

Effect of cholecalciferol and levo carnitine on plasma glucose, plasma insulin and insulin resistance in type 2 diabetic rats

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Abstract

Objective: To compare the effects of combined and individual supplementation of cholecalciferol and levo carnitine on plasma glucose, plasma insulin and insulin resistance in type 2 diabetic rats.

Methods: The randomised controlled trial was conducted at the Department of Physiology, Army Medical College, Rawalpindi, between October 2010 and April 2011. It comprised 80 healthy Sprague Dawley rats who were divided into four groups (n = 20 each). Rats were fed high-fat diet for 2 weeks followed by an intraperitoneal injection of streptozocin to induce type 2 diabetes mellitus. Group I served as diabetic control; group II was given cholecalciferol; group III; levo carnitine; and group IV was administered cholecalciferol and levo carnitine together. After 6 days of supplementation, terminal intracardiac blood extraction was done and samples were analysed for fasting plasma glucose and plasma insulin. Insulin resistance was calculated by homeostatic model assessment for insulin resistance. SPSS 17.0 was used for statistical analysis.

Results: Fasting plasma glucose levels were significantly decreased ($p < 0.001$) in the combined supplementation group compared to the diabetic control and individual supplementation groups. Combined supplementation showed a significant increase in fasting plasma insulin levels when compared with diabetic control and levo carnitine groups ($p < 0.001$), and the effect of combined supplementation on ameliorating insulin resistance was significantly better ($p < 0.001$) as compared to the individual supplementation of cholecalciferol and levo carnitine.

Conclusions: The combined supplementation of cholecalciferol and levo carnitine for 6 days markedly improved the glycaemic control, insulin secretion and insulin resistance in type 2 diabetic rats on high-fat diet. A prolonged supplementation by both the compounds along with caloric restriction may yield a more promising outcome.

Keywords: Plasma glucose, Insulin, Insulin resistance, Cholecalciferol, Levo carnitine, Type 2 diabetes mellitus. (JPMA 63: 374; 2013)

Introduction

Type 2 diabetes mellitus (T2DM) occurs as a result of insulin resistance leading to impaired glucose metabolism and insulin deficiency due to β cell exhaustion.¹ The insulin resistance has been attributed to an abnormal insulin molecule, an excessive amount of circulating antagonists or target tissue defects including down regulation and abnormal structure of insulin receptors. Decreased insulin sensitivity results in diminished glucose transport and its utilisation by target cells.²

Cholecalciferol is a fat soluble vitamin, which has been studied for its possible role in improving insulin sensitivity of target tissues in T2DM. Cholecalciferol binds to vitamin D receptor (VDR), which is a steroid hormone

nuclear receptor that mediates transcriptional activation of genes responsible for the synthesis of insulin receptors and the insulin molecule itself. By stimulating the expression of insulin receptors and transcriptional activation of human insulin gene, cholecalciferol has been documented to ameliorate insulin resistance and decreased insulin secretion which are both documented hallmarks of T2DM.³

Levo carnitine is a vitamin-like compound which is abundantly present in mammalian plasma and tissues. It has been documented that levo carnitine supplementation significantly improves insulin sensitivity and stimulates glucose oxidation in type 2 diabetic patients, thereby improving glycaemic control in T2DM.⁴ It has been shown to regulate hepatic glucose metabolism by suppressing glucose over-production in high fructose fed animal models. These properties have been shown to reduce insulin resistance in experimental models of T2DM.⁵

The plausible roles of cholecalciferol and levo carnitine

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in T2DM have been evaluated separately, with controversial outcomes. Studies have reported an improvement in glycaemic control with these drugs, but no study has so far been conducted on evaluating the effects of combined supplementation of these drugs on glycaemic control, insulin secretion and insulin resistance. Considering this, the present study was designed to compare the effects of combined supplementation of cholecalciferol and levo carnitine with their individual supplementation on plasma glucose, plasma insulin and insulin resistance in type 2 diabetic Sprague Dawley rats.

