Does the Epigenome Hold Clues to Leptin-associated Hypertension in Obesity?

Obesity is a disease caused by many factors, including genetic, environmental, and behavioral, and is an expanding health crisis both in developed and developing countries. Obesity also accounts for most essential hypertension cases, and the role of leptin and the sympathetic nervous system in obesity-associated hypertension is particularly intriguing (1). Leptin is a neuroendocrine hormone (product of the *ob* gene) secreted mainly by adipose tissue and functions as an essential regulator of metabolism and fat accumulation (2). Animals with mutations in the *ob* gene are obese and lose weight upon leptin administration (3). Leptin levels are increased in patients who are obese and are directly proportional to the percentage of fat mass in the body (4). Leptin acts primarily on the brain, but the role of leptin in peripheral tissues is increasingly appreciated. Leptin mediates a metabolic response by acting as an afferent signal to the brain to regulate the body's energy stores. In addition, it may also affect blood pressure and contribute to the occurrence of hypertension in obesity through sympathetic activation in the circulatory system or at the renal level (1). Leptin acts through its receptor to activate the downstream signaling pathways. There are multiple leptin receptor isoforms produced because of alternative splicing, of which the long isoform (LepRb) is the most well studied (5). LepRb belongs to the IL-6 receptor family of cytokine receptors, which signal via JAK2 (6).

Interestingly, in patients who are obese, the high circulating leptin levels may not have the beneficial central metabolic responses that mediate weight loss but may instead have harmful effects on arterial pressure and renal sympathetic nerve activity. This phenomenon, termed selective leptin resistance, contributes to obesity-associated hypertension (7, 8). Accordingly, it is vital to identify the mechanisms by which leptin contributes to obesity-associated hypertension to help guide optimal therapies in this patient population.

In this issue of the *Journal*, Yeung and colleagues (pp. 214–221) build on their previous work to delineate the molecular mechanisms by which leptin regulates *Trpm7* (transient receptor potential melastatin 7) expression in the carotid body (CB) glomus cells (9). The central hypothesis of this project was that leptin epigenetically regulates *Trpm7*. The CB functions as a chemosensory organ and is located at the bifurcation of the carotid artery, where it plays a significant role in the response to hypoxia. The glomus cells within the CB are the chemoreceptors that detect hypoxia, hypercapnia, or acidosis. These cells then transmit to the central medullary centers through the carotid sinus nerve to activate the sympathetic nervous system (10). This study used the undifferentiated rat pheochromocytoma (PC12) cells stably expressing LEPRb ($PC12^{LEPRb}$) as an *in vitro* model system for the CB glomus cells. PC12 cells have similar acute responses to hypoxia as glomus cells and express JAK2 and *Trpm7*.

The authors have previously reported that leptin increased the carotid sinus nerve response to hypoxia, and this was reversed by nonselective blockers of Trp (transient receptor potential) channels (11). They subsequently showed that among the Trp channels, *Trpm7* are highly expressed in CB and colocalized with LepRb in CB glomus cells. TRPM7 belongs to the TRP ion channel family; it conducts Ca^{2+} and Mg^{2+}. It is unique among ion channels, as it also contains a functional kinase domain (12). TRPM7 function across the vesicle membrane is essential in small synaptic-like vesicle fusion and is critical for sympathetic neurotransmitter release (13). They also showed that *Trpm7* mRNA was decreased and noted *Trpm7* promoter hypermethylation in CB in mice deficient in LepRb, and leptin-elicited cation current in CB glomus cells and leptin-induced hypertension was ameliorated by *Trpm7* blockers or silencing (10). Logically, the current study builds on this by identifying the precise signaling pathway by which leptin activates *Trpm7* channels.

The authors first document that $PC12^{LEPRb}$ cells transfected with the *Trpm7* reporter show increased promoter activity both at baseline and in response to leptin. Next, they highlight the epigenetic regulation of *Trpm7* by leptin. They show that leptin exposure caused demethylation of *Trpm7* promoter at specific CpG sites putative for pSTAT3, p50, and p65 binding. Next, they show evidence for the JAK2/STAT3 signaling pathway in mediating the leptin-induced change in *Trpm7* promoter demethylation using the specific JAK2/STAT3 inhibitor (SD1008). Upon exposure to SD1008, there was a significant reduction in the *Trpm7* mRNA level, promoter activity, and promoter demethylation. They then proceed to specify the *Trpm7* promoter regions showing increased binding of pSTAT3 and PoIIIA upon exposure to leptin. Recognizing that histone modifications can act together with changes in DNA methylation to modulate gene expression, they subsequently identify the histone modifications that also play a role in leptin-mediated epigenetic changes leading to increased *Trpm7* expression. Specifically, leptin induced trimethylation of H3K4 (H3K4M3) and acetylation of H3K27 (H3K27Ac) and decreased trimethylation of H3K27 (H3K27M3). These changes were also reversed by the JAK2/STAT3 inhibitor (SD1008). In the final set of studies, using chromatin immunoprecipitation–qPCR, the authors specify the epigenetic modifications at the three previously identified differentially demethylated clusters of *Trpm7* promoter regions by leptin leading to increased binding of pSTAT3 and/or PoIIIA.

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Collectively, Yeung and colleagues provide compelling data to show that leptin activation of *Trpm7* channels in the $PC12^{LEPRb}$ cells (as a model for CB glomus cells) is mediated through epigenetic modifications through promoter demethylation and posttranslational histone modifications and is mediated by the LEPRb-dependent STAT3 activation. Some limitations of this study need to be considered. This was an *in vitro* study using the modified PC12 cell line as a surrogate for the CB glomus cells, but the reasons to do so are well justified. There are demonstrable differences in leptin biology between animals and humans. In contrast to data from animal studies, leptin treatment does not affect sympathetic nervous system activity, heart rate, or blood pressure (14). The study also identified putative binding sites for p50 and p65 at the CpG sites for *Trpm7*; therefore, other transcription factors like NF-κB may also modulate leptin-mediated *Trpm7* expression.

The main finding of the article, that leptin-mediated increase in the *Trpm7* expression is mediated through epigenetic mechanisms (DNA methylation and histone modifications) and through activation of the pSTAT3-JAK2 signaling pathway, is new and may have broad implications. Finally, the findings of Yeung and colleagues suggest that epigenetic modifications of *Trpm7* may represent a potential therapeutic target in obesity-associated hypertension associated with high leptin levels. ■

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