

A single injection of vitamin D₃ improves insulin sensitivity and β -cell function but not muscle damage or the inflammatory and cardiovascular responses to an acute bout of resistance exercise in vitamin D-deficient resistance-trained males

Damoon Ashtary-Larky^{1,2}, Alireza Kheirollah³, Reza Bagheri⁴, Mohammad Ali Ghaffari³, Seyyed Ali Mard⁵, Seyed Jalal Hashemi⁶, Iman Mir⁷ and Alexei Wong^{8*}

¹Department of Clinical Biochemistry, School of Medicine, Abvaz Jundishapur University of Medical Sciences, Abvaz, 61357-15794, Iran

²Health Research Institute, Diabetes Research Center, Abvaz Jundishapur University of Medical Sciences, Abvaz, 61357-15794, Iran

³Department of Clinical Biochemistry, School of Medicine, Cellular & Molecular Research Center, Abvaz Jundishapur University of Medical Sciences, Abvaz, 61357-15794, Iran

⁴Department of Exercise Physiology, University of Isfahan, Isfahan, 81746-73441, Iran

⁵Department of Physiology, School of Medicine, Physiology Research Center (PRC), Abvaz Jundishapur University of Medical Sciences, Abvaz, 61357-15794, Iran

⁶Research Center for Infectious Diseases of Digestive System, Abvaz Jundishapur University of Medical Sciences, Abvaz, 61357-15794, Iran

⁷Department of Biostatistics and Epidemiology, School of Health, Abvaz Jundishapur University of Medical Sciences, Abvaz, 61357-15794, Iran

⁸Department of Health and Human Performance, Marymount University, Arlington, VA 22207, USA

(Submitted 26 June 2019 – Final revision received 10 October 2019 – Accepted 22 October 2019 – First published online 8 November 2019)

Abstract

Vitamin D deficiency is now a recognised problem affecting multiple physiological functions. The aim of the present study was to evaluate the effect of a single dose of vitamin D₃ injection on the inflammatory, muscular damage, metabolic and cardiovascular responses to an acute bout of resistance exercise (RE) in vitamin D-deficient resistance-trained males. Blood samples from fourteen vitamin D-deficient resistance-trained males were obtained during two separate trials: lower vitamin D (LVD) and higher vitamin D (HVD, after vitamin D₃ injection). Metabolic, inflammatory, muscle damage and cardiovascular markers were evaluated at baseline, immediately and 1 h after RE. There were significant trial-by-time interactions for insulin and homeostatic model assessment of insulin resistance (HOMA-IR) which significantly ($P < 0.05$) declined for 1 h after RE in the HVD trial compared with the LVD trial. Homeostasis model assessment of β -cell function (HOMA- β) declines at 1 h post-RE in the HVD trial. There was also a time effect for blood sugar which significantly ($P < 0.05$) decreased and for creatine kinase, lactate dehydrogenase and IL-6 which increased significantly 1 h post-RE in both trials. There were no significant changes in other inflammatory and cardiovascular markers following both trials. A single injection of vitamin D₃ improved insulin resistance and β -cell function following RE in previously vitamin D-deficient resistance-trained males. Conversely, the injection did not change muscle damage and the inflammatory response to acute RE. Intramuscular vitamin D replacement may have key implications for the promotion of glucose metabolism and lowering the risk of diabetes in vitamin D-deficient individuals.

Key words: Resistance exercise: Immunology: Glucose metabolism: Nutrition

Vitamin D deficiency is now recognised as a worldwide problem, which even affects athletic populations⁽¹⁾. Previous findings show associations between vitamin D deficiency and some chronic conditions such as CVD, cancer, type 2 diabetes and neurological disorders^(2,3). Vitamin D plays an important role

in immunomodulation, which can be beneficial not only in treatment and in management of inflammatory diseases but also for exercise-related inflammation^(1,4). Besides the immune system, literature also suggests that vitamin D has effects on muscular function through its role in muscle cell maturation, growth, repair

Abbreviations: BP, blood pressure; BS, blood sugar; CK, creatine kinase; HOMA-IR, homeostatic model assessment of insulin resistance; HR, heart rate; HVD, higher vitamin D; LDH, lactate dehydrogenase; LVD, lower vitamin D; RE, resistance exercise.

* **Corresponding author:** Alexei Wong, email awong@marymount.edu

and mitochondrial function, which potentially links this secosteroid to physical capability⁽⁵⁾. Indeed, prior results indicate that low levels of vitamin D can directly impair both muscle strength and sports performance⁽⁶⁾. Therefore, improving vitamin D deficiency seems essential to achieve optimal physiological effects during and following exercise^(7,8).

It is well established that acute resistance exercise (RE) enhances glucose uptake and increases insulin action⁽⁹⁾. Additionally, acute RE can induce muscle damage and prompt a temporary inflammatory response, which is dependent on the intensity and duration of the session⁽¹⁰⁾. As a response, creatine kinase (CK) levels are increased, triggering the release of pro-inflammatory cytokines such as TNF- α and IL-1⁽¹¹⁾. It is also known that the plasma level of the anti-inflammatory myokine IL-6 increases during intense or prolonged exercise⁽¹²⁾, producing beneficial metabolic effects and contributing to the maintenance of glucose homeostasis and exercise-induced lipolysis⁽¹²⁾. Recent findings indicate that vitamin D₃ supplementation attenuates exercise-induced muscle damage in rats⁽¹³⁾. Furthermore, vitamin D supplementation resulted in an improved resistance and sensitivity to insulin in different populations⁽¹⁴⁾. However, the effects of a vitamin D₃ injection on the inflammatory and metabolic response to an acute bout of RE in vitamin D-deficient athletic cohorts have not been previously evaluated. Furthermore, very little is identified about vitamin D improvement and its physiological effects among athletes and fitness enthusiasts. These represent a gap in knowledge that must be addressed. Therefore, the objective of the present study was to evaluate the effects of a single vitamin D₃ injection on the metabolic, inflammatory and muscular damage responses to an acute bout of RE in vitamin D-deficient resistance-trained males. We hypothesised that a single vitamin D₃ injection would improve the metabolic, inflammatory and muscular damage markers following acute RE.