Materials and methods

These lab-based randomised controlled trial was conducted at the Department of Physiology, Army Medical College, Rawalpindi, from October 2010 to April 2011, and comprised 80 Sprague Dawley rats (250±50g), aged 60-90 days, which were normally behaving and were not having any pre-existing ailment, as reported by the animal supervisor of the National Institute of Health (NIH), Islamabad. The subjects were picked through non-probability convenient sampling. A formal approval from the ethical committee of the Army Medical College was taken before the commencement of the experiment. Pre-existing diabetes was ruled out with the help of blood glucose testing with glucometer. The rats were kept in a temperature-controlled environment, and a 12:12-h light-dark cycle was maintained. All the 80 rats were fed high fat diet (HFD) for 2 weeks. At the end of the 2nd week, a single intraperitoneal injection of streptozocin (35 mg/kg) was administered following which the rats continued on HFD for the 3rd week.⁶ At the end of the 3rd week, 1.5ml tail vein blood was aseptically taken after an overnight fast of 12 hours, and put in sodium fluoride tubes. The samples were analysed for fasting plasma glucose and insulin levels, which confirmed the development of T2DM in all the rats. Insulin resistance was calculated by homeostatic model assessment for insulin resistance (HOMA-IR). Plasma glucose level > 220mg/dl was taken as the cut-off value for the confirmation of T2DM.⁷ After the induction and confirmation of T2DM, the rats were randomly divided into four equal groups (n=20 each) using random number tables:

Group I (Diabetic control) comprised diabetic rats which continued on HFD for another 6 days (4th week of study) and no drug was administered to this group; Group II (Cholecalciferol group) comprised diabetic rats which continued on HFD and cholecalciferol was administered subcutaneously (10 ng/100 g) daily for 6 days;⁸ and Group III (Levo carnitine) comprised diabetic rats which

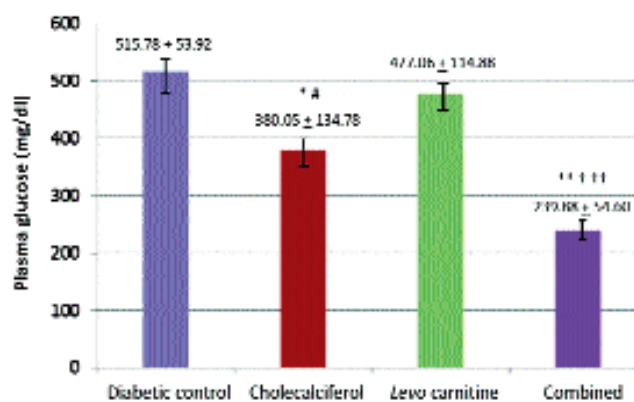
continued on HFD and levo carnitine was administered intraperitoneally (200mg/kg) daily for 6 days;⁹ and Group IV (Combined supplementation) comprised diabetic rats which continued on HFD and both cholecalciferol (10 ng/100g) and levo carnitine (200mg/kg) were administered daily for 6 days.^{8,9}

At the end of the 4th week, the rats were kept on overnight fast (12 hours). Rats were anaesthetised by keeping them in a chamber containing ether soaked cotton, for 3-5 minutes. Terminal blood samples (4-5 ml) were taken by intracardiac extraction with 18-gauge needles attached to 5ml syringes. The samples were put in sodium fluoride tubes and were centrifuged for 15 minutes at 4000rpm (Eppendorf centrifuge 5810R, Germany). Plasma was pipetted out of the sodium fluoride tubes and transferred to Eppendorf tubes. Plasma glucose was estimated by glucose oxidase method (GLUCOSE - L, Minias Globe Diagnostics, Italy). Plasma insulin levels were estimated with insulin (Rat) ELISA (DRG Instruments GmbH, Germany). Insulin resistance was calculated by HOMA-IR.¹⁰

The data was analysed on SPSS version 17.0. The mean and standard deviation (SD) values were calculated for quantitative variables i.e. plasma glucose, plasma insulin and insulin resistance. Data within the groups were analysed by using one-way analysis of variance (ANOVA), followed by post-hoc Tukey HSD. $P \leq 0.05$ was considered statistically significant.

