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Original article

Vitamin D3 intake as regulator of insulin degrading enzyme and insulin receptor phosphorylation in diabetic rats

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ABSTRACT

Insulin-degrading enzyme (IDE, insulysin) is a rate–limiting enzyme in the insulin degradation process. It is an intracellular 110-kDa thiol zinc-metalloendopeptidase located in the cytosol, peroxisomes, endosomes and cell surface. IDE catalyzes degradation of several small proteins including insulin, amylin and β-amyloid protein. In addition, insulin clearance was expressed as a target in the treatment of type 2 diabetes given the role of hyperinsulinemia in the pathogenesis of insulin resistance. In this study, fourtyadult male Wistar albino rats were used, thirty rats received 20% fructose in drinking water (HFW) for six weeks to induce diabetes. Subsequently, these rats developed significantly higher body weights, dyslipidemia, hyperglycemia and insulin resistance compared to their controls. Significant increase in the levels of serum glucagon, IDE in liver tissue along with an inhibition of insulin receptor phosphorylation were also observed. Concurrent oral administration of vitamin D3 along with HFW resulted in significant decrease of serum glucose, total cholesterol, triacylglycerol and LDL-C levels. Vitamin D alleviated also insulin resistance, where both IDE, glucagon levels showed significant decrease along with activation of insulin receptor phosphorylation. Normal rats, received vitamin D3 only demonstrated non significant changes of the studied biomarkers. We concluded that vitamin D3 ameliorated insulin resistance and hyperinsulinemia in diabetic rat model received HFW through reduction of IDE and activation of insulin receptor phosphorylation.

1. Introduction

Increased consumption of fructose is closely related to the incidence of obesity [1]. This could be mostly attributed to stimulation of insulin secretion and absence of GLUT5 (glucose transporter) in the pancreatic β-cell [1]. The metabolism in turn bypasses the main glycolysis pathway which converts glucose-6-phosphate to fructose-1,6-biphosphate by phosphofructokinase enzyme [2]. These two factors are counteracted with glucose which stimulates the secretion of insulin and conversion of glucose to glycogen. Previous study indicated that the higher calorie intake would establish the development of metabolic syndrome in experimental rat models [3].

Last years the relationship between vitamin D effect, insulin secretion and insulin resistance has been extensively investigated. Locally in the pancreas, vitamin D activation occurs through 1 α-hydroxylase enzyme. Subsequently, the active form directly through its receptors or through intracellular calcium regulation may help in insulin secretion [4,5]. Vitamin D increases insulin sensitivity through its muscle cell receptors or increasing the sensitivity of the latter to insulin. Modulation of peroxisome proliferator activated receptor (PPAR)-δ, regulation of extracellular calcium are additional effects of vitamin D [6–10]. Accordingly vitamin D may affect insulin secretion and sensitivity as well; both factors contribute to the pathogenesis of type 2 diabetes.

Biological evidences implicating a potential influence of vitamin D on glucose homeostasis are many [11], including the presence of specific vitamin D receptors on pancreatic β-cells [12] and other tissues, such as vascular endothelial cells, neurons, immune cells, osteoblasts and myocytes [13]. In addition, vitamin D response element is present in the human insulin gene promoter [14]. 1,25 $(OH)_2$ D directly activates transcription of the human insulin receptor gene [15], activates peroxisome proliferator activator receptor–δ [16], stimulates the expression of insulin receptor and enhances insulin-mediated glucose transport *in vitro* [17]. The insulin receptor (IR) is a heterotetramer consisting of two α-subunits and two β-subunits that are linked by disulphide

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bonds. Insulin binds to α subunit of IR and activates the tyrosine kinase in the β-subunit leading to auto phosphorylation of the β-subunit [18]. Most of the metabolic and anti-apoptotic effects of insulin are mediated by the signaling pathway involving the phosphorylation of the insulin receptor substrate proteins and the activation of certain kinases [19].

Growing evidence from human observational studies indicates that vitamin D deficiency is positively associated with metabolic diseases such as musculoskeletal, cardiovascular, neoplastic diseases and Type 2 diabetes [13], however, the mechanisms by which vitamin D mediates these actions remain unknown. A possible mechanism is that vitamin D receptor is a nuclear receptor that dimerizes with retinoid X receptors (RXRs) in adipose tissues like PPAR-γ [20], enhancing its expression during adipogenesis [21]. However, this is still controversial due to the differences in dosage and species in previous studies [22,23].

Vitamin D status is also associated with glucose metabolism. In a systematic review and meta-analysis, Forouhi et al. [24] found that only prospective studies showed inverse associations between circulating vitamin D levels and incidence of Type 2 diabetes. This is in agreement with data recorded by the USA National Health and Nutrition Examination Survey [25]. However, George et al. [26] demonstrated that in randomized trials of vitamin D supplementation no evidence to recommend vitamin D for patients with type 2 diabetes or impaired glucose intolerance for treating diabetes and/or improving glycemic control. Therefore, it remains unclear whether vitamin D supplementation is beneficial or not for type 2 diabetes. However low vitamin D status based on serum 25-OH-D levels was highly associated with the prevalence of Type 2 diabetes in most studies. Therefore, much of the uncertainty may be due to a possible disconnection between vitamin D intake and its status.

