Insulin-sensitizing effects of vitamin D repletion mediated by adipocyte vitamin D receptor: Studies in humans and mice

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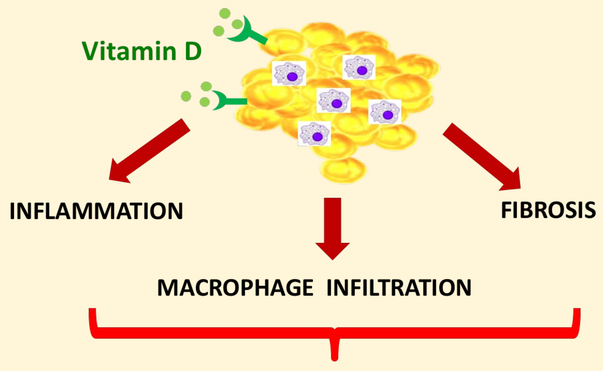
BACKGROUND

Vitamin D deficiency and type 2 diabetes

- Despite epidemiologic evidence linking vitamin D deficiency with insulin resistance and type 2 diabetes, much controversy exists regarding whether vitamin D repletion has beneficial metabolic effects.

Adiposity and Insulin Resistance



- Adipose tissue inflammation and fibrosis appear to be mediated by adipocytes and contribute to insulin resistance in obesity. Vitamin D [25(OH)D] has anti-inflammatory and anti-fibrotic effects in various tissues.

- Expression of the vitamin D receptor (VDR) in both adipocytes and macrophages suggests that 25(OH)D could mediate paracrine effects in adipose tissue, and might explain epidemiological associations between 25(OH)D deficiency and insulin resistance.

AIMS

We designed parallel studies in humans and rodents to define the effects of vitamin D on adipose tissue inflammation and fibrosis, and on systemic insulin resistance.

METHODS-Humans studies

Variables	Vitamin D Group (N=11)	Placebo Group (N=8)	P-value
Age (years)	42.0 ± 3.2	47.3 ± 4.1	0.33
BMI (kg/m²)	34.1 ± 1.3	34.6 ± 2.0	0.83
Fasting glucose (mg/dL) *(normal < 100mg/dl)*	109.6 ± 3.8	100.8 ± 3.6	0.11
Fasting insulin (uU/mL)	20.4 ± 2.0	20.6 ± 3.0	0.96
HOMA-IR *(normal < 2)*	5.5 ± 0.6	5.2 ± 0.8	0.74
25-(OH)D level at baseline (ng/mL) *(normal > 30ng/ml)*	13.9 ± 1.1	12.0 ± 1.7	0.36

- We performed a randomized, double-blinded placebo-controlled trial to examine the effects of repleting vitamin D levels to >30 ng/ml in 25(OH)D-deficient (<20 ng/ml), insulin resistant, overweight-to-obese humans (n=19).

METHODS-Humans studies

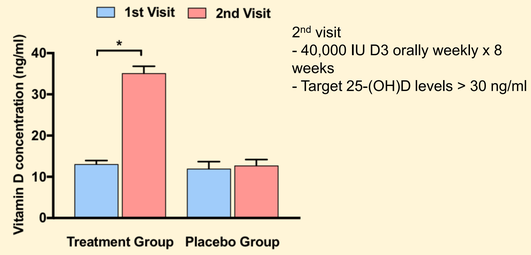


Fig 1. *25 (OH) D repletion from the 1st to the 2nd visits*

- Whole-body insulin action was undertaken with stepped euglycemic (~90mg/dL) hyperinsulinemic clamp studies, both before (1st visit) and after administration of vitamin D or placebo (2nd visit). Adipose tissue fibrosis and inflammation were quantified by 'real-time' rt-PCR and immunofluorescence in subcutaneous abdominal adipose tissue.

