Vitamin D3 supplementation in controlling metabolic changes associated with non-alcoholic steatohepatitis (NASH)

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Abstract

Background Supplementation with vitamin D3 was previously demonstrated to have a number of positive effects in models of non-alcoholic steatohepatitis (NASH). In this study, metabolic changes associated with vitamin D3 supplementation in rats kept on choline-deficient (CD) diet were studied.

Methods Animals were randomly assigned into three groups: a control group (CG, n = 5) with adequate level of dietary choline content, a CD group (CDG, n = 5) which was kept on CD-diet, and a vitamin D3 supplementation group (CDVDG, n = 15) with CD-diet plus IP vitamin D3 injection. To explore the effects of different vitamin D3 doses on the progression of NASH, the animals in CDVDG group were randomly assigned into three subgroups with the vitamin D3 dosage of 1, 5, or 10 μg/kg of body weight administered IP twice a week (CDVDG1, CDVDG5 and CDVDG10, respectively).

Results After 12 weeks of the experiment period, as compared with the CG, the CDG had an increase in the serum fasting insulin (29.50±0.34 mU/L vs 26.49±0.76 mU/L, $p < 0.05$), HOMA-IR (homeostasis model of assessment for insulin resistance index) (11.07±0.20 vs 9.07±0.34, $p < 0.05$) and leptin (569.71±38.97 ng/L vs 408.24±43.71 ng/L, $p < 0.01$), with no significant difference in the serum fasting blood glucose (8.44±0.11 mM vs 7.70±0.22 mM, $p = 0.07$). As compared with the CDG, the CDVDG had significantly decreased fasting blood glucose (CDVDG5: 7.70±0.03 mM vs 8.44±0.11 mM, $p < 0.05$), fasting insulin (CDVDG1, 5, 10 vs CDG: 27.20±0.91 μIU/mL, 22.94±0.90 μIU/mL, 21.47±0.77 μIU/mL vs 29.50±0.34 μIU/mL, respectively, $p<0.05$, $p<0.01$, $p<0.01$), HOMA-IR (CDVDG5, 10 vs CDG: 7.85±0.32, 7.56±0.59 vs 11.07±0.20, respectively, $p<0.01$, $p<0.05$) and leptin (CDVDG5 vs CDG: 445.87±14.98 ng/L vs 569.71±38.97 ng/L, $p<0.05$) (Fig. 1). In addition, serum fasting blood glucose, insulin, HOMA-IR and leptin levels in the CDVDG were significantly decreased upon treatment with vitamin D3 in a dose-dependent manner.

Conclusions These results show that vitamin D3 supplementation could effectively control the critical metabolic parameters known to be affected by NASH. The findings suggest that VD3 supplementation could be an effective measure for the treatment of patients with clinical NASH.

Background

With the rate of occurrence of up to 30%, non-alcoholic fatty liver disease (NAFLD) represents a serious public health concern. In particular, its potential to progress into non-alcoholic steatohepatitis (NASH) may have serious consequences for the affected individuals. The liver of patients affected by NASH is characterised by steatosis, intralobular inflammation, hepatocellular ballooning, perisinusoidal fibrosis in zone 3 and, often, susceptibility to liver tumors. Furthermore, the patients show metabolic abnormalities such as obesity, insulin resistance, fasting hyperglycemia, dyslipidemia, and altered adipokine profile.

The need for detailed studies of the pathologies associated with NASH and appropriate therapeutic interventions led to the development of several animal models of this disease [1]. Although the models do reflect certain features of human NASH, none replicates the full spectrum of this condition in humans.