Results

Fasting plasma glucose (mg/dl) of rats at the end of the

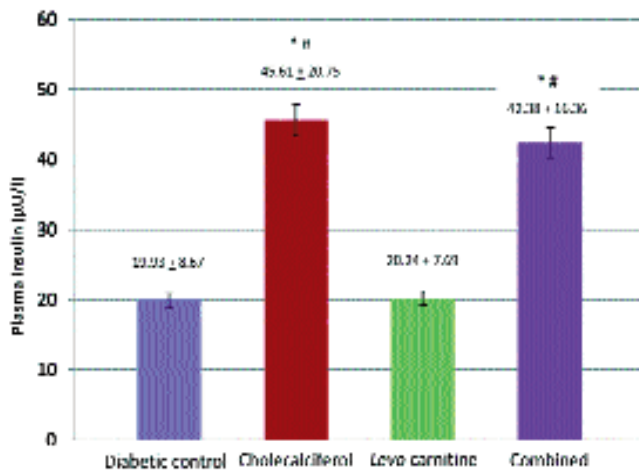


*: $p < 0.001$ compared to diabetic control. #: $p = 0.011$ compared to levo carnitine.

**: $p < 0.001$ compared to diabetic control. †: $p < 0.001$ compared to cholecalciferol.

††: $p < 0.001$ compared to levo carnitine.

Figure-1: Fasting plasma glucose levels amongst the groups after drug supplementation (end of 4th week).



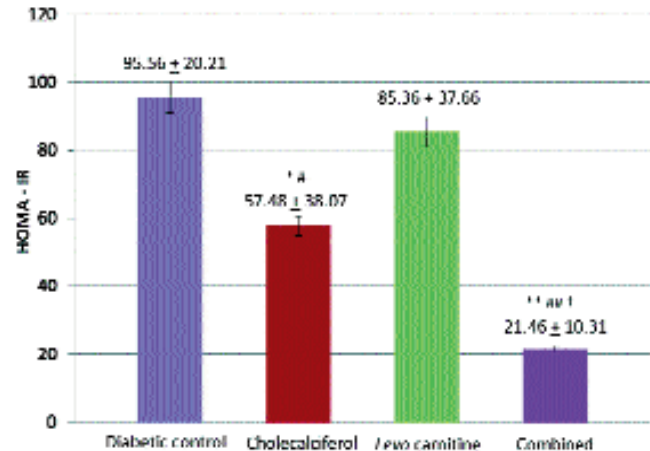
* $p < 0.001$ compared to diabetic control.

$p < 0.001$ compared to levo carnitine.

Figure-2: Fasting plasma insulin levels amongst the groups after drug supplementation (end of 4th week).

4th week (after drug supplementation) showed a significant statistical difference ($p < 0.05$) amongst all the groups (Figure-1). The combined supplementation revealed a highly significant effect ($p < 0.001$) on glycaemic profile compared to the diabetic controls (Table-1). Individual supplementation with cholecalciferol markedly decreased ($p < 0.001$), whereas levo carnitine did not affect ($p > 0.05$) plasma glucose levels compared to the diabetic controls. The glycaemic control exerted by the combined supplementation of both the drugs was significantly pronounced ($p < 0.001$) compared to that of individual supplementation of either drug.

The comparison of fasting plasma insulin levels ($\mu\text{U/l}$) amongst various groups after the supplementation of drugs showed a significant statistical difference ($p < 0.001$) (Figure-2). The combined supplementation



* $p < 0.001$ compared to diabetic control.

$p < 0.017$ compared to levo carnitine.

** $p < 0.001$ compared to diabetic control.

$p < 0.001$ compared to cholecalciferol.

