Sex Differences in Limb Ischemia Recovery Following Conditional Endothelial Overexpression of Cept1

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Objectives: Choline enthanolamine phosphotransferase 1 (CEPT1), is essential for endothelial de novo lipogenesis (DNL) and is elevated in disease peripheral arterial segments. Women are more sensitive to diet-induced DNL, and those with diabetes have higher incidence of severe peripheral arterial disease. We therefore hypothesized that women may have higher CEPT1 content in diseased peripheral arteries and that murine conditional endothelial overexpression of CEPT1 may lead to more favorable outcomes.

Methods: CEPT1 expression was evaluated in diseased peripheral plaque of seven women and nine men. A murine endothelial CEPT1 overexpression model was engineered with Cre-induced expression of a CEPT1 transgene inserted in a C57Bl/6J background. Endothelial CEPT1 overexpression was evaluated using reverse transcriptase polymerase chain reaction. Male (n = 5) and female (n = 5) received streptozotocin and unilateral femoral artery ligation. Limb perfusion, appearance, and use were then evaluated. Gastrocnemius was stained with hematoxylin and eosin, and endothelial isolectin. Muscle fiber size and microvascular density were analyzed using the Student t test.

Results: Compared with males, female patients have higher CEPT1 in peripheral segments (P < .05; Figure, A). Murine endothelial CEPT1 overexpression had a seven-fold increase in expression compared with control (Figure, B). Compared with female mice, males demonstrated improved perfusion (P < .005; Figure, C, D) limb function (P < .005; Figure, E, F, G), but both has adequate muscle microvascular density (Figure, H).

Conclusions: This study reveals a gender difference in peripheral arterial CEPT1 expression and ischemic recovery following overexpression. Differences do not seem to be directly associated with angiogenesis and may be due to underlying DNL metabolic consequences. These findings may have important implications for the prevalence of severe peripheral arterial disease in diabetic women.

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Sex Hormones Mediate Sex Differences in Hemodynamics and Inflammation during Arteriovenous Fistula Maturation

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Background: Arteriovenous fistulae (AVF) fail to mature, that is dilate and thicken, in women more than in men, leading to inferior outcomes and decreased utilization in women. As our mouse AVF model

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recapitulates human AVF maturation, including decreased maturation and patency in female mice, we hypothesize that sex hormones mediate sex differences during AVF maturation.

Methods: C57Bl/6 mice (aged 9-11 months) were treated with sham or aortocaval AVF surgery; some were pretreated with gonadectomy 1 week before surgery. AVF diameter was measured using ultrasound examination (days 0-21). Blood was collected for fluorescent activated cell sorting and tissue examined using immunofluorescence or enzyme-linked immunosorbent assay (days 2-7; wall thickness was assessed using histology (day 21).

Results: At baseline, female and male mice had similar inferior vena cava (IVC) diameters (P = 0.61) and wall thicknesses (P = 0.491). After AVF creation, female mice had an increased normalized IVC diameter (P = 0.0012), flow velocity (P = 0.003), and shear stress (P = 0.002, days 3-21; n = 10); however, there was no difference in wall thickness (female: 15.8 ± 0.2 μm; male: 12.4 ± 2.3 μm; P = 0.008; n = 10). There were also increased circulating CD11b+ macrophages in female compared with male mice (31.8% ± 4.0% vs 18.7% ± 1.9%; P = 0.01; day 3; n = 22) and CD4+ T cells (57.9% ± 2.9% vs 34.5% ± 6.1%; P = 0.001). In the AVF wall there were increased CD45+ cells on immunofluorescence in females (22.5 ± 15 cells/high-power field vs 15 ± 2 cells/high-power field; P = 0.03; day 3; n = 4) as well as increased IL-10 immunoreactivity (3.96 ± 0.44 ng/μg vs 2.49 ± 0.47 ng/μg; P = 0.076). In mice with gonadectomy, baseline IVC diameter, velocity, and shear stress were similar between female and male mice. After gonadectomy, male mice had increased AVF wall thickness than intact males (24.2 ± 1.6 μm vs 12.4 ± 2.3 μm; P = 0.014). Conversely, female mice had decreased thickness than intact females (6.1 ± 0.6 μm vs 15.8 ± 0.2 μm; P = 0.005). There was a loss of differences among circulating immune cells after gonadectomy (CD11b+; P = 0.082; CD4+; P = 0.705).

Conclusions: Sex differences in hemodynamics and inflammation are present during venous remodeling, whereas these differences disappear after gonadectomy. Sex hormones mediate AVF maturation and suggest that hormone receptor signaling may be a target to improve AVF maturation.


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MOF Expression Regulates Interferon β in Diabetic Wound Macrophages and Impairs Tissue Repair

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Objective: Following tissue injury, monocytes are recruited to the site of injury and differentiate into proinflammatory macrophages (Mφs). As wound healing progresses, these Mφs transition to an anti-inflammatory phenotype and promote tissue repair. Conversely, in type 2 diabetic (T2D) wounds the transition of Mφs to an anti-inflammatory phenotype does not occur. This causes Mφs to remain in a chronic inflammatory state, effectively preventing wound resolution. The molecular mechanisms controlling Mφ plasticity are not fully understood. Our prior work has focused on epigenetic-based mechanisms that lead to persistent expression of inflammatory genes. Specifically, we have identified that the histone acetyltransferase MOF is involved in regulating wound Mφ phenotype.

Methods: Using mice that are deficient in MOF in their monocytes/macrophages (MOFflLyz2cre), we identified that tumor necrosis factor α receptor signaling induces MOF transcription in wound macrophages and that MOF is increased in T2D wound Mφs. We also found that MOF acetylates interferon regulatory factor 3 (IRF3) in Mφs, resulting in repression of downstream genes, including interferon β (IFNβ).

Results: This is important; we have previously shown that IFNβ expression is decreased in T2D wounds and that IFNβ is critical for the downregulation of inflammatory genes in Mφs during the transition from a proinflammatory to an anti-inflammatory phenotype.

Conclusions: Taken together, these data suggest that tumor necrosis factor α-induced MOF expression in wound macrophages regulates IFNβ via IRF3.