[18,94]**.

SIRT-1, through its dependent transduction pathways, is a well-established insulin sensitizer **[95]**. It plays a critical role in glycemic control; mediated via its activation to pathways involved in cellular energy metabolism, glucose uptake, and glucose production inhibition **[96]**. Upregulation of SIRT-1 was associated with enhancement of insulin-stimulated glucose uptake and downregulation of IRS 1 phosphorylation which consequently improved insulin signaling **[95,97]**. Moreover, SIRT-1 could activate AMPK **[98]** and interact with it resulting in activation of the pathways that control cellular energy metabolism, mitochondrial biogenesis and glucose uptake **[96]**. Consistently, studies suggested that activating AMPK could prevent and/or ameliorate IR **[99]**.

Interestingly, treatment with VD3 not LC could increase the SIRT1 levels in skeletal muscles of VD3-aged versus aged group. In agreement, an in vitro study revealed that VD3 could upregulate glucose uptake in myotubes, mediated by SIRT1/IRS1/GLUT4 signaling cascade **[100]**. Consistently, the study of Manna et al., **[101]** signified the ability of VD3 to upregulate the SIRT1/AMPK/IRS 1/GLUT4 cascade, which could mediate the promoting effect of VD3 on glucose uptake in diabetic mice model. Thus the increment of SIRT1 by VD3 might be a fundamental path to modulate glucose homeostasis, improving IR in this rat model.

A large body of evidence suggested that intramyocellular TGs were associated with skeletal muscle IR **[102,103]** and accompanied by reduced glucose utilization and downregulation of insulin signaling through the phosphatidylinositol 3-kinase/Akt pathway **[104-106]**.

Another key finding of our study was that TGs levels were significantly increased in the muscles of aged rats compared with YC ones, which was in concomitance with previous studies **[1,107]**. An imbalance between fatty acid synthesis and oxidation with decreased fatty acid oxidation in skeletal muscle is one of the major factors leading to lipid accumulation and IR with aging **[1]**.

On the contrary, results herein revealed that both VD3 and LC significantly reduced TGs accumulation in the muscle of VD3-aged and LC-aged rats versus aged ones, with more prominent effect of LC in this respect. Our findings were in parallel with previous studies **[1,34,43,59]**.

The effect of either VD3or LC could be attributable to their well- documented capabilities to activate AMPK **[41,108,109]**. AMPK had a pivotal role in regulating the skeletal muscle fatty acid metabolism with its ability to enhance skeletal muscle fatty acid oxidation and long chain fatty acid flux into the mitochondria, improving IR **[108]**. Additionally, Vit. D insufficiency was elucidated to be significantly and inversely associated with the degree of fat infiltration in skeletal muscle **[110]**. Vitamin D3 could reverse the intra muscular lipid infilteration through suppressing the expression of Sterol Regulatory Element-Binding Protein (SREBP)-1c **[59]**, which was shown to be increased in aging skeletal muscle **[111]**. SREBP-1c is a key transcription factor that regulates de novo lipogenesis in insulin-sensitive tissues including the muscle

[112]. Aberrant activation of SREBP-1c leads to lipid accumulation, IRS-1 suppression, and subsequent muscular IR [113]. Meanwhile, the most obvious mechanism of TG lowering effect by LC is its influence on the influx of fatty acids to the mitochondria. L carnitine acts as a cofactor in β-oxidation, facilitating long chain fatty acid entrance into mitochondria in the form of acyl-carnitine esters and the exit of acetyl groups from mitochondria to the cytosol [37]. Thereby, the ability of LC to reduce muscular lipid accumulation through enhancing the mitochondrial fatty acid oxidation capacity, may be one of its important insulin sensitizing mechanisms in this rat model.

The main cellular mechanism which decreases blood glucose after glucose load is insulin-stimulated glucose transport into skeletal muscle. The principle glucose transporter mediating this uptake is GLUT-4, hence it is a key determinant of glucose disposal [65]. Our results showed that GLUT 4 mRNA expression was significantly decreased in aged versus YC rats, which agree previous studies linking aging induced IR with defective skeletal muscle mRNA expression [94,114].

Several researches reported that the level of GLUT4 mRNA expression in skeletal muscle regulates total body glucose homeostasis and correlated with muscle glucose uptake capacity and whole body glucose disposal [115,116]. A decreased GLUT-4 mRNA level was reported in IR patients [117]. Moreover, Transgenic ablation of GLUT-4 in muscle results in IR and impaired glucose tolerance [116]. In contrast, the increase in muscle GLUT-4 protein expression enhanced insulin sensitivity in vitro [118]. Overexpression of GLUT-4 in muscle of genetically diabetic mice attenuates IR and promotes glycemic control by increasing both basal and insulin-stimulated glucose transport [119]. Accordingly, these reports denote that skeletal muscle glucose transport is a rate-limiting step for whole body glucose disposal, suggesting a role for upregulation of GLUT-4 expression in mitigating the aging induced IR.