† $p < 0.001$ compared to levo carnitine.

HOMA-IR: Homeostatic model assessment for insulin resistance.

Figure-3: HOMA-IR amongst the groups after drug supplementation (end of 4th week).

significantly increased the plasma insulin levels in T2DM rats ($p < 0.001$). The effect of combined supplementation in increasing the plasma insulin levels was not statistically different ($p > 0.05$) from the effect of individual supplementation of cholecalciferol. Cholecalciferol increased the plasma insulin levels significantly ($p < 0.001$) compared to levo carnitine ($p > 0.05$). Therefore, cholecalciferol supplementation increased plasma insulin levels when given in combination or alone, whereas levo carnitine did not have any effect on plasma insulin levels of T2DM rats.

HOMA-IR after drug supplementation showed a significant ($p > 0.001$) difference across the groups (Figure-3). The individual and combined supplementation of cholecalciferol and levo carnitine

Table: Comparison (Post Hoc) of fasting plasma glucose, plasma insulin and HOMA-IR amongst various groups after drug supplementation.

Parameters Comparison between groups	Plasma glucose (mg/dl)		Plasma insulin ($\mu\text{U/l}$)		HOMA - IR	
	Mean difference	P-value	Mean difference	P-value	Mean difference	P-value
Diabetic control vs Cholecalciferol	135.73	< 0.001	-25.68	< 0.001	38.07	0.001
Diabetic control vs Levo Carnitine	38.73	0.585	-0.31	1.000	10.19	0.685
Diabetic control vs Combined	275.90	< 0.001	-22.44	< 0.001	74.09	< 0.001
Cholecalciferol vs Levo Carnitine	-97.00	0.011	25.37	< 0.001	-27.88	0.017
Cholecalciferol vs Combined	140.17	< 0.001	3.23	0.893	36.02	0.001
Levo Carnitine vs Combined	237.17	< 0.001	-22.13	< 0.001	63.90	< 0.001

HOMA-IR: Homeostatic model assessment for insulin resistance.

revealed a significant effect ($p < 0.001$), whereas individual supplementation of levo carnitine revealed no significant effect ($p > 0.05$) on reducing insulin resistance when compared to the diabetic controls. The combined supplementation of both the drugs revealed a significant decrease in insulin resistance compared to the use of individual drugs ($p < 0.001$).

Discussion

The animal model of T2DM described by Srinivasan et al. in 2005 was used in this study because it closely resembled the natural course and metabolic characteristics of the disease. This model had been documented to manifest the similar response to standard anti-diabetic drugs as in human T2DM. We induced T2DM in HFD rats by injecting a single dose of streptozocin (35mg/kg) which reduced the β cell mass in islets of Langerhans and resulted in frank hyperglycaemia and insulin resistance.⁶

The development of T2DM in all the groups of the study was confirmed by estimating plasma glucose and HOMA-IR at the end of the 3rd week. The glucose levels in all the groups ranged between 513-534mg/dl and HOMA-IR was found to be >2 (confirmative of T2DM). Cholecalciferol has been documented to decrease the plasma glucose levels in T2DM Sprague Dawley rats.^{11,12} In this study, cholecalciferol (10 ng/100g subcutaneously for 6 days), significantly decreased (26.31%) the glucose levels of cholecalciferol treated rats (380.05 mg/dl) compared to the diabetic control (515.78 mg/dl). It has been documented that cholecalciferol (5000 IU/kg body weight/day for 15 days by gastric Gavage method which involves insertion of a feeding tube in the animal being fed to ensure proper supplementation of medication) decreased the blood glucose levels to near-normal values in alloxan-induced diabetic rats.¹³ In a study conducted on obese Wistar rats, cholecalciferol (12.5 μ g/kg body weight for 14 days by gastric Gavage) was found to decrease plasma glucose concentrations by as much as 40%.¹⁴ It is evident that the difference in the duration of supplementation and route of administration of cholecalciferol lead to a better glucose-lowering effect in these studies compared to ours.