Experimental methods

Participants

Fourteen resistance-trained healthy males between the ages of 20 and 30 years old volunteered for the present study. Participants

were recruited from a group of individuals referred to a sport nutrition clinic (Ahvaz, Iran). All participants regularly performed RE, at least 3 d a week, for at least 2 years prior to the study. Participants were vitamin D deficient (25-hydroxycholecalciferol (25(OH)D) level < 20 ng/ml), but had no history of diabetes, cardiovascular, inflammatory or thyroid diseases or were taking any medications or nutritional supplements that could affect the inflammatory and/or metabolic response. Additionally, participants did not smoke, consume alcohol or had dietary changes in the year before the study. All subjects gave written informed consent, and the ethical committee of Ahvaz Jundishapur University of Medical Sciences approved the protocol (Ethic code: IR.AJUMS.REC.1394.212).

Study design. We used a non-randomised within-subject cross-over design (Fig. 1), in which each participant served as their own control⁽¹⁵⁾. Besides a familiarisation session, participants were evaluated in two different occasions, in which they participated in acute RE bouts 4 weeks after normal saline (lower vitamin D (LVD) trial) and vitamin D₃ (higher vitamin D (HVD) trial) injections. The experiments were conducted in the afternoon, in temperature-controlled room (23°C) and at the same time of the day (± 2 h). Participants abstained from caffeinated drinks for 12 h and avoided intense exercise 72 h before each testing session. Anthropometric measurements were collected upon entering the laboratory, before each RE session. Thereafter, blood samples, heart rate (HR) and blood pressure (BP) measurements were collected at rest, immediately and 1 h after acute RE. Four weeks before the first RE session, 1 ml of normal saline was injected. Approximately 24 h after the first RE session, an intramuscular injection of 7500 μ g vitamin D₃ was performed on each participant. A second RE session was conducted 4 weeks after vitamin D₃ injection; this period allowed for the improvement in the vitamin D values of the participants (Fig. 1)⁽¹⁶⁾.

Since diet plays an important role in metabolic and systemic inflammation, food records were taken 3 d before each RE trial. To equalise macronutrient intake in exercise day, pre-exercise meals and its contents calculated based on each subject's body weight (Table 1).

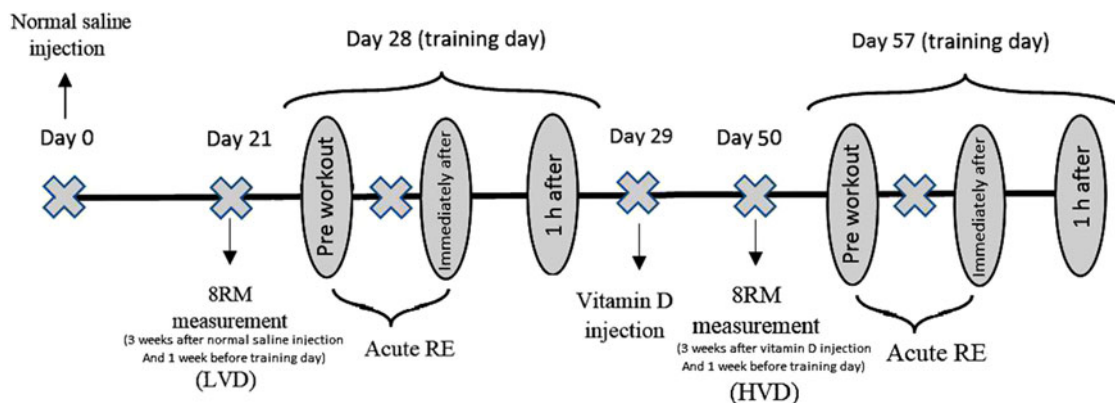


Fig. 1. Study design. HVD, high vitamin D; LVD, low vitamin D; RE, resistance exercise; RM, repetitions maximum.

Table 1. Amount of macronutrient intake on exercise days

	Amount	Food items	Time of day (hours)
Waking up	–	–	09.00
Breakfast (g/kg)	Carbohydrate: 2 Protein: 0.2 Fat: 0.1	Bread, low-fat cheese, honey, fruit (apple), low-fat milk	09.30
Pre-exercise meal (g/kg)	Carbohydrate: 3 Protein: 0.6 Fat: 0.2	Rice, chicken breast, low-fat yogurt, oil	12.00 (noon)
Pre-exercise snack (g/kg)	Carbohydrate: 1 Protein: – Fat: –	Fruit (banana)	14.00
Training session	–	–	16.00

8 Repetition maximum testing. One week before each acute RE trial, participants underwent 8 repetition maximum (RM) testing to prescribe RE intensity during the trials. The 8RM was assessed for the nine exercises used in acute RE, as described in previous research⁽¹⁷⁾.

Acute resistance exercise protocol. In both trials, acute RE consisted of three sets of eight repetitions (using 8RM load) and 1-min rest between sets and exercises. The exercises included barbell bench press, dumbbell bench press, incline barbell press, incline dumbbell fly, cable crossover, barbell curl, incline dumbbell curl, lying triceps extension and close-grip bench press. If needed, the participants were spotted to complete the eight repetitions in each set⁽¹⁸⁾. The acute RE protocol was adapted from the previous literature^(18,19).

Anthropometric measurements. Body composition was measured using a precision body composition analyser (inbody 270, Biospace) as previously described⁽²⁰⁾.