Noteworthy, VD3 or LC administration significantly increased the GLUT-4 mRNA expression in skeletal muscle of treated -aged versus aged rats which lie in parallel with previous studies [41,58, 120,121]. As regarding the molecular mechanisms, VD3-induced upregulation of GLUT-4 expression, might be attributable to the increased muscle SIRT-1 levels observed herein, which in turn could up regulate IRS-1 leading to enhancement of GLUT-4 translocation from intracellular vesicles into the cell membrane [100]. Moreover, vit D3 and LC could activate AMPK either directly [41,108] or through increasing adiponectin levels [88]. AMPK was shown to enhance GLUT-4 expression [75] and act as a signal for GLUT4 translocation from intracellular vesicles into the cell membrane which consequently increased glucose uptake inside the muscle with improvement of insulin sensitivity [122,123].

Conclusion:

The present study demonstrated that both VD3 and LC supplementation were equally effective in attenuating the age-related IR. This might be mediated by their anti-inflammatory effects; their partial increment of serum adiponectin levels; their up regulation to the skeletal muscle GLUT-4 expression as well as their reduction to skeletal muscle lipid accumulation. The anti-inflammatory and TGs lowering effects of LC were more prominent than VD3, while, VD3 alone could increase skeletal muscle SIRT1 levels. Nevertheless, the overall effect of VD3 and LC on IR-related parameters were nearly equal. Thus, VD3 or LC may be a possible candidate for alleviating the age-induced IR, thus may provide a possible therapeutic intervention in the elderly.

References

1- LIU L., YAO L., WANG S., CHEN Z., HAN T., MA P., et al.: 6-Gingerol Improves Ectopic Lipid Accumulation, Mitochondrial Dysfunction, and Insulin Resistance in Skeletal Muscle of Ageing Rats: Dual Stimulation of the AMPK/PGC-1 α Signaling Pathway via Plasma Adiponectin and Muscular AdipoR1. Mol. Nutr. Food Res., Mar., 63 (6): e1800649, 2019.

2- MORENO M., ORDOÑEZ P., ALONSO A., DÍAZ F., TOLIVIA J. and GONZÁLEZ C.: Chronic 17 beta-estradiol treatment improves skeletal muscle insulin signaling pathway components in insulin resistance associated with aging. Age (Dordr), 32 (1): 1-13, 2010.

3- AKINTOLA A.A. and VAN HEEMST D.: Insulin, aging, and the brain: Mechanisms and implications. Front Endocrinol, (Lausanne), 6, 13, 2015.

4- CARRASCOSA J.M., ANDRÉ S.A., ROS M., BOGÓ NEZ E., ARRIBAS C., FERNÁNDEZ-AGULLÓ T., et al.: Development of insulin resistance during aging: Involvement of central processes and role of adipokines. Curr. Prot. Pept. Sci., 12: 305-15, 2011.

5- GUARNER-LANS V., RUBIO-RUIZ M.E., PÉREZ-TORRES I. and BAÑOS De MACCARTHY G.: Relation of aging and sex hormones to metabolic syndrome and cardiovascular disease. Exp. Gerontol., 46: 517-23, 2011.

6- De TATA V.: Age-related impairment of pancreatic Beta-cell function: Pathophysiological and cellular mechanisms. Front Endocrinol (Lausanne), 5: 138, 2014.

7- HOWCROFT T.K., CAMPISI J., LOUIS G.B., SMITH M.T., WISE B., WYSS-CORAY T., et al.: The role of

inflammation in age-related disease. Aging (Albany NY), 5: 84-93, 2013.

8- INOUE A., CHENG X.W., HUANG Z., HU L., KIKUCHI R., JIANG H., et al.: Exercise restores muscle stem cell mobilization, regenerative capacity and muscle metabolic alterations via adiponectin/AdipoR1 activation in SAMP10 mice. J. Cachexia Sarcopenia Muscle, 8 (3): 370-85, 2017.

9- WARAGAI M., HO G., TAKAMATSU Y., SHIMIZU Y., SUGINO H., SUGAMA S., et al.: Dual-therapy strategy for modification of adiponectin receptor signaling in aging-associated chronic diseases. Drug Discov. Today, 23 (6): 1305-11, 2018.

10- JIANG L., YAO L., YANG Y., KE D., BATEY R., WANG J6, et al.: Jiangzhi Capsule improves fructose-induced insulin resistance in rats: Association with repair of the impaired sarcolemmal glucose transporter-4 recycling. J. Ethnopharmacol., 24 (194): 288-98, 2016.

11- SAFDAR A., HAMADEH M.J., KACZOR J.J., RAHA S., DEBEER J. and TARNOPOLSKY M.A.: Aberrant mitochondrial homeostasis in the skeletal muscle of sedentary older adults. PLoS One, 24; 5 (5): e10778, 2010.

12- JOSEPH A.M., ADHIHETTY P.J., BUFORD T.W., WOHLGEMUTH S.E., LEES H.A., NGUYEN L.M., et al.: The impact of aging on mitochondrial function and biogenesis pathways in skeletal muscle of sedentary high- and low-functioning elderly individuals. Aging Cell, 11 (5): 801-9, 2012.

13- HAIGIS M.C. and SINCLAIR D.A.: Mammalian sirtuins: Biological insights and disease relevance. Annu. Rev. Pathol., 5: 253-95, 2010.

14- PARDO P.S. and BORIEK A.M.: The physiological roles of Sirt1 in skeletal muscle. Aging (Albany NY), 3 (4): 430-7, 2011.

15- YOSHINO J., MILLS K.F., YOON M.J. and IMAI S.: Nicotinamide mononucleotide, a key NAD(+) intermediate, treats the pathophysiology of diet-and age-induced diabetes in mice. Cell Metab., 5; 14 (4): 528-36, 2011.