They can, however, be used in verifying hypotheses on the pathogenesis of NASH and in performing interventional studies. Atherogenic diet leads to the progressive formation of steatosis, inflammation, and fibrosis. However, the animal fed on this diet were systematically insulin sensitive, albeit they showed hepatic insulin resistance. Therefore, this model appears to differ from human NASH in metabolic status. The choline-deficient (CD) model shows low fasting blood glucose, peripheral insulin sensitivity, low serum insulin and leptin levels, which appear to be more consistent with what is observed in human NASH. The obvious limitation of this model is the lack of weight gain by the animals, which mean that not the full spectrum of the human NASH features can be observed in the animals. Still, the model can be very good for more probing studies of metabolic changes associated with NASH. It is because higher fasting glucose level and insulin/ leptin resistance are important metabolic factors on the NASH progression. In our recent study [7], we have established a typical model of NASH liver with a CD-diet, increased hepatic lipid content, inflammation, lipid peroxidation and fibrosis, but no significant changes in body weight and lipid profile.

The role of vitamin D in the development and progression of NASH remains a topic of debates in the literature. While many authors suggest that Vitamin D_3 (VD_3) supplementation decreases blood glucose levels, ameliorates insulin/leptin resistance and NASH progression [2–5], others point to the lack of association between plasma vitamin D levels and blood glucose, insulin resistance, or the severity of NASH [6]. The role of vitamin D supplementation remains poorly studied, however, and deserves further investigation.

We recently reported [7] that supplementation of vitamin D_3 ameliorated lipid accumulation, lipid peroxidation, inflammation, hepatocyte apoptosis and fibrosis in the liver caused by CD-diet with alteration of the levels of serum 25-(OH)D_3 and hepatic VDR, but higher doses of VD_3 may lead to adverse effects.

In the present study, we further studied the metabolic changes in animals kept on CD-diet and discussed the effect of VD_3 supplementation on the NASH progression in this model.

Methods

Animals and experimental treatments

All experimental procedures on animals were approved by the Ethics Committee of Yanbian University. The Ethical Guidelines of the China Association of Laboratory Animal Care were strictly followed in the experiments.

Six-week-old male Wistar rats (150–180 g) were obtained from Yisi Experimental Animal Technology Company (Changchun, China). After a 1-week acclimation period, the animals were randomly assigned into three groups: a control group (CG, n = 5) with adequate level of dietary choline content, a CD group (CDG, n = 5) which was kept on CD-diet, and a VD_3 supplementation group (CDVDG, n = 15) with CD-diet

plus IP VD_3 injection. The choline-adequate diet and the CD-diet were both the L-amino acid-defined diets based on AIN93 standard [8], but had different choline contents (1000 mg/kg (TP 1R810) and 0 mg/kg (TP 1R800, Trophic Animal Feed High-Tech Company, China), respectively).

To explore the effects of different doses of VD_3 on the progression of NASH, the animals in CDVDG group were randomly assigned into three subgroups (CDVDG1 (n = 5), CDVDG5 (n = 5) and CDVDG10 (n = 5)), and subjected to IP injections of VD_3 (General Pharmaceutical Ltd, China) with a dosage of 1, 5, or 10 μg/kg of body weight, respectively, twice a week (Tuesdays and Saturdays). The same amount of saline (10 ml/kg) was administrated IP to the rats in the CG and CDG groups.

At the end of the experimental period (12 weeks), the rats were fasted for 12–13 h and, under anaesthesia with ether, blood was withdrawn from abdominal aorta. Thereafter, the animals were euphanized (CO_2 gas in an induction chamber). The blood was snap frozen in liquid nitrogen and stored at − 80 °C (Froilabo Bio-Memory BM 690, France) until analysed.

Measurement of serum fasting blood glucose, fasting insulin, HOMA-IR and leptin
Serum levels of fasting blood glucose (FBG) were detected by Hexokinase (HK) method using Modular PPI testing system (Roche Diagnostics, Mannheim, Germany).
Serum levels of fasting insulin (FINS) were measured using rat insulin ELISA kit (Xinlebio, China).
Homeostasis model of assessment for insulin resistance index (HOMA-IR) = (FBG × FINS)/22.5 [9].
Serum levels of leptin (LEP) were measured using rat leptin ELISA kit (Xinlebio, China).
Statistical analysis
Data were presented as means ± standard error of mean (SEM). For multiple comparisons, one-way analysis of variance (ANOVA) was used. In case ANOVA showed an overall significant difference, post hoc analysis was performed with LSD's test. Differences were considered significant at $p < 0.05$.