Blood pressure measurements. BP, both systolic and diastolic BP, was measured in the supine position by an automatic BP monitor (Jawon Medical). Rate pressure product was estimated by systolic BP \times HR. The HR was obtained utilising HR monitor (Beurer, PM70). Mean arterial pressure was calculated according to the formula: $(2 \text{ diastolic BP} + \text{systolic BP})/3$.

Biochemical and inflammatory analysis. A volume of 5 ml of blood samples was collected from the cubital vein of subjects. Venous blood was also drawn immediately after the exercise and 1 h after the exercise. Blood sugar (BS), CK and lactate dehydrogenase (LDH) were measured by the spectrophotometric method (Pars Azmoon Inc.) using an auto-analyser (Hitachi). Inter- and intra-assay CV were $<2.2\%$ for glucose, 1.6% for CK and 1.2% for LDH. Fasting serum insulin is assayed by an ELISA kit (Diaplus Inc.) with intra- and inter-assay CV of 4.9 and 8% , respectively. Insulin resistance and pancreatic β -cell function were estimated using homeostasis model assessment (HOMA)^(21,22). The 25(OH)D level of each sample was measured by an ELISA kit (Bioactiva Diagnostica). The secreted cytokine

levels including IL-6, IL-10 and TNF- α in the serum samples were determined using commercially available Sandwich ELISA kits (Eastbiopharm). All inter- and intra-assay CV for the inflammatory markers were <12 and $<10\%$, respectively.

Statistical analysis

An estimation of an appropriate sample size was conducted using the G*Power analysis software⁽²³⁾. Our rationale for sample size was based on a previous study, which evaluated changes in glucose, insulin and HOMA-IR scores after acute RE in twelve young men⁽²⁴⁾. The results of the present study showed significant declines in glucose, insulin and HOMA-IR following RE. Using the equation for effect size = (mean before – mean after acute RE)/the pooled standard deviation, the present study revealed an effect size of $0.19 = ((91.48 - 82.98)/45.8)$, $0.36 = ((4.87 - 4.69)/0.5)$ and $0.35 = ((1.10 - 0.96)/0.4)$ for glucose, insulin and HOMA-IR, respectively. Based on $\alpha = 0.05$, a power $(1 - \beta)$ of 0.80 and an effect size = 0.4 (highest approximate effect size for our variables), a sample size of at least eleven participants was needed to have sufficient power to detect significant changes in BS, insulin and HOMA-IR after acute RE among our two study trials. The Kolmogorov–Smirnov test was used to check the normality of the observations. Differences in mean values for each variable between groups were compared by a 2×3 ANOVA with repeated measures, followed by LSD test for pairwise comparisons. Pearson correlation coefficients were utilised to examine the relationships between intensity of exercise and all baseline anthropometric indices, metabolic, inflammatory and BP changes during exercise. Statistical significance was considered as $P < 0.05$. All data are presented as mean values and standard deviation. The SPSS version 19.0 was used for analysing the data.

Results

Effects of vitamin D injection on anthropometrics and exercise load

Participants' baseline characteristics and exercise load for each trial are presented in Table 2. Other than vitamin D level, no significant differences were observed in baseline characteristics between trials ($P > 0.05$). Additionally, there were no significant differences in anthropometric values and exercise load between trials.

Effects of vitamin D injection on the metabolic responses to resistance exercise

Metabolic responses to RE are shown in Fig. 2. There were significant trial-by-time interactions for insulin and HOMA-IR which significantly ($P < 0.05$) declined 1 h after RE in the HVD trial when compared with LVD trial. Only a time effect was detected for HOMA-IR immediately and 1 h after RE in the LVD trial. HOMA-B levels were significantly ($P < 0.05$) reduced 1 h after RE in the HVD trial when compared with LVD trial; however, no significant intra-trial differences were achieved ($P = 0.07$). There was also a time effect for BS which significantly ($P < 0.05$) decreased 1 h after RE in both the LVD and HVD trials.

Table 2. Participant characteristics and exercise load for each trial (Mean values and standard deviations)

Variables	LVD		HVD		P
	Mean	SD	Mean	SD	
Anthropometric characteristics					
Age (years)	24.3	2.4	24.3	2.4	–
Weight (kg)	78.1	6.2	78.3	6.6	0.597
Height (cm)	176.4	6.9	176.4	6.9	–
BMI (kg/cm ²)	25.1	1.4	25.1	1.4	0.692
LBM (kg)	36.5	3.4	36.9	3.7	0.133
FM (kg)	14.1	2.8	13.8	3.3	0.232
TBW (kg)	46.8	4.0	47.2	4.3	0.169
FFM (kg)	63.9	5.5	64.3	6.0	0.191
PBF (%)	18.1	3.2	17.7	3.7	0.169
WC (cm)	83.9	4.0	83.4	3.8	0.308
HC (cm)	96.8	4.8	96.6	4.3	0.636
25(OH)D (ng/ml)	14.0	3.9	22.3	5.5	<0.001
Exercise load					
Barbell bench press	75.7	15.7	74.6	15.7	0.426
Dumbbell bench press	42.9	12.4	38.9	16.2	0.068
Incline barbell press	52.9	17.4	52.5	16.4	0.793
Incline dumbbell fly	21.1	6.3	22.5	6.4	0.104
Cable crossover (kg)	37.9	11.9	42.1	11.2	0.111
Barbell curl (kg)	29.7	6.3	28.6	7.4	0.272
Incline dumbbell curl	22.5	5.8	21.4	6.3	0.385
Lying triceps extension	28.6	8.2	28.6	6.9	1.000
Close-grip bench press	52.5	18.1	51.8	17.5	0.612

LVD, low vitamin D; HVD, high vitamin D; LBM, lean body mass; FM, fat mass; TBW, total body water; FFM, fat-free mass; PBF, percentage body fat; WC, waist circumference; HC, hip circumference; 25(OH)D, 25-hydroxycholecalciferol.