16- BANKS A.S., KON N., KNIGHT C., MATSUMOTO M., GUTIERREZ-JUAREZ R., ROSSETTI L., GU W. and ACCILI D.: SirT1 gain of function increases energy efficiency and prevents diabetes in mice. Cell Metab, 8 (4): 333-41, 2008.

17- HOUMARD J.A., WEIDNER M.D., DOLAN P.L., LEGGETT-FRAZIER N., GAVIGAN K.E., HICKEY M.S., TYNDALL G.L., et al.: Skeletal muscle GLUT4 protein concentration and aging in humans. Diabetes, 44 (5): 555-60, 1995.

18- MOHAMED J.S., WILSON J.C.1, MYERS M.J., SISSON K.J. and ALWAY S.E.: Dysregulation of SIRT-1 in aging mice increases skeletal muscle fatigue by a PARP-1-dependent mechanism. Aging (Albany NY), 6 (10): 820-34, 2014.

19- CANTO C. and AUWERX J.: AMP-activated protein kinase and its downstream transcriptional pathways. Cell Mol. Life Sci., 67 (20): 3407-23, 2010.

20- ZISMAN A., PERONI O.D., ABEL ED MICHAEL M.D., MAUVAIS-JARVIS F., LOWELL B.B., et al.: Targeted disruption of the glucose transporter 4 selectively in muscle causes insulin resistance and glucose intolerance. Nature Medicine, 6 (8): 924-8, 2000.

21- CHITTARI H.V.: Vitamin D and diabetes mellitus. Hormones (Athens), 13 (2): 163-81, 2014.

22- MEEHAN M. and PENCKOFER S.: The Role of Vitamin D in the Aging Adult. J. Aging Gerontol., 2 (2): 60-71, 2014.

23- JOHNSON J.A., GRANDE J.P., ROCHE P.C. and KUMAR R.: Immunohistochemical localization of the 1 25(OH)2D3 receptor and calbindin D28k in human and rat pancreas, Am. J. Physiol., 267 (3): E356-E360, 1994.

24- ROSEN C.J., ADAMS J.S., BIKLE D.D., BLACK D.M., DEMAY M.B., MANSON J.E., et al.: The non skeletal effects of Vitamin D: An endocrine society scientific statement, Endocr. Rev., 33: 456-92, 2012.

25- KELISHADI R., SALEK S., SALEK M., HASHEMIPOUR M. and MOVAHEDIAN M.: Effects of Vitamin D supplementation on insulin resistance and cardiometabolic risk factors in children with metabolic syndrome: A triple-masked controlled trial. J. Pediatr. (Rio J.), 90: 28-34, 2014.

26- SUN X., CAO Z.B., TANISAWA K., ITO T., OSHIMA S., et al.: Vitamin D supplementation reduces insulin resistance in Japanese adults: A secondary analysis of a double-blind, randomized, placebo-controlled trial. Nutr. Res., 36: 1121-9, 2016.

27- NADA A.M. and SHAHEEN D.A.: Cholecalciferol improves glycemic control in type 2 diabetic patients: A 6-month prospective interventional study. Ther .Clin. Risk Manag., 13: 813-20, 2017.

28- ABDEL-REHIM W.M., EL-TAHAN R.A., EL-TARAWY M.A., SHEHATA R.R. and KAMEL: The possible antidiabetic effects of Vitamin D receptors agonist in rat model of type 2 diabetes. Mol. Cell Biochem., 450 (1-2): 105-12, 2019.

29- CEFALO C.M.A., CONTE C., SORICE G.P., MOFFA S., SUN V.A., CINTI F., et al.: Effect of Vitamin D Supplementation on Obesity-Induced Insulin Resistance: A Double-Blind, Randomized, Placebo-Controlled Trial. Obesity (Silver Spring), 26 (4): 651-7, 2018.

30- MOUSA A., NADERPOOR N., De COURTEN M.P., TEEDE H., KELLOW N., WALKER K., SCRAGG R. and De COURTEN B.: Vitamin D supplementation has no effect on insulin sensitivity or secretion in Vitamin D-deficient, overweight or obese adults: A randomized placebo-controlled trial. Am. J .Clin. Nutr., 105 (6): 1372-81, 2017.

31- SITIAN H.E., SONGCHENG Y.U., ZHOU Z., WANG C., WU Y. and LI W.: Effect of Vitamin D supplementation on fasting plasma glucose, insulin resistance and prevention of type 2 diabetes mellitus in non-diabetics: A systematic review and meta-analysis Biomed Rep., 8 (5): 475-84, 2018.

32- FULEIHAN G.E.H., BADDOURA R., HABIB R.H., HALABY G., ARABI A., RAHME M. and KASSIR M.F.: Effect of Vitamin D replacement on indexes of insulin resistance in overweight elderly individuals: A randomdmized controlled trial. American Journal of Clinical Nutrition, 104 (2): 315-23, 2016.

33- GEORGE P.S., PEARSON E.R. and WITHAM M.D.: Effect of Vitamin D supplementation on glycaemic control and insulin resistance: A systematic review and meta-analysis, Diabet. Med., 29: 142-50, 2012.

34- RAJASEKAR P. and ANURADHA C.V.: Effect of L-Carnitine on Skeletal Muscle Lipids and Oxidative Stress in Rats Fed High-Fructose Diet. Exp. Diabetes Res., 2007: 72741, 2007.