Results

After 12 weeks of the experiment period, as compared with the CG, the CDG had an increase in the serum FINS (29.50 ± 0.34 mU/L vs 26.49 ± 0.76 mU/L, $p < 0.05$), HOMA-IR (11.07 ± 0.20 vs 9.07 ± 0.34, $p < 0.05$) and leptin (569.71 ± 38.97 ng/L vs 408.24 ± 43.71 ng/L, $p < 0.01$), with no significant difference in the serum FBG (8.44 ± 0.11 mM vs 7.70 ± 0.22 mM, $p = 0.07$).

As compared with the CDG, the CDVDG had significantly decreased FBG (CDVDG5: 7.70 ± 0.03 mM vs 8.44 ± 0.11 mM, $p < 0.05$), FINS (CDVDG1, 5, 10 vs CDG: 27.20 ± 0.91 μIU/mL, 22.94 ± 0.90 μIU/mL, 21.47 ± 0.77 μIU/mL vs 29.50 ± 0.34 μIU/mL, respectively, $p < 0.05$, $p < 0.01$, $p < 0.01$), HOMA-IR (CDVDG5, 10 vs CDG: 7.85 ± 0.32, 7.56 ± 0.59 vs 11.07 ± 0.20, respectively, $p < 0.01$, $p < 0.05$) and leptin (CDVDG5 vs CDG: 445.87 ± 14.98 ng/L vs 569.71 ± 38.97 ng/L, $p < 0.05$) (Fig. 1). In addition, serum FBG, FINS, HOMA-IR and leptin levels in the CDVDG were significantly decreased upon treatment with VD_3 in a dose-dependent manner (respectively, $p < 0.01$, $p < 0.01$, $p < 0.01$, $p < 0.05$), but the dosage of 10 μg of VD_3 per kg of body weight could not further decrease the serum FBG, FINS, HOMA-IR and leptin levels.

These results show that VD_3 supplementation could effectively decrease the FBG, FINS, HOMA-IR and leptin, suggesting that VD_3 ameliorates FBG and insulin/leptin resistance caused by CD-diet.

Discussion

The results of this study indicate that VD_3 may reduce blood glucose levels and improve the CD-diet - induced NASH metabolic syndrome in rats by improving insulin and leptin resistance.

NASH is a disease closely related to metabolism, stress and genetic environment. Fasting hyperglycemia, insulin and leptin resistance are important metabolic factors in NASH progression. Low VD levels have been recently reported to be associated with metabolic syndrome and high leptin levels [10]. Previous studies [7] have shown that VD_3 significantly reduces lipid and lipid peroxidation, inflammation, apoptosis, and fibrosis in the liver of CD-diet-induced NASH rats, but the changes in NASH metabolism were not clear.

Insulin resistance (IR) is considered to be the central link in the "first strike" process of NASH pathogenesis, while LEP is a NASH adipocytokine that promotes inflammation and fibrosis. Therefore, IR and LEP play an important role in the pathogenesis of NASH [11]. In recent years, phototherapy has been reported to improve insulin and LEP resistance in NASH rats induced by CD-diet combined with iron supplementation diet [2]. It was also demonstrated that VD regulates insulin sensitivity [12], and VD supplementation reduces FBG and HOMA-IR levels and increases serum VD levels [13]. Some scholars believe that VD_3 supplementation fails to improve IR in patients with NAFLD [14]. However, recent reports [15] show that the effect of supplementation with VD_3 on NASH was significantly better than that of NAFLD, and it was suggested that supplementation with VD_3 slightly changes the fasting blood glucose and insulin levels in NAFLD patients. Several experimental studies point towards a direct role of VD in modulating liver inflammation and fibrogenesis and improving hepatic response to insulin, likely through the binding to specific VD receptor expressed by different cell types in the liver [16–19]. The results of this study show thatVD_3 slightly reduces serum HOMA-IR and leptin levels in NASH rats induced by the CD-diet and improves insulin and leptin resistance. The issue of supplementation of VD to address insulin and leptin resistance remains to be studied on a large scale.