There were no significant changes in the level of C-peptide following RE in both LVD and HVD trials.

Effects of vitamin D injection on the muscle damage, inflammatory and cardiovascular responses to resistance exercise

Muscle damage, inflammatory and cardiovascular responses are shown in Table 3. Muscle damage markers, CK and LDH increased significantly ($P < 0.05$) immediately and 1 h after RE in both trials. However, differences between trials were insignificant (time-effect only).

Amongst the inflammatory biomarkers analysed, significant increases ($P < 0.05$) were observed in the level of IL-6 immediately and 1 h after RE in both trials. A significant rise was also seen in the ratio of IL-6:IL-10 in the LVD trial only, but no significant differences between trials were observed. There were no significant changes in the level of TNF- α , IL-10 and the ratio of TNF- α :IL-10 in both the LVD and HVD trials.

Systolic BP, HR, rate pressure product and pulse pressure significantly ($P < 0.05$) increased immediately and returned to baseline 1 h after RE in both trials. There were no significant differences in response to these variables between trials (time-effect only). Diastolic BP stayed unchanged in both trials.

Discussion

The present study aimed at examining the effect of a single intramuscular high dose of vitamin D₃ injection of 7500 μ g on inflammatory, muscular damage, metabolic and cardiovascular

responses to an acute bout of RE in previously vitamin D-deficient resistance-trained males. Our finding showed that the injection declined insulin levels, improved insulin resistance and β -cell function following RE. Conversely, a single injection of vitamin D₃ did not change muscle damage, cardiovascular and the inflammatory responses to acute RE in previously vitamin D-deficient resistance-trained males.

Skeletal muscle tissue is responsible for most insulin-dependent glucose uptake in humans. It was previously reported that the capacity of glucose uptake mediated by insulin is directly associated with the muscle mass and adversely related to fat mass⁽²⁵⁾. In our study, a strong inverse relationship was observed between reduced BS levels after exercise with muscle mass but not fat mass. In comparison with the endurance exercise, there is little information in relation to the potential effects of RE on insulin sensitivity or glucose resistance in vitamin D-deficient athletic populations. Based on our findings, in both trials, the amount of insulin and HOMA-IR declined significantly immediately after RE, but only remained reduced in the HVD trial after 1 h of the exercise. Although not significant, β -cell function (HOMA- β) (approximately 94, $P = 0.07$ intra-group) showed a trend to decline in the HVD trial, reaching a significant between-group difference 1 h post-RE. Our results agree with the notion that the function of β -cell does not significantly change during an intense session and immediately after an exercise session^(15,26). Koopman *et al.* examined the effect of an intense session of RE on insulin resistance. According to the study, BS and insulin levels did not change significantly during 24 h after an exercise, but insulin sensitivity improved following RT⁽²⁷⁾. In another study, Magkos *et al.* reported that a session of RT improved insulin resistance through the reduction in HOMA-IR index, and this reduction was observed in insulin and blood glucose levels⁽²⁸⁾. The researchers noted that the reduction of insulin resistance is associated with energy expenditure during exercise⁽¹⁵⁾. GLUT-4 protein expression plays an important role in skeletal muscle capacity to stimulate insulin-dependent glucose transport⁽²⁹⁾. It seems that overexpression of this protein in a prolonged exercise session can cause the declining effects of insulin resistance; and the impacts of improvement in insulin resistance during a single bout of RE are related to increase in GLUT4 translocation⁽³⁰⁾. Following this translocation, membranous appearance of the proteins will be significantly increased. It has been previously shown that vitamin D supplementation and vitamin D levels could play a role in improving insulin sensitivity⁽³¹⁾. While based on our literature search, no studies have investigated the effect of supplementation with vitamin D on the metabolic response and insulin resistance during a RE session, it seems that moderating effects of the vitamin D are associated with nuclear receptor of this vitamin⁽³⁰⁾.

LDH and CK isoenzymes are muscle injury biomarkers⁽³²⁾. Various studies have shown that after intense exercise, changes in serum levels of enzymes and muscle isoenzymes in athletes and normal individuals can be observed^(33,34). An increase in the activity of CK may be caused by metabolic and mechanical reasons. In fact, metabolically exhausted muscle fibres show a reduction in resistance of membrane after an increase in free intracellular Ca ions that stimulate the activation of K channels. Other mechanisms could be the localised tissue damage with the

