Effects of the anti-inflammatory drug salsalate on bone turnover in type 2 diabetes mellitus

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For the TINSAL-T2D Study Team

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Introduction

Adults with type 2 diabetes (T2D) have an increased risk of fractures [1]. This increased risk, despite normal bone mineral density (BMD) [2, 3], has led to investigation of deficits

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in diabetic skeletal properties. Skeletal dynamics are reduced in T2D [4, 5], possibly as a result of inflammation [6]. Inflammation plays an important role in T2D, as obesity activates the transcription-factor-nuclear-factor- κ B (NF- κ B), which increases the risk for T2D [7]. Inflammation also compromises skeletal remodeling by reducing bone formation and increasing bone resorption [8], possibly via activation of receptor- activator of nuclear-factor- κ -B ligand (RANKL) [9]. RANKL and NF- κ B may act as hormones, exerting effects at sites distant from where they are produced. We analyzed stored samples from the TINSAL-T2D trial [10], where it was found that salsalate, a prodrug of salicylate which reduces NF- κ B activity [11, 12], decreased HbA_{1c} levels [10]. We hypothesized that reducing inflammation in T2D would rebalance the bone remodeling process.

Methods

TINSAL-T2D was a RCT in 108 T2D patients who were randomized to placebo or salsalate (3.0, 3.5 or 4.0 g/day) for 14 weeks [10]. Serum calcium, albumin, PTH, and 25-hydroxyvitamin D were measured at baseline and bone turnover markers (BTM) including P1NP, bone specific alkaline phosphatase (BSAP), osteocalcin, serum C-telopeptide (s-CTx), and tartrate-resistant acid phosphatase-5b (TRAP-5b) were measured at 0, 4, and 14 weeks in 78 participants who did not differ from the overall cohort (data not shown). All provided written Informed Consent. Calciotropic indices, adiponectin, CRP, C-peptide, and insulin were measured as previously described [10, 13].

The primary endpoint was the change in osteocalcin. Between-group differences in the time course of osteocalcin change were tested with linear mixed models for repeated measures; fixed effects were entered for treatment dose, time, and time-by-dosage group interaction, and the baseline value of the dependent variable was entered as a continuous covariate. Differences in the response to salsalate on other indices were analyzed in similar models. Spearman correlation was used to correlate changes in glycemic indices, inflammatory cytokines, and changes in BTM.

Results

Salsalate ($n = 62$) and placebo ($n = 16$) groups did not differ with regard to age, gender, race, BMI, renal or thyroid function, glycemic indices (HbA_{1c}: 7.6 ± 0.2 vs 7.8 ± 0.2 %), adiponectin, C-reactive protein, calciotropic indices (data not shown), or BTM (Table 1). Insulin (12.2 ± 1 vs 20.2 ± 5 μ U/ml; $p < 0.05$) and c-peptide (1.0 ± 0.1 vs 1.3 ± 0.2 ng/ml; $p < 0.05$) levels were lower in the salsalate group; the linear mixed models analyses were adjusted for this. Glycemic and inflammatory changes were the same as in the parent-trial [10]. Osteocalcin levels increased by 10.2 % (placebo-corrected) at 14 weeks (Table 1; $p = 0.05$ between groups). When the salsalate doses were analyzed separately, a dose– response increase was seen in the two higher dosages (data not shown). Analysis by gender (Table 1), with the salsalate doses combined demonstrated an increase in osteocalcin in men at 4 ($p < 0.001$) and 14 weeks ($p < 0.001$) which persisted after adjustment for baseline HbA_{1c} and BMI, and after continuous adjustment for the time-dependent changes in HbA_{1c}. In women, the change in osteocalcin was not significant, although the analysis by gender did not reveal a difference.

Baseline PTH and 25(OH)D levels did not predict the BTM responses. Although mean CRP levels did not change, there was a trend toward an inverse relationship between the change in CRP and osteocalcin ($r = -0.26$, $p = 0.06$). Changes in fasting glucose, HbA_{1c}, and adiponectin did not correlate with changes in osteocalcin. There was no change in P1NP or BSAP in any of the groups.

Serum CTx levels increased at 14 weeks (Table 1; $p = 0.04$). When the three doses were analyzed separately, the increase in serum CTx levels occurred at the higher dosages (data not shown). Analysis by gender again showed an increase of serum CTx levels in men at 14 weeks which persisted after adjustment for baseline HbA_{1c}, while, the change in s-CTx was not significant in women. TRAP-5b levels did not change in any of the groups. There was an inverse association between the change in fasting glucose between 0 and 14 weeks in all salsalate-treated patients with the change in s-CTx ($r = -0.3$, $p = 0.03$). Changes in HbA_{1c} and adiponectin did not correlate with changes in s-CTx.

Discussion

In this small study of BTM response to salsalate treatment in T2D, we found a marginal increase in osteocalcin and a larger increase in s-CTx. Suggestions of a beneficial skeletal effect include the dose response of osteocalcin to salsalate, as well as the tendency toward an inverse relationship between osteocalcin and CRP. While the osteocalcin change might have been due to decreases in the placebo group, these declines were not significant. Furthermore, stabilization from further loss could also be of clinical benefit. Although osteocalcin levels at baseline were lower in both groups because of the known effects of obesity and glucose to lower osteocalcin [14, 15], the increase in osteocalcin with salsalate persisted even after adjusting for baseline HbA_{1c} and BMI.

The relatively greater increase in s-CTx as compared to osteocalcin could suggest that salsalate is not beneficial for bone. An alternative explanation for the increase in s-CTx may be a coupled increase in bone remodeling as inflammation is reduced. That osteocalcin and s-CTx were elevated at 14 weeks, the longest point of salsalate exposure, suggests that the effects on BTM may increase with duration of exposure. Finally, the lack of change in serum TRAP might be more reflective of osteoclast number and not activity.