A dose of 200mg/kg levo carnitine was administered intraperitoneally daily for 6 days. In this study the plasma glucose levels in levo carnitine-treated group (477.06 mg/dl) were found to be reduced by 7.5% ($p > 0.05$) compared to the diabetic controls (515.78mg/dl). These results were not consistent with

the findings of studies in which administration of levo carnitine decreased blood glucose level in inbred strain of rats.^{15,16} The most potent effect (53.49%) in lowering plasma glucose level was observed in the combined supplementation group (239.88 mg/dl). Although this value was close to the cut-off value (220 mg/dl) of plasma glucose to establish T2DM in our study, the combined administration of cholecalciferol and levo carnitine could not develop normoglycaemia. It is suggested that the combined administration of these drugs along with the commonly used oral hypoglycaemic agents (OHAs) may help in reducing the required dose of the latter and this effect may be more beneficial if the diet is also regulated. To our knowledge no study has so far been conducted on T2DM rats in which the combined supplementation of cholecalciferol and levo carnitine was given.

Insulin secretion has been documented to increase consequent to cholecalciferol supplementation in response to glucose tolerance testing in T2DM rats with reduced insulin secretion.¹¹ In another experimental T2DM model, obese Wistar rats were found to have increased insulin secretion upon cholecalciferol supplementation.¹⁴ Similar results were observed in a study conducted on alloxan-induced T2DM rats in which the insulin secretion was increased up to 98% after daily administration of cholecalciferol.¹³ In our study, the level of insulin secretion in cholecalciferol-treated group was enhanced by 114.54% (45.61 μ U/l) compared to the diabetic controls (19.93 μ U/l). This is due to the fact that cholecalciferol enhances the insulin secretion by transcriptional activation of insulin production by the pancreatic β cells.¹⁷

We employed HOMA-IR for the calculation of insulin resistance in our study, which is a reliable surrogate index that provides an approximation of formal measures of insulin resistance when applied to rats and mice as they do in humans.¹⁸ Reports on association between insulin resistance and serum cholecalciferol levels have been inconsistent. Several cross-sectional studies in humans have shown negative association between serum cholecalciferol levels and fasting plasma glucose and HOMA-IR, whereas a few have provided either no or minimal association. The difference in this relationship is likely due to the differences in subject populations and different methods to determine insulin secretion. A significant positive association between serum cholecalciferol levels and oral glucose tolerance test (OGTT) induced insulin secretion was reported in East

London Asians at risk for T2DM.¹⁹ On the other hand, no association was documented between serum cholecalciferol levels and meal-induced insulin secretion in men with T2DM.²⁰ It is possible that cholecalciferol is unable to augment insulin secretion in uncontrolled T2DM subjects who have already exhausted their insulin secretory capacity. Analysis of the National Health and Nutrition Examination Survey 1989-1994 (NHANESIII) disclosed that serum cholecalciferol levels were inversely associated with the risk of diabetes and help to measure insulin resistance.²¹ Interventional human studies have reported contradictory results between cholecalciferol supplementation and HOMA-IR. A non-significant decrease in HOMA-IR was found in Bulgarian women with T2DM on oral cholecalciferol supplementation for one month.²² HOMA-IR was found to have been improved in American adults, with daily oral supplementation of cholecalciferol for 3 years.²³ No change in HOMA-IR of T2DM Norwegian adults was observed who were treated with weekly supplementation of cholecalciferol for 6 months.²⁴ It is possible that the difference in duration of intervention between these studies led to the contradictory outcome of results. To our knowledge, no study has so far been conducted to assess the effects of combined supplementation of cholecalciferol and levo carnitine on insulin resistance. In our study the effect of combined supplementation was highly significant ($p < 0.001$) compared to the individual supplementation of the drugs on insulin resistance. The significant improvement of HOMA-IR in our study showed a positive contribution of cholecalciferol in normalising the plasma glucose levels, and levo carnitine in shuttling the fatty acids in to the mitochondria for oxidation, suggesting the contribution of both the compounds in improving glycaemic control. HOMA-IR was significantly ($p < 0.05$) decreased by cholecalciferol supplementation compared to the diabetic control. The finding that cholecalciferol had a significant effect in lowering HOMA-IR was consistent with the findings of Norman et al.²⁵ A similar outcome was found in another study conducted on Sprague Dawley rats.²⁶ Levo carnitine has been shown to improve insulin resistance in a randomised controlled study on humans. However, these subjects were given a low-calorie diet throughout the study period.²⁷ In a human randomised controlled trial, levo carnitine supplementation has been shown to improve HOMA-IR significantly.²⁸ We found that levo carnitine did not have any significant effect on HOMA-IR ($p > 0.05$).