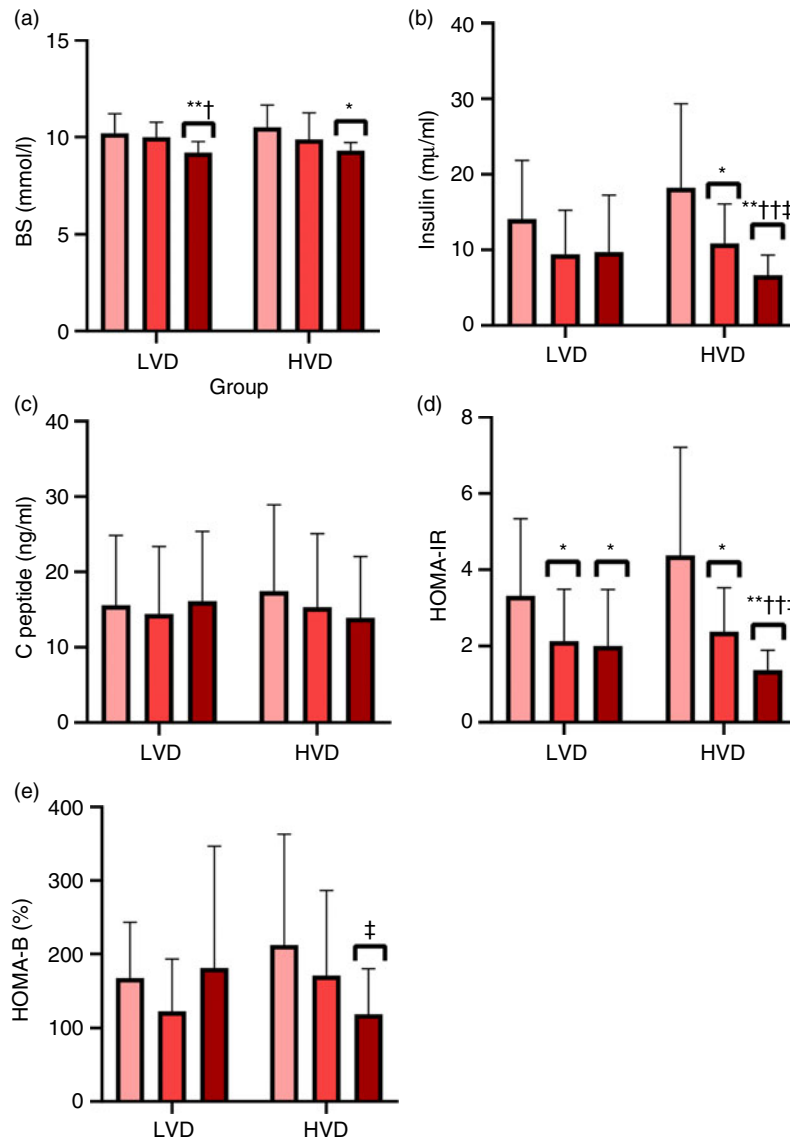


Fig. 2. Metabolic responses to acute resistance exercise in the LVD (low vitamin D) and HVD (high vitamin D) interventions. (a) BS, blood sugar; (b) insulin; (c) C peptide; (d) HOMA-IR, homeostasis model of assessment-insulin resistance; (e) HOMA-B, homeostasis model of assessment- β -cell function assessment of insulin secretory capacity. * $P < 0.05$ and ** $P < 0.01$ compared with the values of pre workout. † $P < 0.05$ and †† $P < 0.01$ compared with the values of immediately after workout, respectively. ‡ $P < 0.05$ compared with LVD at the same time period. □, Preworkout; ■, immediately after; ▨, 1 h after.

sarcomeric destruction of the Z line⁽³⁴⁾. In our study, there was also a significant increase in CK activity in both conditions immediately after and 1 h after exercise compared with baseline. Unlike CK that increased within 1 h following exercise, LDH activity was declined after exercise. While the LDH activity was reduced after 1 h compared with immediately after RE in both trials, only on the LVD, the LDH activity recovered back to baseline. Based on our results, the increased CK activity was positively associated with RE intensity (8RM load). This relationship between RE intensity and CK changes was not observed in LDH. Rodrigues *et al.* reported that after a session of the RT, CK activity increased for 48 h and went down 72 h after the exercise. In the present study, the level of LDH activity did not change significantly⁽³⁵⁾. According to the study by Güzel *et al.*, the CK activity increased immediately after RT and will be increased

continuously until 24 h then will be reduced⁽³⁶⁾. Although the role of vitamin D supplementation on muscle tissue function is well known⁽³⁷⁾, effects of this supplementation on muscle damage during exercise have been less studied. In the only study conducted to date, Choi *et al.* reported vitamin D supplementation in rats with muscle damage induced by high-intensity aerobic exercise (30 min/d and 5 d/week for 8 weeks) that could decrease the CK activity compared with the control group and its rate statistically reached a level similar to a non-exercise group as well⁽¹³⁾. Discrepancies between this observation and our results may be related to a higher dose of vitamin D₃ (25 µg/kg per d vitamin D₃) and a longer duration of the exercise protocol in the study by Choi *et al.*⁽¹³⁾. Furthermore, animal studies are often poor predictors of human outcomes⁽³⁸⁾. Nevertheless, future studies evaluating muscle damage in

Table 3. Muscle damage, inflammatory and cardiovascular responses to acute resistance exercise (Mean values and standard deviations)

Variables		Pre workout		Immediately after		1 h after		Intra	Between-group P
		Mean	SD	Mean	SD	Mean	SD		
CK (U/l)	LVD	196.95	100.35	282.5**	157.7	311.6**	183.3	0.001‡	0.171
	HVD	228.39	155.38	304.5**	214.6	309.5**	223.2	0.002‡	
LDH (U/l)	LVD	281.57	17.12	314.73**	29.7	293.35††	30.28	<0.001‡	0.405
	HVD	271.02	35.89	309.5**	45.39	289.8*††	51.4	<0.001‡	
SBP (mmHg)	LVD	115.85	10.97	126.4**	12.91	110.28††	8.83	<0.001‡	0.868
	HVD	114.07	9.12	123.51*	4.44	109.0††	9.54	0.005‡	
DBP (mmHg)	LVD	75.42	10.05	77.92	11.67	73.64	7.95	0.337	0.391
	HVD	73.42	5.4	79.92	14.23	77.57	13.36	0.269	
HR (beats/min)	LVD	77.78	13.43	114.1**	12.09	80.92††	12.63	<0.001‡	0.458
	HVD	75.21	8.86	109.2**	13.84	80.64††	8.95	<0.001‡	
RPP (mmHg)	LVD	8938	1291	14483**	2414	8899††	1406	<0.001‡	0.414
	HVD	8554	1001	13521**	2602	8748††	860	<0.001‡	
PP (mmHg)	LVD	40.4	9.1	48.5*	15.3	36.6†	11.9	0.017‡	0.436
	HVD	40.642	8.91	43.571**	13.7	31.428††	12.2	0.003‡	
MAP (mmHg)	LVD	88.9	9.4	94.1	9.7	85.8†	6.0	0.022‡	0.477
	HVD	86.9	5.4	94.4	12.7	88.0	10.7	0.125	
IL-6 (pg/ml)	LVD	0.48	0.31	0.64**	0.38	0.59**	0.32	<0.001‡	0.372
	HVD	0.40	0.22	0.53**	0.29	0.5*	0.27	0.004‡	
TNF- α (pg/ml)	LVD	0.5	0.25	0.56	0.26	0.52	0.24	0.089	0.653
	HVD	0.46	0.28	0.5	0.28	0.49	0.28	0.325	
IL-10 (pg/ml)	LVD	0.29	0.17	0.34	0.23	0.3	0.17	0.258	0.347
	HVD	0.3	0.18	0.32	0.22	0.27	0.15	0.104	
IL-6:IL-10 ratio	LVD	1.57	0.57	2.06*	0.42	2.02*	0.33	0.017‡	0.862
	HVD	1.46	0.42	2.02	0.77	1.99	0.92	0.059	
TNF- α :IL-10 ratio	LVD	1.87	0.74	2.04	0.87	1.9	0.52	0.7	0.698
	HVD	1.55	0.28	1.8	0.61	1.79	0.34	0.257	