35- SHAHOUZEHI B., BARKHORDARI K., AMINIZADEH S. and MASOUMI-ARDAKANI Y.: Effect of L-carnitine administration on serum insulin and adiponectin levels, and AMPK, APPL1 and PPARγ gene expression in STZ-induced diabetic rat liver. Ukr. Biochem. J., 89 (6): 48-55, 2017.

36- BERNARD A., RIGAULT C., MAZUE F., Le BORGNE F. and DEMARQUOY J.: L-carnitine supplementation and physical exercise restore age-associated decline in some mitochondrial functions in the rat. J. Gerontol. A Biol. Sci. Med. Sci., 63 (10): 1027-33, 2008.

37- XIA Y., LI Q., ZHONG W., DONG J., WANG Z. and WANG C.: L-carnitine ameliorated fatty liver in high-calorie diet/STZ-induced type 2 diabetic mice by improving mitochondrial function. Diabetology & Metabolic Syndrome, 3: 31, 2011.

38- MINGRONE G., GRECO A.V., CAPRISTO E., BENEDETTI G., GIANCATERINI A., De GAETANO A., et al.: L-carnitine improves glucose disposal in type 2 diabetic patients. J. Am. Coll. Nutr., 18: 77-82, 1999.

39- MINGRONE G.: Carnitine in type 2 diabetes. Ann. N. Y. Acad. Sci., 1033: 9-107, 2004.

40- RINGSEIS R., KELLER J. and EDER K.: Role of carnitine in the regulation of glucose homeostasis and insulin sensitivity: Evidence from in vivo and in vitro studies with carnitine supplementation and carnitine deficiency. Eur. J. Nutr., 51: 1-18. 2012.

41- MOSTAFA A.F. and ADEL M.: L-carnitine Increases Skeletal Muscle GLUT4 Expression and Improves Metabolic Control in Type 2 Diabetes. Bull. Egypt. Soc. Physiol. Sci., 34 (3): 386-403, 2015.

42- SCHOONEMAN M.G., HOUTKOOPER R.H., HOLLAK C.E., WANDERS R.J., VAZ F.M., SOETERS M.R. and HOUTEN S.M.: The impact of altered carnitine availability on acylcarnitine metabolism, energy expenditure and glucose tolerance in diet-induced obese mice. Biochim. Biophys. Acta., 1862 (8): 1375-82, 2016.

43- AMIN S.N., HUSSEIN U.K., YASSA H.D., HASSAN S.S. and RASHED L.A.: Synergistic actions of Vitamin D and metformin on skeletal muscles and insulin resistance of type 2 diabetic rats. J. Cell Physiol., 233 (8): 5768-79, 2018.

44- ELATTAR S., ESTAPHAN S., MOHAMED E.A., EL-ZAINY A. and NAGUIB M.: The protective effect of 1alpha, 25-dihydroxyvitamin d3 and metformin on liver in type 2 diabetic rats. J. Steroid Biochem. Mol. Biol., 173: 235-44, 2017.

45- RANJBAR KOHAN N., NAZIFI S., TABANDEH M.R. and ANSARI LARI M.: Effect of L-Carnitine Supplementation on Apelin and Apelin Receptor (Apj) Expression in Cardiac Muscle of Obese Diabetic Rats. Cell J., 20 (3): 427-34, 2018.

46- OKADA N., KOBAYASHI S., MORIYAMA K., MIYATAKA K., ABE S., SATO C. and KAWAZOE K.: Helianthus tuberosus (Jerusalem artichoke) tubers improve glucose tolerance and hepatic lipid profile in rats fed a high-fat diet. Asian Pac J. Trop. Med., 10 (5): 439-43, 2017.

47- PURVES R.D.: Optimum numerical integration methods for estimation of area-under-the-curve (AUC) and area-under-the-moment-curve (AUMC). J. Pharmacokinet. Biopharm., 20 (3): 211-26, 1992.

48- NOVELLI E.L., DINIZ Y.S., GALHARDI C.M., EBAID G.M., RODRIGUES H.G., MANI F., et al.: Anthropometrical parameters and markers of obesity in rats. Lab. Anim., 41: 111-9, 2007.

49- LOWRY O.H., ROSENBROUGH N.J., FARR A.L. and RANDALL R.J.: Protein measurement with the Folin phenol reagent, J. Biol. Chem., 193: 265-75, 1951.

50- TRINDER P.: Determination of blood glucose using 4-amino phenazone as oxygen acceptor. J. Clin. Pathol., 22 (2): 246, 1969.

51- MATTHEWS D.R., HOSKER J.P., RUDENSKI A.S., NAYLOR B.A., TREACHER D.F. and TURNER R.C.: Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia, 28: 412-9, 1985.

52- LIVAK K.J. and SCHMITTGEN T.D.: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C (T)) Method. Methods, 25 (4): 402-8, 2001.

53- CHANG A.M. and HALTER J.B.: Aging and insulin secretion. Am. J. Physiol. Endocrinol. Metab., 284 (1): 7-12, 2003.

54- NAKHANAKUP C., MOUNGMEE P., APPELL H.J. and DUARTE J.: Regular physical exercise in patients with type II diabetes mellitus. Eur. Rev. Aging. Phys. Act., 31: 10-9, 2006.

