Does vitamin D status contribute to caveolin-1-mediated insulin sensitivity in skeletal muscle? Reply to Boucher BJ [letter]

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Abbreviations

1,25(OH)₂D₃ 1,25-Dihydroxycholecalciferol
VDR Vitamin D receptor

To the Editor: Vitamin D deficiency is a significant risk factor for type 2 diabetes, although the effect of vitamin D supplementation for treatment of type 2 diabetes is still under debate [1–3]. It has also been reported that vitamin D receptor (VDR) is present in caveolae [4]. Dr Boucher has therefore posed two important questions [5] in response to our study [6] on caveolin-1 in an ageing-dependent non-obese type 2 diabetic animal model (JYD mouse): (1) whether insulin sensitivity could be improved by vitamin D in JYD mice; and (2) whether such an improvement might be due to an upregulation of caveolin-1 production affected by vitamin D.

To answer Dr Boucher's first question, we measured glucose tolerance and insulin sensitivity in 30-week-old

JYD mice treated with 1,25-dihydroxycholecalciferol [1,25(OH)₂D₃], the hormonal form of vitamin D. Figure 1a shows that 1,25(OH)₂D₃-treated JYD mice had significantly improved glucose clearance compared with control mice, and 1,25(OH)₂D₃-treated mice had significantly lower glucose levels in response to insulin injection compared with control mice (Fig. 1b); this suggests that insulin sensitivity was improved. These results agree with previous reports that supplementation with 1,25(OH)₂D₃ improves insulin sensitivity and glucose tolerance [7, 8].

To answer Dr Boucher's second question, we performed western blot analysis on adipose tissue and skeletal muscle from 1,25(OH)₂D₃-treated JYD mice. We found that the level of caveolin-1, the principal component of caveolae, was not affected by 1,25(OH)₂D₃ treatment in JYD mice (Fig. 2), indicating that the positive effect of vitamin D on insulin sensitivity is affected via a mechanism other than caveolin-1 upregulation.

As it has been reported that *Vdr* mRNA expression is upregulated by 1,25(OH)₂D₃ [9, 10], we studied the expression of *Vdr* mRNA in the skeletal muscle and adipose tissue of JYD mice treated with 1,25(OH)₂D₃ and found that *Vdr* mRNA content in skeletal muscle, but not in adipose tissue, was upregulated by 1,25(OH)₂D₃ treatment (Fig. 3). Although some reports have shown that the tissue-specific regulation of VDR levels are influenced by the state of differentiation of the cells, serum parathyroid hormone level and extracellular calcium concentration [11], the mechanisms for this tissue-specific upregulation of *Vdr* mRNA in JYD mice after 1,25(OH)₂D₃ treatment remain to be determined. 1,25(OH)₂D₃ exerts its actions by binding to VDR, which acts as a nuclear receptor or a cell surface receptor. The nuclear VDR is a ligand-dependent nuclear transcription factor and regulates the transcription of the target genes. The

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Fig. 1 Glucose and insulin tolerance tests in vitamin D-treated JYD mice. Thirty-week-old male JYD mice were injected daily with 12 nmol/kg body weight 1,25(OH)₂D₃ (circles) or ethanol in phosphate buffered saline (control; diamonds) i.p. for 4 weeks. Age-matched, non-diabetic female JYD mice served as a positive control (triangles). **a** For glucose tolerance tests, fasted mice (16 h) were injected with glucose (2 g/kg, i.p.). **b** For insulin tolerance tests, fed mice were injected with insulin (1 U/kg body weight i.p.). Glucose was measured in the tail vein blood at 0, 15, 30, 60, 90 and 120 min after injection using a glucometer. Values are mean±SEM, *n*=6 per group. **p*<0.05 for 1,25(OH)₂D₃-treated vs control mice

rapid non-transcriptional responses of 1,25(OH)₂D₃ are thought to be mediated by the surface VDR [12]. Thus, the mechanism of action of vitamin D in the improvement of insulin resistance is mediated not only through regulation of

Fig. 2 Levels of caveolin in adipose tissues and skeletal muscle of vitamin D-treated JYD mice. Thirty-week-old male JYD mice were injected daily with 12 nmol/kg body weight 1,25(OH)₂D₃ (VIT D) or ethanol in phosphate buffered saline (CON) i.p. for 4 weeks; adipose tissue (ADI) and skeletal muscle from the hindlimb (MUS) were isolated. **a** Levels of caveolin-1 protein (CAV-1) were analysed by western blotting. Actin and tubulin were used as loading controls. **b** The fold-change relative to the control band on western blots was quantitated by densitometric analysis. CON, black bar; VIT D, white bar. Each bar is the mean±SEM (*n*=4)

signalling induction pathways by the cell surface VDR, but also through transcriptional regulation by nuclear VDR. Regardless of how insulin resistance is improved through VDR, our results suggest that 1,25(OH)₂D₃ treatment may affect the insulin sensitivity of JYD mice by VDR upregulation in skeletal muscle tissues, but not by caveolin-1 status.