CK, creatine kinase; LDH, lactate dehydrogenase; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; HR, heart rate; RPP, rate-pressure product; MAP, mean arterial pressure; LVD, low vitamin D; HVD, high vitamin D.

* $P < 0.05$ and ** $P < 0.01$ compared with the values of pre workout time.

† $P < 0.05$ and †† $P < 0.01$ compared with the values of immediately after workout, respectively.

‡ Values are significantly different ($P < 0.05$).

vitamin D-deficient individuals after oral or intramuscular vitamin D₃ should focus on using higher doses and longer duration than our present investigation.

Based on our results, a single injection of high-dose vitamin D₃ did not improve inflammatory response to a RT session. Although the immunomodulatory effect of vitamin D is well known⁽⁴⁾, this is the first study to evaluate the effect of a single dose of vitamin D₃ injection on inflammatory response following an acute RE session. In agreement with our findings, an 8-week intervention of aerobic exercise combined with vitamin D supplementation in rats produced no changes in serum levels of IL-6 and TNF- α . Although other anti-inflammatory effects, such as a reduction in the phosphorylation of AMP-activated protein kinase (MAPK) pathway, proteins (AMPK, p38, Extracellular signal-regulated protein kinases 1 and 2, I κ B and I κ B kinase) and expression of IL-6 and TNF- α were shown⁽¹³⁾.

Our findings also show that regardless of vitamin D level, a RT session could increase the level of IL-6 and the ratio of the IL-6:IL-10, but other inflammatory markers remain unchanged. Consistent with our results, Phillips *et al.* reported that the level of IL-6 immediately after RT increased and returned to the baseline following 6 h⁽³⁹⁾. In another study, there was no any change in the serum level of IL-1 β , 2, 5, 6, 8, 10 and TNF- α following a RT session. However, the mRNA of IL-1 β , IL-6, IL-8 and TNF- α was up-regulated⁽⁴⁰⁾. It seems that increased levels of IL-6 are caused by contraction of muscle fibres, which induce different metabolic effects⁽⁴¹⁾. Increased levels of IL-6 due to exercise

cause the stimulation of lipolysis and fat oxidation and involvement in glucose homeostasis, particularly through the induction of glycogenesis⁽⁴¹⁾. Based on our results, other inflammatory cytokines and index did not change significantly in different times in both conditions. This unchanging could be due to the biological role of IL-6, which based on it, increased levels of IL-6 induced by muscle contraction during intense exercise have strong anti-inflammatory effects and could cause a decrease in TNF- α , inhibition of the TNF-induced insulin resistance and an increase in the anti-inflammatory IL-1ra IL-10^(12,41).

In the present study, similar changes showed in BP, HR and the rate pressure product at rest and in response to exercise were seen in both trials. Therefore, intervention by vitamin D had no effect on the cardiovascular profile. Our findings agree with previous systematic reviews and meta-analyses that do not support the use of vitamin D for the improvement of resting cardiovascular health and function, due to the inconsistent findings of interventional studies and the need for larger-scale studies⁽⁴²⁻⁴⁴⁾. Therefore, our present outcomes reinforce the notion that vitamin D supplementation does not change resting cardiovascular markers. Moreover, our results add further information on this area as to the best of our knowledge, this is the first investigation to assess cardiovascular changes post-exercise after vitamin D level improvement.

Our subjects were resistance-trained healthy Iranian males. Previous studies in Iran and other Middle Eastern countries

reported a high prevalence of vitamin D deficiency and insufficiency, despite a high level of sun exposure in that region of the world^(45–47). For instance, the prevalence of vitamin D deficiency/insufficiency in adults was reported as 61.4 and 87.8% in Kuwait and Saudi Arabia, respectively^(48,49). The high prevalence of vitamin D deficiency in Iranians may be due to various factors such as the presence of different skin pigments within the population, low dietary vitamin D intake, clothing preference and genetic factors such as special vitamin D receptor polymorphisms. Vitamin D deficiency may also result from low Ca intakes, involved in the vitamin D catabolism^(50,51). Importantly, our findings may have potential clinical implications not only in countries with low sun exposure but also in countries with elevated sunshine levels and a high prevalence of vitamin D deficiency, such as those in the Middle East.

Our study has some limitations such as a small sample size, although our power calculation was suitable to detect significant differences between groups in metabolic markers. Additional limitations include the short duration and the use of a single dose of vitamin D₃, which warrants further studies with longer interventions and different doses of vitamin D₃ with a focus on understanding the relationship between changes in metabolic and anti-inflammatory parameters after RE.