55- SANTOS J.M., RIBEIRO S.B., GAYA AR, APPELL H.J. and DUARTE J.A.: Skeletal muscle pathways ofcontraction-enhanced glucose uptake. Int. J. Sports Med., 29 (10): 785-94, 2008.

56- MORENO M., SILVESTRI E., De MATTEIS R., De LANGE P., LOMBARDI A., GLINNI D., et al.: 3, 5-Diiodo-L-thyronine prevents high-fat-diet-induced insulin resistance in rat skeletal muscle through metabolic and structural adaptations. FASEB J., 25 (10): 3312-24, 2011.

57- ABOOD A.M. and ALGHAMDI B.S.: Oxytocin supplementation alleviates age-related insulin resistance through down regulation of pro-inflammatory cytokine gene expression. Biomedical Research, 28 (5): 2209-15, 2017.

58- ZAYED E.A., AIN SHOKA A.A., EL SHAZLY A.K. and ABD EL LATIF H.A.: Improvement of insulin resistance via increase of GLUT4 and PPARγ in metabolic syndrome-induced rats treated with omega-3 fatty acid or L-carnitine. J. Biochem. Mol. Toxicol., 32 (11): e22218, 2018.

59- BENETTI E., MASTROCOLA R., CHIAZZA F., NIGRO D., D'ANTONA G., BORDANO V., et al.: Effects of Vitamin D on insulin resistance and myosteatosis in diet-induced obese mice. PLoS One, 17; 13 (1): e0189707, 2018.

60- AMIN K.A. and NAGY M.A.: Effect of Carnitine and herbal mixture xtract on obesity induced by high fat diet in rats: Diabetol. Metab. Syndr., 1: 17, 2009.

61- REFAIE M.R., SAYED-AHMED N.A., BAKR A.T., ABDEL AZIZ Y.M., EL KANNISHI H.M. and ABDEL-GAWADD S.S.: Aging inevitable risk factor for insulin resistance. Journal of Taibah University Medical Sciences, 1 (1): 30-41, 2006.

62- FINK R.I., WALLACE P. and OLEFSKY J.M.: Effects of aging on glucose-mediated glucose disposal and glucose transport. Journal of Clinical Investigation, 77: 2034-41, 1986.

63- TALAEI A., MOHAMADI M., and ADGI Z.: The effect of Vitamin D on insulin resistance in patients with type 2 diabetes. Diabetol. Syndr., 5: 8, 2013.

64- De TATA V.: Age-related impairment of pancreatic Betacell function: Pathophysiological and cellular mechanisms. Front Endocrinol (Lausanne), 5: 138, 2014.

65- FARKHONDEH T., ABEDIC F. and SAMARGHANDIAN S.: Chrysin attenuates inflammatory and metabolic disorder indices in aged male rat. Biomedicine & Pharmacotherapy, 109: 1120-5, 2019.

66- ROTTER V., NAGAEV I. and SMITH U.: Interleukin-6 (IL-6) induces insulin resistance in 3T3-L1 adipocytes and is, like IL-8 and tumor necrosis factor-alpha, overexpressed in human fat cells from insulin-resistant subjects. J. Biol. Chem., 278 (46): 45777-84, 2003.

67- RAZNY U., KIEC-WILK B., WATOR L., POLUS A., DYDUCH G., SOLNICA B., et al.: Increased nitric oxide availability attenuates high fat diet metabolic alterations and gene expression associated with insulin resistance. Cardiovasc. Diabetol., 10: 68, 2011.

68- PLOMGAARD P., BOUZAKRI K., KROGH-MADSEN R., MITTENDORFER B., ZIERATH J.R. and PEDERSEN B.K.: Tumor necrosis factor-alpha induces skeletal muscle insulin resistance in healthy human subjects via inhibition of Akt substrate phosphorylation. Diabetes, 54 (10): 2939-45, 2005.

69- AGUIRRE V., UCHIDA T., YENUSH L., DAVIS R. and WHITE M.F.: The c-Jun NH (2)-terminal kinase promotes insulin resistance during association with insulin receptor substrate-1 and phosphorylation of Ser. J. Biol. Chem., 275: 9047-54, 2000.

70- GUAL P., LE MARCHAND-BRUSTEL Y. and TANTI J.F.: Positive and negative regulation of insulin signaling through IRS-1 phosphorylation. Biochimie, 87: 99-109, 2005.

71- AGUIRRE V., WERNER E.D., GIRAUD J., LEE Y.H., SHOELSON S. and WHITE M.F.: Phosphorylation of Ser307, in insulin receptor substrate-1 blocks interactions with the insulin receptor and inhibits insulin action. J. Bio. Chem., 277: 1531-7, 2002.

72- STEINBERG G.R., MICHELL B.J., VAN DENDEREN B.J., WATT M.J., CAREY A.L., FAM B.C., et al.: Tumor necrosis factor alpha-induced skeletal muscle insulin resistance involves suppression of AMP-kinase signaling. Cell Metab., 4: 465-74, 2006.

73- JING M., CHERUVU V.K. and ISMAIL-BEIGI F.: Stimulation of glucose transport in response to activation of distinct AMPK signaling pathways. Am. J. Physiol. Cell Physiol., 295 (5): C1071-82, 2008.