We appreciate Dr Boucher's valuable comments on our work and we hope this letter answers some of the questions raised.

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Fig. 3 mRNA expression levels of *Vdr* in skeletal muscle and adipose tissues of vitamin D-treated JYD mice. Thirty-week-old male JYD mice were injected daily with 12 nmol/kg body weight 1,25(OH)₂D₃ (VIT D) or ethanol in phosphate buffered saline (CON) i.p. for 4 weeks; tissue (ADI) and skeletal muscle from the hindlimb (MUS) were isolated. Total RNA was prepared using TRIzol reagents. **a** mRNA expression level of *Vdr* was determined by RT-PCR using a specific *Vdr* primer. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was the loading control. **b** The fold-change relative to the control band was quantitated by densitometric analysis. CON, black bar; VIT D, white bar. Each bar is the mean±SEM (*n*=4)

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

References

- Huang Y, Ishizuka T, Miura A et al (2002) Effect of 1 alpha, 25-dihydroxy vitamin D₃ and vitamin E on insulin-induced glucose uptake in rat adipocytes. *Diabetes Res Clin Pract* 55:175–183
- Borissova AM, Tankova T, Kirilov G, Dakovska L, Kovacheva R (2003) The effect of vitamin D₃ on insulin secretion and peripheral insulin sensitivity in type 2 diabetic patients. *Int J Clin Pract* 57:258–261
- Zittermann A (2003) Vitamin D in preventive medicine: are we ignoring the evidence? *Br J Nutr* 89:552–572
- Huhtakangas JA, Olivera CJ, Bishop JE, Zanello LP, Norman AW (2004) The vitamin D receptor is present in caveolae-enriched plasma membranes and binds 1 α, 25(OH)₂-vitamin D₃ in vivo and in vitro. *Mol Endocrinol* 18:2660–2671
- Boucher BJ (2009) Does vitamin D status contribute to caveolin-1-mediated insulin sensitivity in skeletal muscle? *Diabetologia* DOI: 10.1007/s00125-009-1478-5
- Oh YS, Khil LY, Cho KA et al (2008) A potential role for skeletal muscle caveolin-1 as an insulin sensitivity modulator in ageing-dependent non-obese type 2 diabetes: studies in a new mouse model. *Diabetologia* 51:1025–1034
- Nagpal J, Pande JN, Bhartia A (2009) A double-blind, randomized, placebo-controlled trial of the short-term effect of vitamin D₃ supplementation on insulin sensitivity in apparently healthy, middle-aged, centrally obese men. *Diabet Med* 26:19–27
- Zhou QG, Hou FF, Guo ZJ, Liang M, Wang GB, Zhang X (2008) 1,25-Dihydroxyvitamin D improved the free fatty-acid-induced insulin resistance in cultured C2C12 cells. *Diabetes Metab Res Rev* 24:459–464
- Naveh-Manly T, Marx R, Keshet E, Pike JW, Silver J (1990) Regulation of 1,25-dihydroxyvitamin D₃ receptor gene expression by 1,25-dihydroxyvitamin D₃ in the parathyroid in vivo. *J Clin Invest* 86:1968–1975
- Tang X, Meng H (2009) Osteogenic induction and 1,25-dihydroxyvitamin D₃ oppositely regulate the proliferation and expression of RANKL and the vitamin D receptor of human periodontal ligament cells. *Arch Oral Biol* 54:625–633
- Zineb R, Zhor B, Odile W, Marthe RR (1998) Distinct, tissue-specific regulation of vitamin D receptor in the intestine, kidney, and skin by dietary calcium and vitamin D. *Endocrinology* 139:1844–1852
- Ceglia L (2008) Vitamin D and skeletal muscle tissue and function. *Mol Aspects Med* 29:407–414