In the oral glucose tolerance test, there was an independent negative correlation between $25(OH)D_3$ concentration and blood glucose concentration. The prevalence of metabolic syndrome components in the low VD group was significantly higher than that in the non-low VD group [20]. VD_3 has been reported to reduce blood glucose levels in type 2 diabetic rats [3]. The mechanism may be related to VD_3 promoting insulin secretion by increasing Ca^{2+} levels in pancreatic beta cells through non-genomic effects [21]. It may also be related to the down-regulation of TLR4 expression through antigen-presenting cells, such as dendritic cells and macrophages, so as to reduce Thl-mediated inflammatory response and autoimmune damage and improve the inflammatory damage of islets [22]. It has also been reported that VD_3 increases the number of insulin receptors and up-regulates the insulin receptor gene, thereby

increasing the glucose uptake capacity of the liver and improving glucose metabolism [23]. In this study, FBG was slightly increased in rats ($p = 0.07$), After VD_3 supplementation, FBG level was decreased in a dose-dependent manner, which is consistent with the previous reports. However, recent experiments with glucose-stimulated insulin responses have shown that later supplementation may be relatively ineffective in patients with VD deficiency and type 2 diabetes [24]. The precise modulatory role of VD in hepatic and islet functions has not been determined and needs further research.

No significant weight gain or lipid changes in CD-diet-induced NASH rats were observed throughout our study. However, we have observed pathogenesis processes similar to NASH in humans, such as liver lipid accumulation, insulin and leptin resistance, fasting hyperglycemia, lipid peroxidation, hepatocyte apoptosis, liver tissue inflammation, hepatic stellate cells (HSC) activation, and partial early liver fibrosis. Our results demonstrate the role of VD_3 in inhibiting the progression of NASH in rats induced by CD-diet by increasing the expression of VD receptor in liver tissue. This prompts VD_3 to prevent NASH progress, especially for non-overweight NASH patients, while its high doses are associated with adverse effects. Recently, Heaney [25] pointed out that a possible reason for the inconsistent efficacy of previous VD_3 supplementation approaches is the lack of studies on dose-response relationship.

Conclusions

In conclusion, our results suggest that VD_3 supplementation is expected to be an effective measure for the treatment of patients with clinical NASH.

Abbreviations

ANOVA - analysis of variance

CD - choline-deficient

CDG - CD group

CDVDG – CD group with vitamin D_3 supplementation

CG – control group

FBG - fasting blood glucose

FINS - fasting insulin

HK - hexokinase

HOMA-IR - homeostasis model of assessment for insulin resistance index

HSC - hepatic stellate cells

IP – intraperitoneal

LEP - leptin

NAFL - non-alcoholic fatty liver

NAFLD - non-alcoholic fatty liver disease

NASH - non-alcoholic steatohepatitis

SEM - standard error of mean

VD – vitamin D

VD_3 – vitamin D_3

Declarations

Ethics approval and consent to participate: All experimental procedures on animals were approved by the Ethics Committee of Yanbian University.

Consent for publication: Not applicable

Availability of data and materials: Not applicable

Competing interests: None

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Authors' contributions: HH and XS done most part of experimental work and wrote the manuscript. MC was responsible for planning the experimental work and general quality control. XY and XP were involved in data collection and analysis. All authors have read and approved the manuscript.