Conclusion

The study not only substantiated previous studies of individual efficacy of cholecalciferol and levo carnitine, but also suggested that combined supplementation of both the drugs had a significant effect of decreasing insulin resistance ($p < 0.001$). However, keeping in view the limited number of rats in the study, it is concluded that increasing the number of rats and duration of the supplementation of both cholecalciferol and levo carnitine may yield more promising prospects in this area.

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References

1. Cali AM, Man CD, Cobelli C, Dziura J, Seyal A, Shaw M, et al. Primary defects in beta-cell function further exacerbated by worsening of insulin resistance mark the development of impaired glucose tolerance in obese adolescents. *Diabetes Care* 2009; 32: 456-61.
2. Czech A, Taton J, Piatkiewicz P. Cellular glucose transport disturbances in the pathogenesis and therapy of type 2 diabetes mellitus. *Endokrynol Pol* 2010; 61: 292-302.
3. Pittas AG, Lau J, Hu FB, Dawson Hughes B. The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. *J Clin Endocrinol Metab* 2007; 92: 2017-29.
4. Rajasekar P, Anuradha CV. Effect of L-carnitine on skeletal muscle lipids and oxidative stress in rats fed high-fructose diet. *Experimental Diabetes Res* 2007; 72741: 1-8.
5. Coker RH, Williams RH, Yeo SE, Kortebein PM, Bodenner DL, Kern PA, et al. The impact of exercise training compared to caloric restriction on hepatic and peripheral insulin resistance in obesity. *J Clin Endocrinol Metab* 2009; 94: 4258-66.
6. Srinivasan K, Viswanad B, Asrat L, Kaul CL, Ramarao P. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. *Pharmacol Res* 2005; 52: 313-20.
7. Yassin MM, Mwafy SN. Protective potential of glimepiride and nerium oleander extract on lipid profile, body growth rate, and renal function in streptozotocin-induced diabetic rats. *Turk J Biol* 2007; 31: 95-102.
8. Dudeja PK, Wali RK, Klitzke A, Sitrin MD, Brasitus TA. Correction of enhanced Na^{+} - H^{+} exchange of rat small intestinal brush-border membranes in streptozotocin-induced diabetes by insulin or 1,25-dihydroxycholecalciferol. *J Clin Invest* 1991; 87: 1755-62.
9. Cavazza C, inventor Composition for the prevention of muscle fatigue and skeletal muscle adaptation of strenuous exercise. United States of America, 2003.
10. Yokohama H, Emoto M, Fujiwara S, Motoyama K, Morioka T, Komatsu M. Quantitative insulin sensitivity check index and the reciprocal index of homeostasis model assessment in normal range weight and moderately obese type 2 diabetic patients. *Diabetes Care* 2003; 26: 2426-32.
11. Cade C., Norman A.W. Vitamin D3 improves impaired glucose tolerance and insulin secretion in the vitamin D deficient rat in vivo. *Endocrinology* 1986; 119: 84-90.
12. Danescu LG, Levy S, Levy J. Vitamin D and diabetes mellitus.