Insulin degrading enzyme (IDE) is a potential drug target in the treatment of type 2 diabetes. It controls circulating insulin through a degradation-dependent clearance mechanism in multiple tissues [27]. Circulating insulin is determined by the balance of insulin secretion, clearance and a reduction of the latter as a mechanism which deals with hyperinsulinemia. IDE enzyme activity is induced by zinc, inhibited by copper, aluminum and nitric oxide [28,29]. Inactivation of IDE by gene knockout induces hyperinsulinemia and insulin resistance in mice as previously reported [30]. Thus, the control of hyperinsulinemia is a result of improved insulin sensitivity in the peripheral tissues [31].

The present article describes the biological plausibility behind the potential association between vitamin D administration, type 2 diabetes and the feasibility of modulating IDE protein level in addition to insulin receptor phosphorylation as a new therapeutic strategy for the treatment of type 2 diabetics.

2. Materials and methods

2.1. Animals and ethics

Adult male Wistar albino rats (weighing 160 ± 20 g) were housed in stainless steel cages in a controlled environment (23 °C and with a 12-h light/dark cycle). All experimental procedures were performed according to the guidelines of the Animal Care and Use Committee of Zagazig University, Egypt.

2.2. Experimental design

Fourty maleWistar albino rats were randomly assigned into the following four groups, n = 10/each (a) Normal control group: received rat chow diet, normal tap water and no treatment, (b) Diabetic control group: received rat chow diet, 20% fructose in drinking water (HFW) [3] and no treatment, (c) vitamin D group (Diabetic): received rat chow diet, fructose 20% in drinking water and vitamin D3 (oral drops, Novartis Co, Switzerland) in a dose level of 170 IU/week, p.o [32]. Vitamin D group (sham): Normal group received rat chow diet and Vitamin D3 in a dose level of 170 IU/week, p.o.

2.3. Blood and tissue sampling

The rats were fasted overnight and supplemented with only tap water, blood samples were obtained at the baseline and at end of the experiment *via* orbital vein of anaesthetized rats . . . Animals were weighed before collection of blood samples. Blood was centrifuged at 4500 rpm and serum samples were immediately analyzed for glucose. The remaining serum was frozen at −80 °C and stored for further analysis of lipid profile, insulin and glucagon. Rats were then killed by decapitation and the liver was excised, washed with cold saline, kept in liquid nitrogen and stored at −80 °C for measurement of IDE protein level.

2.4. Biochemical measurements

Colorimetric kits (Spinreact, Spain) were used for determination of serumtotal cholesterol (TC), triacylglycerol (TAG) and high density lipoprotein cholesterol (HDL-C) levels. Low density lipoprotein cholesterol (LDL-C) was calculated from friedwald formula: LDL-C = TC − (TAG/5 + HDL-C). Enzyme-linked immunosorbent assay (ELISA) kits for rat insulin, glucagon (serum), insulin receptor phosphorylation in hepatic cell lysate [33] and IDE in the liver were purchased from SPI bio, Montigny Le Bretonneux, France; CUSABIO, China; Sigma Aldrich, MO, USA; and Cloud-Clone Crop, assembled by USCN Life Science Inc., Wuhan, China, respectively. We have followed exactly the recommendations of the manufacturer's procedures. Liver tissue extracts were prepared by homogenizing tissue in Cytobuster Protein Extraction Reagent (Millipore, Billerica, MA 01821) for assessment of IDE protein using ELISAkit.

2.5. Quantitative real time polymerase chain reaction (q-PCR) for insulin degrading enzyme (IDE)

Total RNA was isolated from the livers of animals in the studied groups using Qiagen tissue extraction kit (Qiagen, USA), and RNA was converted into cDNA using high capacity cDNA reverse transcription kit (Fermentas, USA). The primers sequence for IDE and GAPDH genes are shown in Table 1. The cDNA was subjected to amplification and analysis using the TaqMan Master Mix (Applied Biosystems, Foster City, CA, USA) for quantitative RT-PCR. GAPDH was used as the reference gene. The assay was run on Step One™ Real-Time PCR System version 3.1 (Applied Biosystems, USA) according to the manufacturer's instructions.

2.6. Statistical analysis

Statistical analysis was done using the InStat 2.04 statistical package (Graph Pad InStat). Data are presented as means ± standard deviation (SD) for six rats/group. ANOVA test was used to compare between studied groups. $P < 0.05$ was considered to be statistically significant.

Table 1
Shows primers sequence for all studied genes in the work.

Gene symbol	Primers sequence
IDE	F: 5′-GTCCTGTTGTTGGAGAGTTCCCATGTCA-3′ R: 5′-GGGGAATCTTCAGAGTTTTGCAGCCAT-3′
GAPDH	F: 5′-ACCACAGTCCATGCCATCAC-3′ R: 5′-TCCACCACCCTGTTGCTGTA-3′