In conclusion, a single injection of vitamin D₃ declined insulin and BS levels, improved insulin-resistance and β -cell function following RE in previously vitamin D-deficient resistance-trained males. On the other hand, single injections of 7500 μ g vitamin D₃ did not improve muscle damage and inflammatory response to a RT session. Furthermore, no significant changes in baseline (resting) metabolic, inflammatory and cardiovascular changes were detected after vitamin D level improvement in this population.

Acknowledgements

The authors thank all the subjects and the Diabetes Research Center for their kind cooperation on the biochemical tests.

This work was financially supported by grant CMRC-9412 from the Vice Chancellor for Research Affairs, Cellular and Molecular Research Center, of Ahvaz Jundishapur University of Medical Sciences.

D. A. L. conducted the experiments and wrote the paper; R. B. revised some minor comments throughout the paper; A. W. edited, interpreted data and wrote some content of the paper; A. K. and M. A. G. designed and conceptualized the study; I. M. performed statistical analysis; and S. A. M. and S. J. H. performed analysis and interpretation of data.

The authors declare no conflicts of interest.

References

- Larson-Meyer DE & Willis KS (2010) Vitamin D and athletes. *Curr Sports Med Rep* **9**, 220–226.
- Holick MF (2017) The vitamin D deficiency pandemic: approaches for diagnosis, treatment and prevention. *Rev Endocr Metab Disord* **18**, 153–165.
- Baggerly CA, Cuomo RE, French CB, *et al.* (2015) Sunlight and vitamin D: necessary for public health. *J Am Coll Nutr* **34**, 359–365.
- Guillot X, Semerano L, Saldenber-Kermanac'h N, *et al.* (2010) Vitamin D and inflammation. *Joint Bone Spine* **77**, 552–557.
- Willis KS, Peterson NJ & Larson-Meyer DE (2008) Should we be concerned about the vitamin D status of athletes? *Int J Sport Nutr Exerc Metab* **18**, 204–224.
- Miraj SS, Thunga G, Kunhikatta V, *et al.* (2019) Benefits of vitamin D in sport nutrition. In *Nutrition and Enhanced Sports Performance*, pp. 497–508 [D Bagchi, S Nair and C Sen, editors]. London: Elsevier.
- Garcia LA, Ferrini MG, Norris KC, *et al.* (2013) 1,25(OH)₂ vitamin D₃ enhances myogenic differentiation by modulating the expression of key angiogenic growth factors and angiogenic inhibitors in C2C12 skeletal muscle cells. *J Steroid Biochem Mol Biol* **133**, 1–11.
- Sinha A, Hollingsworth KG, Ball S, *et al.* (2013) Improving the vitamin D status of vitamin D deficient adults is associated with improved mitochondrial oxidative function in skeletal muscle. *J Clin Endocrinol Metab* **98**, E509–E513.
- Henriksen EJ (2002) Invited review: effects of acute exercise and exercise training on insulin resistance. *J Appl Physiol* **93**, 788–796.
- Ghaffarian M, Ashtary-Larky D, Chinipardaz R, *et al.* (2016) Inflammatory biomarkers' response to two different intensities of a single bout exercise among soccer players. *Iranian Red Crescent Med J* **18**, e21498.
- Brunsgaard H, Galbo H, Halkjaer-Kristensen J, *et al.* (1997) Exercise-induced increase in serum interleukin-6 in humans is related to muscle damage. *J Physiol* **499**, 833–841.
- Petersen AMW & Pedersen BK (2005) The anti-inflammatory effect of exercise. *J Appl Physiol* **98**, 1154–1162.
- Choi M, Park H, Cho S, *et al.* (2013) Vitamin D₃ supplementation modulates inflammatory responses from the muscle damage induced by high-intensity exercise in SD rats. *Cytokine* **63**, 27–35.
- Houmard JA, Tanner CJ, Slentz CA, *et al.* (2004) Effect of the volume and intensity of exercise training on insulin sensitivity. *J Appl Physiol* **96**, 101–106.
- Lloyd JW, Evans KA, Zerfass KM, *et al.* (2016) Effect of an acute bout of aerobic exercise on chemerin levels in obese adults. *Diabetes Metab Syndrome Clin Res Rev* **10**, 37–42.
- Wylon K, Drozdenko G, Krannich A, *et al.* (2017) Pharmacokinetic evaluation of a single intramuscular high dose versus an oral long-term supplementation of cholecalciferol. *PLOS ONE* **12**, e0169620.
- Miranda H, Fleck SJ, Simão R, *et al.* (2007) Effect of two different rest period lengths on the number of repetitions performed during resistance training. *J Strength Cond Res* **21**, 1032–1036.
- De Souza JC, Tibana RA, Cavaglieri CR, *et al.* (2013) Resistance exercise leading to failure versus not to failure: effects on cardiovascular control. *BMC Cardiovasc Disord* **13**, 105.
- George P, Pearson E & Witham M (2012) Effect of vitamin D supplementation on glycaemic control and insulin resistance: a systematic review and meta-analysis. *Diabetic Med* **29**, e142–e150.
- Ghanavati M, Behrooz M, Rashidkhani B, *et al.* (2015) Healthy eating index in patients with cataract: a case-control study. *Iranian Red Crescent Med J* **17**, e22490.
- Ghaffari M-A, Payami S-A, Payami S-P, *et al.* (2016) Evaluation of insulin resistance indices in type 2 diabetic patients treated with different anti-diabetic drugs. *Open J Endocr Metab Dis* **6**, 95.
- Matthews D, Hosker J, Rudenski A, *et al.* (1985) Homeostasis model assessment: insulin resistance and β -cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* **28**, 412–419.