74- MCLAUGIN T., ABBASI F., CHEAL K., CHU J., LAMENDOLA C. and REAVEN G.: Use of metabolic markers to identify overweight individuals who are insulin resistant. Ann. Intern. Med., 139: 802-9, 2003.

75. ZHANG Z., ZHAO M., LI Q., ZHAO H., WANG J. and LI Y.: Acetyl-L-carnitine inhibits TNF-a-induced insulin resistance via AMPK pathway in rat skeletal muscle cells. FEBS Letters, 583: 470-4, 2009.

76- MOORE M.E., PIAZZA A., MCCARTNEY Y. and LYNCH M.A.: Evidence that Vitamin D3 reverses age-related inflammatory changes in the rat hippocampus. Biochem. Soc. Trans., 33: 573-7, 2005.

77- HASHEMI R., MORSHEDI M., JAFARABADI M.A., ALTAFI D., HOSSEINI-ASL S.S and RAFIE-AREFHOSSEINI S.: Anti-inflammatory effects of dietary Vitamin D3 in patients with multiple sclerosis. Neurology Genetics, 4 (6): e278, 2018.

78- HOE E., NATHANIELSZ J., TOH Z.Q., SPRY L., MARIMLA R., ANNE BALLOCH A., et al.: Anti-Inflammatory Effects of Vitamin D on Human Immune Cells in the Context of Bacterial Infection. Journal List Nutrients, 8 (12), 2016.

79- SHAKER A., TABIBI H. and HEDAYATI M.: Effects of L-carnitine supplement on serum inflammatory cytokines, C-reactive protein, lipoprotein (a), and oxidative stress in hemodialysis patients with Lp (a)hyperlipoproteinemia. Hemodialysis International, 14 (4): 498-504, 2010.

80- OZKAN G.O.: The Effects of Vitamin D on Obesity, Insulin Resistance and Type 2 Diabetes. J. Obes .Overweig., 5 (1): 101, 2019.

81- STIO M., MARTINESI M., BRUNI S., TREVES C., MATHIEU C., VERSTUYF A., et al.: The Vitamin D analogue TX 527 blocks NF-kappaB activation in peripheral blood mononuclear cells of patients with Crohn's disease.The Journal of steroid biochemistry and molecular biology, 103 (1): 51-60, 2007.

82- COHEN-LAHAV M., SHANY S., TOBVIN D., CHAIMOVITZ C. and DOUVDEVANI A.: Vitamin D decreases NF-kappa B activity by increasing IkappaBalpha levels. Nephrology, dialysis, transplantation: Official publication of Vitamin D reverses metabolic injury. The European Dialysis and Transplant Association D European Renal Association, 21 (4): 889-97, 2006.

83- DONATH M.Y. and SHOELSON S.E.: Type 2 diabetes as an inflammatory disease. Nature Reviews Immunology, 11 (2): 98-107, 2011.

84- PRADHAN A.D., MANSON J.E., RIFAI N., BURING J.E. and RIDKER P.M.: C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. Journal of the American Medical Association, 286 (3): 327-34, 2001.

85- IWABU M., OKADA-IWABU M., YAMAUCHI T. and KADOWAKI T.: Aiponectin/adiponectin receptor in disease and aging, NPJ Aging Mech. Dis., 1 (15013), 2015.

86- PALMER A.K. and KIRKLAND J.L.: Aging and Adipose Tissue: Potential Interventions for Diabetes and Regenerative Medicine. Exp. Gerontol., 86: 97-105, 2016.

87- OKAMOTO Y., KIHARA S., OUCHI N., NISHIDA M., ARITA Y., KUMADA M., et al.: Adiponectin reduces atherosclerosis in apolipoprotein E-deficient mice. Circulation, 106: 2767, 2002.

88- OKADA-IWABU M., YAMAUCHI T., IWABU M., HONMA T., HAMAGAMI K., MATSUDA K., et al.: A small-molecule AdipoR agonist for type 2 diabetes and short life in obesity. Nature, 503: 493, 2013.

89- KELLY M., RUDERMAN N.B. and TOMAS E.: AMP-activated protein kinase and its regulation by adiponectin and interleukin-6. Scandinavian Journal of Food and Nutrition, 50 (2): 85-91, 2006.

90- RYYNÄNEN J., NEME A., TUOMAINEN T.P., VIRTANEN J.K., VOUTILAINEN S., NURMI T., et al.: Changes in Vitamin D target gene expression in adipose tissue monitor the Vitamin D response of human individuals. Molecular Nutrition & Food Research, 58 (10): 2036-45, 2014.

91- NEYESTANI T.R., NIKOOYEH B., ALAVI-MAJD H., SHARIATZADEH N., KALAYI A., TAYEBINEJAD N., et al.: Improvement of Vitamin D status via daily intake of fortified yogurt drink either with or without extra calcium ameliorates systemic inflammatory biomarkers, including adipokines, in the subjects with type 2 diabetes. The Journal of Clinical Endocrinology and Metabolism, 97 (6): 2005-11, 2012.

92- MINTO C., VECCHIO M.G. and GREGORI D.: Effects of Vitamin D Supplementation on Serum Adiponectin: A Metaregression Analysis of Clinical Trials. The FASEB Journal, 29: LB347, 2015.

93- ODO S., TANABE K. and YAMAUCHI M.: A pilot clinical trial on L-carnitine supplementation in combination with motivation training: Effects on weight management in healthy volunteers. Food Nutr. Sci., 4 (2): 222-31, 2013.

