INFUSION OF INSULIN IN DAIRY COWS INHIBITS NEUTROPHILS PHAGOCYTOSIS OF *STAPHYLOCCOCUS AUREUS* EX VIVO

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Introduction: Dairy cows undergo vast changes in metabolism and energy status during late gestation and early lactation. Likewise, the level of insulin decreases significantly around calving and stays low during the early lactation, after which it slowly increases to the levels seen before gestation (Ingvartsen and Andersen, 2000). Humans suffering from insulin-dependent diabetes often have impaired neutrophil functions (Marhoffer al., 1993; Delamaire et al., 1997). However, infusion of insulin can improve the neutrophils' capacity to phagocytise bacteria ex vivo (Rassias et al., 1999). The objectives of this study were to investigate the effect of high levels of insulin on bovine neutrophils on their 1) number, 2) capacity to phagocytise bacteria and exhibit oxidative burst ex vivo and, 3) expression of adhesion molecules (CD11aCD18, CD11b, CD18, CD62L), insulin growth factor-I receptor (IGF-IR) and insulin receptor (IR) in early and mid lactation.

Methods: A 96-hour hyperinsulinemic-euglycemic clamp was performed in the same 4(3) cows in early (5 weeks) and mid-lactation (17 weeks). Blood samples were taken 2 × before and 2 × during the clamp. The total number of neutrophils in blood was measured by a hemocytometer. Additionally, heat-killed *Staphyloccocus aureus* stained with propidium-iodide (PI) and dihydrorhodamine 123 (DHR) were incubated with whole blood to measure the capacity of neutrophils for phagocytosis and oxidative burst, simultaneously by flowcytometry. Cytochalasin B was added in parallel tubes to block phagocytosis. Finally, whole blood lysis was performed and immunofluorescence stainings made to quantify adhesion molecules, IGF-IR and IR on neutrophils by flowcytometry.

Results: The number of neutrophils was the same in early and mid-lactation (2.84×10^6 versus 2.87 x 106/ml of blood) and did not change during the clamp (2.94×10^6 versus 2.82×10^6). No differences were observed in phagocytosis/oxidative burst in early and mid-lactation. However, the number of bacteria phagocytised by the neutrophils during the infusion of insulin was significantly reduced (mean fluorescence 54.62 vs 65.37, $P<0.01$), whereas the oxidative burst did not change. The levels of CD11aCD18, CD18, CD62L, IGF-IR, and IR on neutrophils were not influenced by lactation stage or clamp status. CD11b increased slightly during the early clamp, but was not investigated in the late clamp.

Conclusion: High levels of insulin do not affect the number of bovine neutrophils in blood or the cells' performance of oxidative burst, but might inhibit the capacity of the neutrophils to phagocytise *S. aureus.* As CD11aCD18, CD11b, CD18, CD62L, IGF-IR, and IR were not reduced during the clamp, none of these seem to be involved in the decreased function of neutrophils seen during the infusion of insulin.

Delamaire et al., 1997, Diabetes Med., 14:29-34; Ingvartsen and Andersen, 2000, J. Dairy Sci, 83: 1573-1597; Marhoffer et al., 1993, Diabetes Res. Clin. Pract., 19:183-188; Rassias et al., 1999, Anesth. Analg., 88:1011-1016; Serlenga et al., 1993, Cytobios, 74:189-195.