It is possible that the observed changes in BTM are attributable to alterations in glucose. However, the increases persisted even after adjusting for changes in HbA_{1c}. Moreover, we observed increases only at the two higher dosages, while reductions in glycemia occurred at all dosages, suggesting that the effects of salsalate on bone remodeling are less pronounced than those on glycemia. Whether blockage of RANKL clinically effects glycemia is unknown. RANKL predicted incident T2D and blockage of RANKL improved glucose tolerance in mouse T2D models [16]. In contrast, denosumab did not affect glycemia in postmenopausal diabetic and pre-diabetic osteoporotic women, although it improved in those without antidiabetic medication [17].

We were likely underpowered to see previously reported differences in turnover markers by gender [18]. Moreover, with the exception of osteocalcin, baseline BTM were higher in the

women. Although menopausal status and HRT treatment were unknown, given the women's age, it is likely that many were in the early postmenopausal period of rapid bone loss, which may have masked any small change due to salsalate.

Limitations of the study include the secondary analysis design along with the small sample size from a larger study not designed to investigate bone remodeling. The observed changes in BTM were small, raising questions about their clinical significance, particularly given the high coefficients of variation in the measurement of BTM. Given that the least significant detectable difference for osteocalcin is 5.48 ng/ml, a future study would need to be powered with a larger sample size (56 placebo and 222 salsalate patients). Our short trial duration might have also limited detection of BTM changes and precluded assessment of BMD changes. While these limitations make it premature to assess a potential influence of salsalate on bone turnover, this proof-of-concept study provides interesting direction for investigation into the relationship between the inflammatory process and skeletal dynamics in T2D.

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Table 1

Biochemical markers of bone turnover $\pm$ SEM

Week	Baseline non-logarithm value	All				Men				Women			
		0	4	14		0	4	14		0	4	14	
Log PINP	Placebo	1.55 $\pm$ 0.02	1.51 $\pm$ 0.02	1.51 $\pm$ 0.02		1.48 $\pm$ 0.02	1.45 $\pm$ 0.02	1.44 $\pm$ 0.02		1.55 $\pm$ 0.04	1.52 $\pm$ 0.04	1.53 $\pm$ 0.05	
	Salsalate	1.53 $\pm$ 0.01	1.49 $\pm$ 0.01	1.51 $\pm$ 0.01		1.49 $\pm$ 0.01	1.44 $\pm$ 0.01	1.46 $\pm$ 0.01		1.62 $\pm$ 0.02	1.57 $\pm$ 0.02	1.58 $\pm$ 0.02	
Log BSAP	Placebo	1.43 $\pm$ 0.02	1.41 $\pm$ 0.02	1.42 $\pm$ 0.02		1.40 $\pm$ 0.02	1.39 $\pm$ 0.02	1.38 $\pm$ 0.02		1.48 $\pm$ 0.03	1.47 $\pm$ 0.03	1.51 $\pm$ 0.03	
	Salsalate	1.43 $\pm$ 0.01	1.39 $\pm$ 0.01	1.40 $\pm$ 0.01		1.41 $\pm$ 0.01	1.35 $\pm$ 0.01	1.37 $\pm$ 0.01		1.48 $\pm$ 0.02	1.45 $\pm$ 0.02	1.47 $\pm$ 0.02	
Osteocalcin (ng/ml)	Placebo	10.8 $\pm$ 0.4	10.7 $\pm$ 0.4	10.1 $\pm$ 0.4		10.26 $\pm$ 0.5	9.32 $\pm$ 0.5	9.27 $\pm$ 0.5		11.77 $\pm$ 0.9	13.27 $\pm$ 0.9	11.90 $\pm$ 1.0	
	Salsalate	10.7 $\pm$ 0.2	11.0 $\pm$ 0.2	11.1 $\pm$ 0.2 ^a		10.00 $\pm$ 0.2	10.59 $\pm$ 0.2 ^b	10.48 $\pm$ 0.2 ^b		12.13 $\pm$ 0.5	11.73 $\pm$ 0.5	12.34 $\pm$ 0.5	
s-CTX (pg/ml)	Placebo	252 $\pm$ 36	276 $\pm$ 40	257 $\pm$ 37		219 $\pm$ 49	245 $\pm$ 52	219 $\pm$ 49		302 $\pm$ 51	309 $\pm$ 51	339 $\pm$ 56	
	Salsalate	248 $\pm$ 19	299 $\pm$ 19	326 $\pm$ 19 ^c		229 $\pm$ 25	282 $\pm$ 25	309 $\pm$ 25 ^d		295 $\pm$ 29	347 $\pm$ 36	380 $\pm$ 31	
Log TRAP-5b	Placebo	0.49 $\pm$ 0.01	0.49 $\pm$ 0.01	0.51 $\pm$ 0.01		0.47 $\pm$ 0.02	0.46 $\pm$ 0.02	0.48 $\pm$ 0.02		0.54 $\pm$ 0.03	0.54 $\pm$ 0.03	0.55 $\pm$ 0.03	
	Salsalate	0.49 $\pm$ 0.01	0.50 $\pm$ 0.01	0.49 $\pm$ 0.01		0.47 $\pm$ 0.01	0.48 $\pm$ 0.01	0.48 $\pm$ 0.01		0.53 $\pm$ 0.01	0.54 $\pm$ 0.01	0.55 $\pm$ 0.02	

^a $p = 0.05$ salsalate versus placebo^b $p = 0.001$ salsalate versus placebo^c $p = 0.04$ salsalate versus placebo^d $p = 0.01$ salsalate versus placebo