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Figures

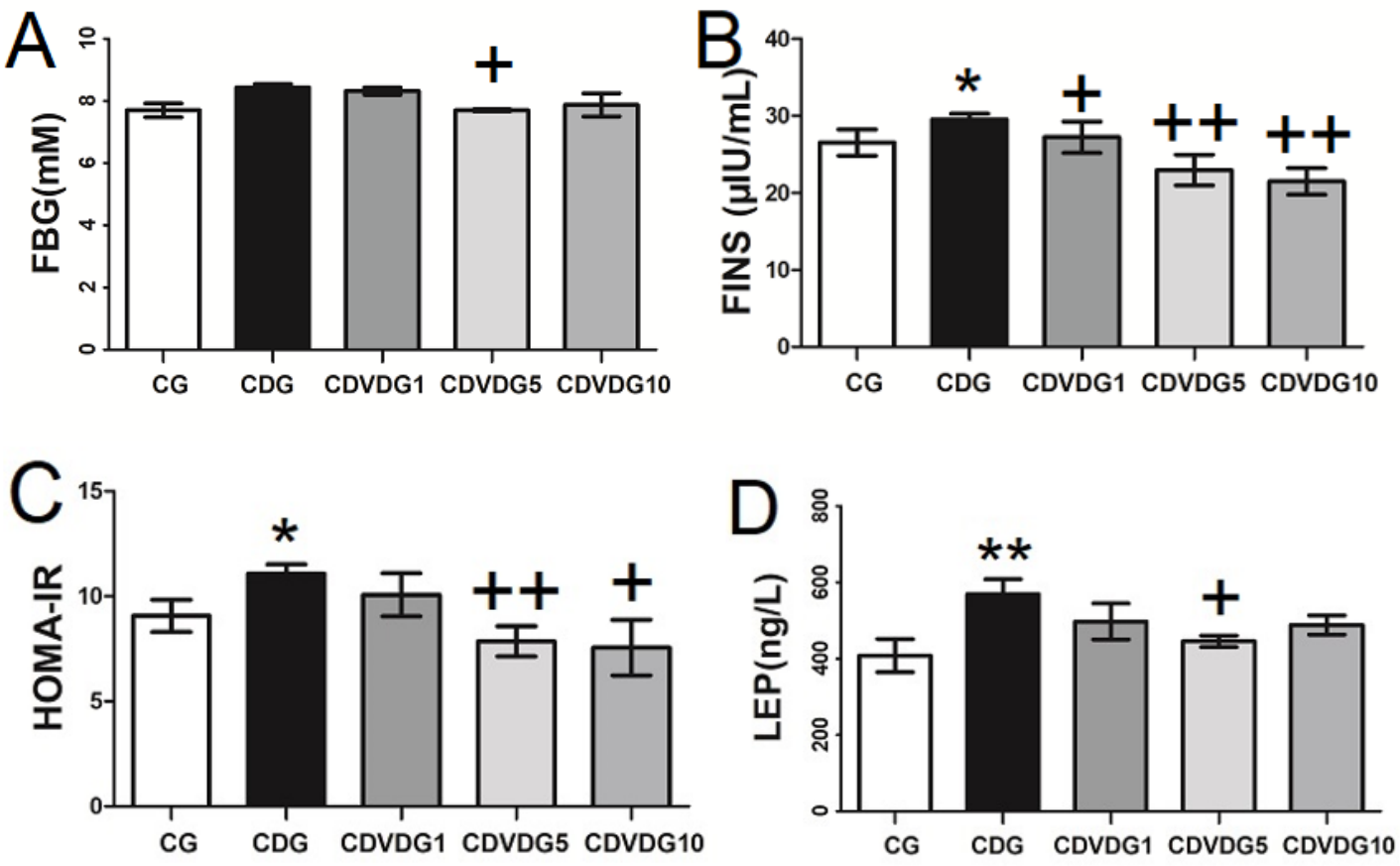

Figure 1

Effects of VD3 supplementation on the serum FBG, FINS, HOMA-IR and leptin. A, B, C and D) serum FBG, FINS, HOMA-IR and leptin levels, respectively. * $p < 0.05$ vs CG; ** $p < 0.01$ vs CG; + $p < 0.05$ vs CDG, ++ $p < 0.001$ vs CDG.

Supplementary Files

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