94- EL AGATY S.M.: Triiodothyronine Improves Age Induced Glucose Intolerance and Increases The Expression of Sirtuin-1 and Glucose Transporter-4 in Skeletal Muscle of Aged Rats. Gen. Physiol. Biophys., 37 (6): 677-86, 2018.

95- SCHENK S., MCCURDY C.E., PHILP A., CHEN M.Z., HOLLIDAY M.J., BANDYOPADHYAY G.K., OSBORN O., BAAR K. and OLEFSKY J.M.: Sirt1 enhances skeletal muscle insulin sensitivity in mice during caloric restriction. J. Clin. Invest., 121 (11): 4281-8, 2011.

96- HARDIE D.G.: AMPK: A target for drugs and natural products with effects on both diabetes and cancer. Diabetes, 62 (7): 2164-72, 2013.

97- SUN C., ZHANG F., GE X., YAN T., CHEN X., SHI X. and ZHAI Q.: SIRT1 improves insulin sensitivity under insulin-resistant conditions by repressing PTP1B. Cell Metab., 6: 307-19, 2007.

98- De LANGE P., CIOFFI F., SENESE R., MORENO M., LOMBARDI A., SILVESTRI E., De MATTEIS R., et al.: Nonthyrotoxic prevention of diet-induced insulin resistance by 3,5-diiodo-L-thyronine in rats. Diabetes, 60 (11): 2730-9, 2011.

99- RUDERMAN N.B., CARLING D., PRENTKI M. and CACICEDO J.M.: AMPK, insulin resistance, and the metabolic syndrome. J. Clin. Invest., 123 (7): 2764-72, 2013.

100- MANNA P., ACHARI A.E. and JAIN S.K.: 1 ,25(OH)2-vitamin D3 upregulates glucose uptake mediated by SIRT1/IRS1/GLUT4 signaling cascade in C2C12 myotubes. Mol. Cell Biochem., 444 (1-2): 103-8, 2018.

101- MANNA P., ACHARI A.E. and JAIN S.K.: Vitamin D supplementation inhibits oxidative stress and upregulate SIRT1/AMPK/GLUT4 cascade in high glucose-treated 3T3L1 adipocytes and in adipose tissue of high fat diet-fed diabetic mice. Arch. Biochem. Biophys., 615: 22-34, 2017.

102- ALBU J.B., KOVERA A.J., ALLEN L., WAINWRIGHT M., BERK E., RAJA-KHAN N., et al.: Independent association of insulin resistance with larger amounts of intermuscular adipose tissue and a greater acute insulin response to glucose in African American than in white nondiabetic women. Am. J .Clin. Nutr., 82: 1210-7, 2005.

103- LEE J.S., PINNAMANENI S.K., EO S.J., CHO I.H., PYO J.H., KIM C.K., et al.: Saturated, but not n-6 polyunsaturated, fatty acids induce insulin resistance: Role of intramuscular accumulation of lipid metabolites. J. Appl. Physiol., 100: 1467-74, 2006.

104- HULVER M.W. and DOHM G.L.: The molecular mechanism linking muscle fat accumulation to insulin resistance. Proc. Nutr. Soc., 63: 375-80, 2004.

105- SHULMAN G.I.: Unraveling the cellular mechanism of insulin resistance in humans: New insights from magnetic resonance spectroscopy. Physiology (Bethesda), 19: 183-90, 2004.

106- MA P., YAO L., LIN X., GU T., RONG X., BATEY R., et al.: Am. J. Transl. Res., 8: 3791, 2016.

107- ZOICO E., ROSSI A., Di FRANCESCO V., SEPE A., OLIOSO D., PIZZINI F., et al.: Adipose tissue infiltration in skeletal muscle of healthy elderly men: Relationships with body composition, insulin resistance, and inflammation at the systemic and tissue level. J Gerontol. A. Biol. Sci. Med. Sci., 65 (3): 295-9, 2010.

108- SAKINAH N.E.: Pharmacodynamics study of cholecalciferol to glut4 protein translocation in muscle fiber of hyperglycemia mice which inducted by streptozotocin.folia medica indonesiana, 49 (3): 134-13, 2013.

109- MASER R.E., LENHARD M.J., POHLIG R.T. and BALAGOPAL P.B.: Pre-heparin lipoprotein lipase mass as a potential mediator in the association between adiponectin and HDL-cholesterol in type 2 diabetes. Journal of Clinical and Translational Endocrinology, 7: 7-11, 2017.

110- GILSANZ V., KREMER A., MO A.O., WREN T.A. and KREMER R.: Vitamin D status and its relation to muscle mass and muscle fat in young women. J. Clin. Endocrinol. Metab., 95 (4): 1595-601, 2010.

111- KIM Y.J., KIM D.H., CHOI J., PARK J.K., JEONG K.S., LEEUWENBURGH C., et al.: Changes in lipid distribution during aging and its modulation by calorie restriction. Age (Dordr)., 31 (2): 127-42, 2009.

112- FERRÉ and FOUFELLE F.: SREBP-1c transcription factor and lipid homeostasis: Clinical perspective. Horm Res., 68 (2): 72-82, 2007.

113- BI Y., WU W., SHI J., LIANG H., YIN W., CHEN Y., et al.: Role for sterol regulatory element binding protein-1 c activation in mediating skeletal muscle insulin resist-

ance via repression of rat insulin receptor substrate-1 transcription. Diabetologia, 57 (3): 592-602, 2014.