23. Faul F, Erdfelder E, Lang A-G, *et al.* (2007) G* Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Res Methods* **39**, 175–191.
24. Kazemi F (2016) The correlation of resistance exercise-induced myostatin with insulin resistance and plasma cytokines in healthy young men. *J Endocrinol Invest* **39**, 383–388.
25. Richter EA & Hargreaves M (2013) Exercise, GLUT4, and skeletal muscle glucose uptake. *Physiol Rev* **93**, 993–1017.
26. Bordenave S, Brandou F, Manetta J, *et al.* (2008) Effects of acute exercise on insulin sensitivity, glucose effectiveness and disposition index in type 2 diabetic patients. *Diabetes Metab* **34**, 250–257.
27. Koopman R, Manders RJ, Zorenc AH, *et al.* (2005) A single session of resistance exercise enhances insulin sensitivity for at least 24 h in healthy men. *Eur J Appl Physiol* **94**, 180–187.
28. Magkos F, Tsekouras Y, Kavouras SA, *et al.* (2008) Improved insulin sensitivity after a single bout of exercise is curvilinearly related to exercise energy expenditure. *Clin Sci* **114**, 59–64.
29. Henriksen EJ, Bourey RE, Rodnick KJ, *et al.* (1990) Glucose transporter protein content and glucose transport capacity in rat skeletal muscles. *Am J Physiol-Endocrinol Metab* **259**, E593–E598.
30. Stockton K, Mengersen K, Paratz JD, *et al.* (2011) Effect of vitamin D supplementation on muscle strength: a systematic review and meta-analysis. *Osteoporos Int* **22**, 859–871.
31. Teegarden D & Donkin SS (2009) Vitamin D: emerging new roles in insulin sensitivity. *Nutr Res Rev* **22**, 82–92.
32. Noakes TD (1987) Effect of exercise on serum enzyme activities in humans. *Sports Med* **4**, 245–267.
33. Priest JB, Oei TO & Moorehead WR (1982) Exercise-induced changes in common laboratory tests. *Am J Clin Pathol* **77**, 285–289.
34. Brancaccio P, Maffulli N & Limongelli FM (2007) Creatine kinase monitoring in sport medicine. *Br Med Bull* **81**, 209–230.
35. Rodrigues BM, Dantas E, de Salles BF, *et al.* (2010) Creatine kinase and lactate dehydrogenase responses after upper-body resistance exercise with different rest intervals. *J Strength Cond Res* **24**, 1657–1662.
36. Güzel NA, Hazar S, Erbas D (2007) Effects of different resistance exercise protocols on nitric oxide, lipid peroxidation and creatine kinase activity in sedentary males. *J Sports Sci Med* **6**, 417.
37. Bischoff H, Borchers M, Gudat F, *et al.* (2001) *In situ* detection of 1, 25-dihydroxyvitamin D receptor in human skeletal muscle tissue. *Histochem J* **33**, 19–24.
38. Bracken MB (2009) Why animal studies are often poor predictors of human reactions to exposure. *J Royal Soc Med* **102**, 120–122.
39. Phillips MD, Mitchell JB, Currie-Eloff LM, *et al.* (2010) Influence of commonly employed resistance exercise protocols on circulating IL-6 and indices of insulin sensitivity. *J Strength Cond Res* **24**, 1091–1101.
40. Buford TW, Cooke MB & Willoughby DS (2009) Resistance exercise-induced changes of inflammatory gene expression within human skeletal muscle. *Eur J Appl Physiol* **107**, 463.
41. Pedersen BK, Steensberg A & Schjerling P (2001) Muscle-derived interleukin-6: possible biological effects. *J Physiol* **536**, 329–337.
42. El-Fakhri N, McDevitt H, Shaikh M, *et al.* (2014) Vitamin D and its effects on glucose homeostasis, cardiovascular function and immune function. *Horm Res Paediatr* **81**, 363–378.
43. Hussin AM, Ashor AW, Schoenmakers I, *et al.* (2017) Effects of vitamin D supplementation on endothelial function: a systematic review and meta-analysis of randomised clinical trials. *Eur J Nutr* **56**, 1095–1104.
44. Agbalalah T, Hughes SF, Freeborn EJ, *et al.* (2017) Impact of vitamin D supplementation on endothelial and inflammatory markers in adults: a systematic review. *J Steroid Biochem Mol Biol* **173**, 292–300.
45. Hovsepian S, Amini M, Aminorroaya A, *et al.* (2011) Prevalence of vitamin D deficiency among adult population of Isfahan City, Iran. *J Health Popul Nutr* **29**, 149.
46. Shahla A, Charehsaz S, Talebi R, *et al.* (2005) Vitamin D deficiency in young females with musculoskeletal complaints in Urmia, northwest of Iran. *Iranian J Med Sci* **30**, 88–90.
47. Rahnavard Z, Eybpoosh S, Homami MR, *et al.* (2010) Vitamin D deficiency in healthy male population: results of the Iranian multi-center osteoporosis study. *Iran J Public Health* **39**, 45.
48. Gaafar M & Badr S (2013) An alarming high prevalence of vitamin D deficiency among healthy adults. *Life Sci J* **10**, 3292–3298.
49. Ardawi M-S, Sibiany A, Bakhsh T, *et al.* (2012) High prevalence of vitamin D deficiency among healthy Saudi Arabian men: relationship to bone mineral density, parathyroid hormone, bone turnover markers, and lifestyle factors. *Osteoporos Int* **23**, 675–686.
50. Salek M, Hashemipour M, Aminorroaya A, *et al.* (2008) Vitamin D deficiency among pregnant women and their newborns in Isfahan, Iran. *Exp Clin Endocrinol Diabetes* **116**, 352–356.
51. Ziaee A, Javadi A, Javadi M, *et al.* (2013) Nutritional status assessment of Minodar residence in Qazvin City, Iran: vitamin D deficiency in sunshine country, a public health issue. *Glob J Health Sci* **5**, 174.