Stepped 'pancreatic clamp' studies

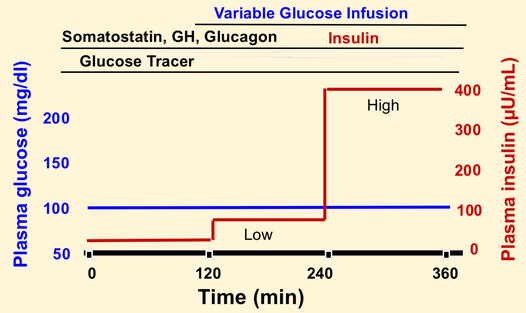


Fig 2. *Schematic representation of euglycemic-hyperinsulinemic clamp studies.*

RESULTS-Humans studies

Insulin Sensitivity Indices

25(OH)D repletion improved hepatic insulin sensitivity in humans, with heightened suppression of endogenous glucose production (EGP) during hyperinsulinemic clamp studies (1.28 ± 0.20 *vs* 0.88 ± 0.18 mg/kg/min, p=0.03). There was no significant change in peripheral glucose uptake (GU) with vitamin D (Fig 3).

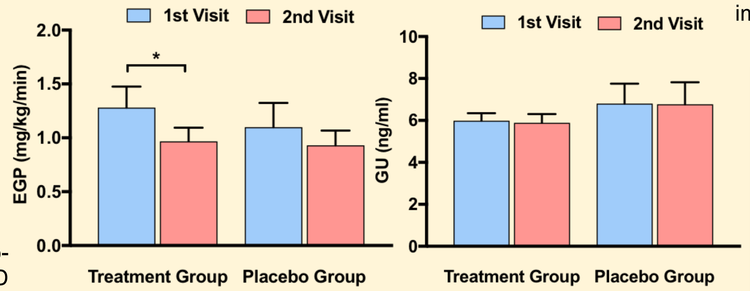


Fig 3. *Effect of Vitamin D repletion on EGP (A) and GU (B)*

Inflammatory Gene Expression in Adipose Tissue

25(OH)D repletion was associated with reductions in adipose tissue gene expression of inflammatory (0.6-0.7-fold decreased expression of *TNF-α, IL-6, iNOS and PAI-1*) factors in whole fat (A) and in adipose tissue macrophage (ATM) (B) ($p<0.05$).

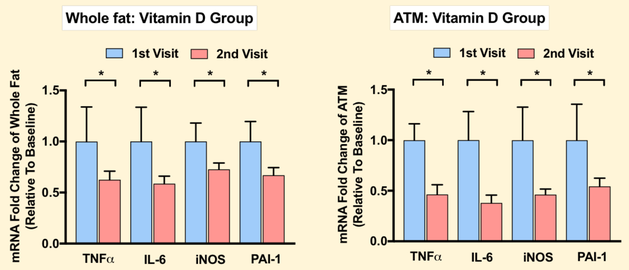


Fig 4. *Effect of Vitamin D repletion on inflammatory gene expression in whole fat (A) and macrophages (B) of adipose tissue.*

Fibrotic Genes Expression in Adipose Tissue

25(OH)D repletion was associated with reductions in adipose tissue gene expression of pro-fibrotic (0.4-0.8-fold decreased expression of *TGF-β1, HiF1α, Collagen I, V, VI and MMP7*) factors ($p<0.05$).

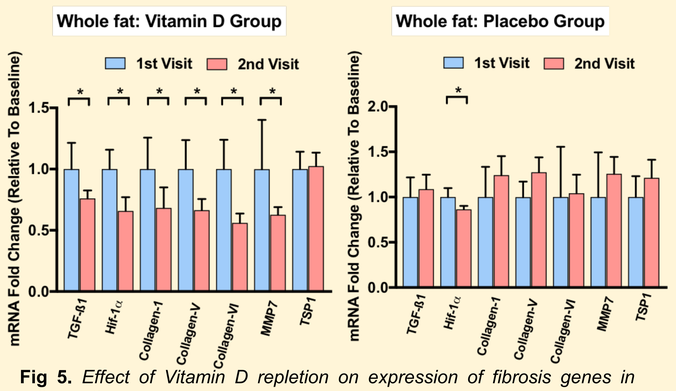


Fig 5. *Effect of Vitamin D repletion on expression of fibrosis genes in adipose tissue*