114- EL GOHARY O.A.: Exercise Training Prevents Age-induced Insulin Resistance in Rats: Effect on Circulating Catecholamines, Inflammatory Cytokines and Skeletal Muscle Glut4 Transporters. American Journal of Biomedical Sciences, 127-39, 2017.

115- TSAO T.S., LI J., CHANG K.S., STENBIT A.E., GALUSKA D., ANDERSON J.E., et al.: Metabolic adaptations in skeletal muscle overexpressing GLUT4: Effects on muscle and physical activity. FASEB Journal, 15 (6): 958-69, 2001.

116- ZISMAN A., PERONI O.D., ABEL ED MICHAEL M.D., MAUVAIS-JARVIS F., LOWELL B.B., et al.: Targeted disruption of the glucose transporter 4 selectively in muscle causes insulin resistance and glucose intolerance. Nature Medicine, 6 (8): 924-8, 2000.

117- DOHM G.L., ELTON C.W., FRIEDMAN J.E., PILCH P.F., PORIES W.J., ATKINSON S.M. and CARO J.F.: Decreased expression of glucose transporter in muscle from insulin-resistant patients. Am. J. Physiol., 260 (3): E459-63, 1991.

118- CHARRON M.J. and KAHN B.B.: Divergent molecular mechanisms for insulin-resistant glucose transport in muscle and adipose cells in vivo. J. Biol. Chem., 265 (14): 7994-8000, 1990.

119- GIBBS E.M., STOCK J.L., MCCOID S.C., STUKENBROK H.A. PESSIN J.E., STEVENSON R.W., et al.: Glycemic improvement in diabetic db/db mice by overexpression of the human insulin-regulatable glucose transporter (GLUT4). J .Clin. Invest., 95 (4): 1512-8, 1995.

120- ZHOU Q.G., HOU F.F., GUO Z.J., LIANG M., WANG G.B. and ZHANG X.: 1,25-Dihydroxyvitamin D improved the free fatty-acid-induced insulin resistance in cultured C2C12 cells. Diabetes/metabolism research and Reviews, 24 (6): 459-64, 2008.

121- MANNA P. and JAIN S.K.: Vitamin D up-regulates glucose transporter 4 (GLUT4) translocation and glucose utilization mediated by cystathionine-gamma-lyase (CSE) activation and H2S formation in 3T3L1 adipocytes. The Journal of Biological Chemistry, 287 (50): 42324, 2012.

122- SANTOS R.S. and VIANNA L.M.: Effect of cholecalciferol upplementation on blood glucose in an experimental model of type 2 diabetes mellitus in spontaneously hypertensive rats and Wistar rats. Clinica. Chimica. Acta., 358: 146-215, 2005.

123- SESHADRI K.G., TAMISELVAN G. and RAJENDRAN A.: Role of Vitamin D in diabetes. J. Endocrinol. Metab., 1: 47-56, 2011.

فيتامين د٣ مقابل الكارنيتين فى تخفيف مقاومة الآنسولين المرتبطة بتقدم العمر فى نموذج الفئران ذو الشيخوخة الطبيعية

الهدف من الدراسة: مقارنة بين دور فيتامين د٣ مقابل الكارنيتين فى التخفيف من مقاومة الآنسولين المرتبطة بتقدم العمر فى نموذج الفئران ذو الشيخوخة الطبيعية.

المواد والطرق: تم تقسيم الفئران إلى ٤ مجموعات. الضابطة صغيرة السن، الضابطة كبيرة السن، كبيرة السن المعالجة بفيتامين د٣، كبيرة السن المعالجة بالكارنيتين. تضمنت الإجراءات التجريبية قياس وزن الجسم، ومؤشر كتلة الجسم (BMI) وإختبارات تحمل الجلوكوز عن طريق الفم (OGTT). كما تم حساب تقييم نموذج التماثل الساكن لمقاومة الآنسولين (HOMA-IR). وأجريت فحوصات الجلوكوز فى الدم ومستويات الآنسولين وكذلك عامل نخر الورم فى المصل (TNF-α) والإنترلوكين-٦ ومستويات الأديبونيكتين. بالإضافة إلى قياس SIRT-1، الدهون الثلاثية (TGs) ومستويات TNF-α إلى جانب ال GLUT 4 mRNA فى نسيج العضلة.

النتائج: كشفت هذه الدراسة أن العلاج بكلا من فيتامين د٣ أو الكارنيتين فى الفئران المعالجة كبيرة السن قد إلى تحسين ملحوظ وبنفس القدر من الكفاءه فى تخفيف مقاومة الآنسولين المرتبطة بالعمر والذى إرتبط بإنخفاض ملحوظ فى مستوى عوامل الإلتهاب وزيادة فى مستوى الأديبونيكتين فى مصل الدم، والذى كان موازياً لتحسين ملحوظ ذى دلالة إحصائية فى الدلالات الكيميائية لنسيج العضله.

الخلاصة: هذه النتائج تشير إلى أن العلاج بفيتامين د٣ أو الكارنيتين لهما نفس القدر من الكفاءة فى التخفيف من مقاومة الآنسولين المرتبطة بتقدم العمر فى نموذج الفئران ذو الشيخوخة الطبيعية.