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# Blockade of receptor activator of nuclear factor-κB (RANKL) signaling improves hepatic insulin resistance and prevents development of diabetes mellitus

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**Hepatic insulin resistance is a driving force in the pathogenesis of type 2 diabetes mellitus (T2DM) and is tightly coupled with excessive storage of fat and the ensuing inflammation within the liver[1–3]. There is compelling evidence that activation of the transcription factor nuclear factor-κB (NF-κB) and downstream inflammatory signaling pathways systemically and in the liver are key events in the etiology of hepatic insulin resistance and β-cell dysfunction, although the molecular mechanisms involved are incompletely understood[3–6]. We here test the hypothesis that receptor activator of NF-κB ligand (RANKL), a prototypic activator of NF-κB, contributes to this process using both an epidemiological and experimental approach. In the prospective population-based Bruneck Study, a high serum concentration of soluble RANKL emerged as a significant ($P < 0.001$) and independent risk predictor of T2DM manifestation. In close agreement, systemic or hepatic blockage of RANKL signaling in genetic and nutritional mouse models of T2DM resulted in a marked improvement of hepatic insulin sensitivity and amelioration or even normalization of plasma glucose concentrations and glucose tolerance. Overall, this study provides evidence for a role of RANKL signaling in the pathogenesis of T2DM. If so, translation to the clinic may be feasible given current pharmacological strategies to lower RANKL activity to treat osteoporosis.**

RANKL (also known as TNFSF11) is a member of the tumor necrosis factor superfamily and, after ligation with its cognate receptor RANK (also known as TNFRSF11a), is a potent stimulator of NF-κB. Notably, both RANKL and RANK are expressed in human liver tissue and pancreatic β-cells[7], and concentrations of the soluble decoy receptor osteoprotegerin (OPG), considered to be a reliable surrogate for the overall activity of this cytokine network, are elevated in patients with T2DM, especially in those with poor glycemic control and complicated disease course[8–11]. RANKL exists in both membrane-bound and biologically active soluble forms, with the latter originating from secretion and cleavage[9,12]. Concentrations of soluble RANKL are elevated in or predictive of various human diseases, including cardiovascular disease, nontraumatic fractures, multiple myeloma, rheumatoid arthritis and inflammatory bowel disease[8–11,13–18]. We determined the distribution of serum concentrations of RANKL and OPG in our study population ($n = 844$) (**Supplementary Fig. 1a**). Soluble RANKL concentrations showed associations with insulin resistance assessed by homeostasis model (HOMA-IR) and Gutt Index values and with the number of metabolic syndrome components clustering in an individual (**Supplementary Data**) but were not related to most standard population characteristics (**Supplementary Table 1**).

Between 1990 and 2005, 78 of the 844 individuals in the study population (9.2%) developed T2DM (incidence rate, 7.2 per 1,000 person years (95% confidence interval (CI) 5.7–8.9)). We determined the baseline characteristics of subjects with and without incident T2DM (**Supplementary Table 2**) and found that the concentrations of soluble RANKL differed considerably between the two groups. In a pooled logistic regression analysis adjusted for age, sex and period

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