Collagen I immunofluorescence in adipose tissue

25(OH)D repletion was associated with decreased collagen I immunofluorescence (61% reduction, p=0.04)

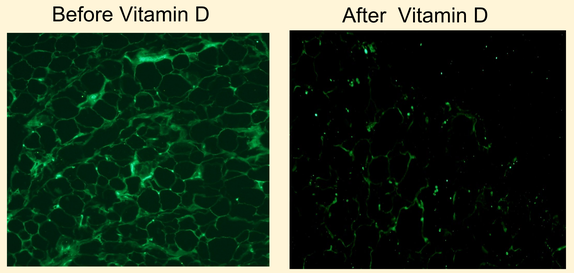


Fig 6. *Collagen I immunofluorescence.*

MOUSE STUDIES

To determine whether vitamin D's effects are mediated through adipocytes, we performed hyperinsulinemic clamp studies (4 mU/kg/min) and adipose tissue analysis in an adipocyte-specific vitamin D receptor knockout (VDR KO) mouse model (Adiponectin-Cre+VDR+/ fl) following high fat diet feeding for 12 weeks.

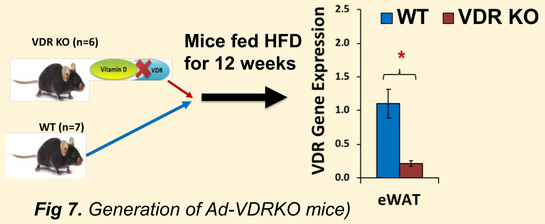


Fig 7. *Generation of Ad-VDRKO mice)*

VDR KO mice exhibited increased hepatic insulin resistance (EGP 10±3 *vs* 3±2 mg/kg/min in WT, p=0.021) *(Fig 8A)* in concert with increase adipose tissue expression of several pro-inflammatory (*Tnf-α*, *iNos*, *Pai-1*, *Mcp*-1 and *F4/80;* 4-10 fold) (Fig 9A), and pro-fibrotic genes (*Tgf-β1*, *Collagen VI*, and *Tsp1;* 2-4 fold*)* (Fig 9B). There were no changes in insulin-mediated glucose uptake (Fig 8B).

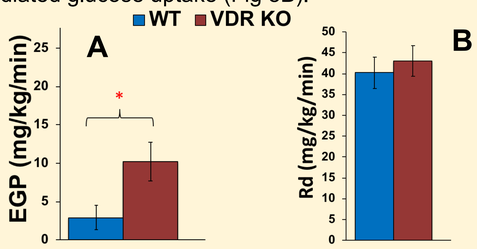
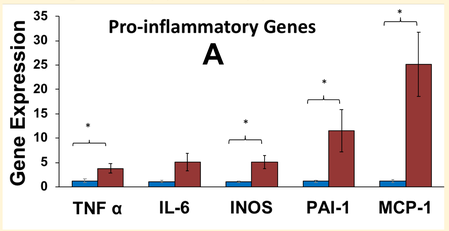


Fig 8. *EGP (A) and Rd (B) VDR KO mice*



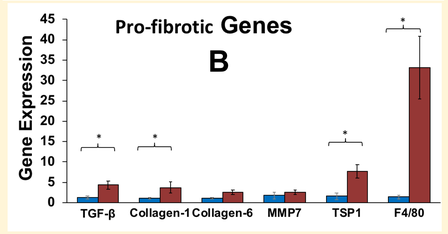


Fig 9. *VDR KO mice display increased adipose tissue inflammation (A) and fibrosis (B)*

SUMMARY & CONCLUSIONS

These complementary human and rodent studies establish a beneficial role of vitamin D to improve hepatic insulin resistance, likely by restraining adipose tissue inflammation and fibrosis. Thus, normalizing 25(OH)D levels could have metabolic benefits in targeted individuals.