- Endocrine 2009; 35: 11-7.
13. Hamden K, Carreau S, Jamoussi K, Miladi S, Lajmi S, Aloulou D, et al. 1 α , 25 dihydroxyvitamin D₃: therapeutic and preventive effects against oxidative stress, hepatic, pancreatic and renal injury in alloxan-induced diabetes in rats. *J Nutr Sci Vitaminol* 2009; 55: 215-22.
 14. de Souza Santos R, Vianna LM. Effect of cholecalciferol supplementation on blood glucose in an experimental model of type 2 diabetes mellitus in spontaneously hypertensive rats and Wistar rats. *Clinica Chimica Acta* 2005; 358: 146-50.
 15. Paulson DJ, Schmidt MJ, Traxler JS, Ramacci MT, Shug AL. Improvement of myocardial function in diabetic rats after treatment with L-carnitine. *Metabolism* 1984; 33: 358-63.
 16. Abdel-aleem S, Karim AM, Zarouk WA, Taylor DA, el-Awady MK, Lowe JE. Reduced effects of L-carnitine on glucose and fatty acid metabolism in myocytes isolated from diabetic rats. *Horm Metab Res* 1997; 29: 430-5.
 17. Boulton PM, Billaudel B, Faure-Dussert A. Influence of vitamin D₃ deficiency and 1,25 dihydroxyvitamin D₃ on de novo insulin biosynthesis in the islets of the rat endocrine pancreas. *J Endocrinol* 1999; 160: 87-95.
 18. Mather K. Surrogate measures of insulin resistance: of rats, mice, and men. *Am J Physiol Endocrinol Metab* 2009; 296: E398-9.
 19. Boucher BJ, Mannan N, Noonan K, Hales CN, Evans SJ. Glucose intolerance and impairment of insulin secretion in relation to vitamin D deficiency in east London Asians. *Diabetologia* 1995; 38: 1239-45.
 20. Orwoll E, Riddle M, Prince M. Effects of vitamin D on insulin and glucagon secretion in non-insulin-dependent diabetes mellitus. *Am J Clin Nutr* 1994; 59: 1083-87.
 21. Scragg R, Holdaway I, Singh V, Metcalf P, Baker J, Dryson E. Serum 25-hydroxyvitamin D₃ levels decreased in impaired glucose tolerance and diabetes mellitus. *Diabetes Res Clin Pract* 1995; 27: 181-8.
 22. Borissova AM, Tankova T, Kirilov G, Dakovska L, Kovacheva R. The effect of vitamin D₃ on insulin secretion and peripheral insulin sensitivity in type 2 diabetic patients. *Int J Clin Pract* 2003; 57: 258-61.
 23. Pittas AG, Lau J, Hu FB, Dawson-Hughes B. The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. *J Clin Endocrinol Metab* 2007; 92: 2017-29.
 24. Jorde R, Figenschau Y. Supplementation with cholecalciferol does not improve glycaemic control in diabetic subjects with normal serum 25-hydroxyvitamin D levels. *Eur J Nutr* 2009; 48: 349-54.
 25. Norman AW, Frankel JB, Heldt AM, Grodsky GM. Vitamin D deficiency inhibits pancreatic secretion of insulin. *Science* 1980; 15: 823-25.
 26. Cade C, Norman AW. Vitamin D₃ improves impaired glucose tolerance and insulin secretion in the vitamin D-deficient rat in vivo. *Endocrinology* 1986; 119: 84-90.
 27. Molino A, Cascino A, Conte C, Ramaccini C, Rossi Fanelli F, Laviano A. Caloric restriction and L-carnitine administration improves insulin sensitivity in patients with impaired glucose metabolism. *J Parenter Enteral Nutr* 2010; 34: 295-59.
 28. Malaguamera M, Gargante MP, Russo C, Anti T, Vacante M, Malaguamera M, et al. L-carnitine supplementation to diet: A new tool in treatment of nonalcoholic steatohepatitis - A randomized and controlled clinical trial. *Am J Gastroenterol* 2010; 24: 356-61.
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