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Physiological role of CD206 macrophages and NAD+ metabolism in obesity and insulin resistance.

Bilal, Muhammad

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M2   Nampt
                                        M2

2   .

                        NAD+   Nampt
NAD+   2

My data showed that transgenic lacking Nampt in M2 macrophages were insulin resistant, with blood glucose levels. Then, I utilized another transgenic mouse lacking M2 receptors that were insulin-sensitive with reduced expression of the inflammatory genes in visceral white adipose tissue. This data further suggests that M2 macrophages are actively involved in regulating glucose metabolism through phenotypic switching. My focus is to investigate the underlying molecular mechanism that regulates these shifting/switching of macrophages, resulting in insulin resistance under high-fat diet-fed conditions. Furthermore, I am generating other M2-related transgenic mice to knock down the M2 signaling conditionally. This will further help me to explore the underlying regulatory mechanism for the treatment of obesity and type 2 diabetes.

Obesity induced type 2 diabetes

adipose tissue M2 macrophages insulin resistant

Obesity is characterized by chronic low-grade inflammation, oxidative stress, increased reactive oxygen species (ROS), and DNA damage, impairing various physiological functions. Macrophages play an integral role in the development of obesity-induced insulin resistance. Obesity induces polarization of macrophages from anti-inflammatory M2 to pro-inflammatory M1 macrophages, thereby causing insulin resistance. Immunometabolism of macrophages also contributes to the development of insulin resistance. Activation of M1 macrophages uses the glycolytic pathway, reduces oxidative phosphorylation, and produces ROS, whereas enhanced fatty acid (FA) utilization and increased mitochondrial respiration with an intact tricarboxylic acid (TCA) cycle were observed in M2 macrophages. M2 macrophages require TCA, whereas M1 macrophages require glycolysis to fulfill their energy demands. Nicotinamide adenine dinucleotide (NAD+) is reported to play an important role in the immunometabolism of macrophages. It is reported that cell-autonomous generated NAD+ regulated immune function during aging and inflammation. In mammalian cells, NAD+ is predominantly synthesized through the salvage pathway, requiring nicotinamide phosphoribosyltransferase (Nampt) (a rate-limiting enzyme). Macrophage-specific de novo NAD+ synthesis restored oxidative phosphorylation and homeostatic immune responses during aging and inflammation. It has been reported that Nampt plays an important role in the polarization of immune cells, i.e., M1 and M2 macrophages.

The purpose of this was to investigate the role of NAD+ in the regulation of immunometabolism of M2 macrophages during the development of diet-induced obesity. My preliminary data showed that Nampt is highly expressed in bone marrow-derived M2 macrophages. However, the effect of Nampt-dependent NAD+ metabolism in M2-like tissue-resident macrophages on adipocyte progenitors has not been elucidated. Further, the effect of the gain- or loss-of-function of Nampt in M2-like macrophages on the fate of adipocyte progenitors (APs) during the progression of obesity is also unclear.

To investigate the physiological role of NAD+ in the regulation of immunometabolism of M2 macrophages during the development of diet-induced obesity, we generated a transgenic mouse model in which Nampt was conditional knock-out from M2 macrophages by targeting CD206+ M2 macrophages. Then I investigate the weight gain and tissue weight in response to high-fat diet (HFD) for 8 weeks. Then, I performed a glucose tolerance test (GTT), insulin tolerance test (ITT), gene expression analysis, etc.
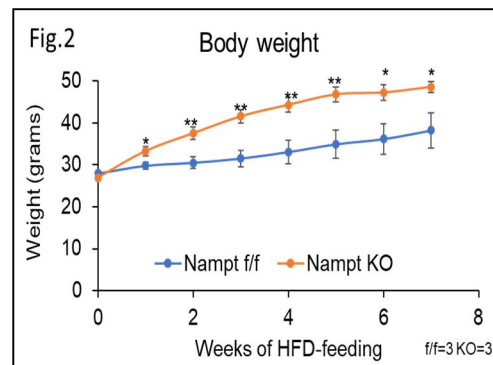
After the successful generation of genetically engineered M2- macrophage-specific Nampt knock-out (KO) transgenic mice, I found that tamoxifen (TAM) administration did not affect the body weight of the chow-fed mice. After the administration of TAM, I performed GTT and ITT. My data showed that there is no significant difference between CD206-specific Nampt-KO mice and control mice (fig.1).

Fig.1

NC fed ipGTT — Nampt ff, Nampt KO (f/f=3 KO=3)
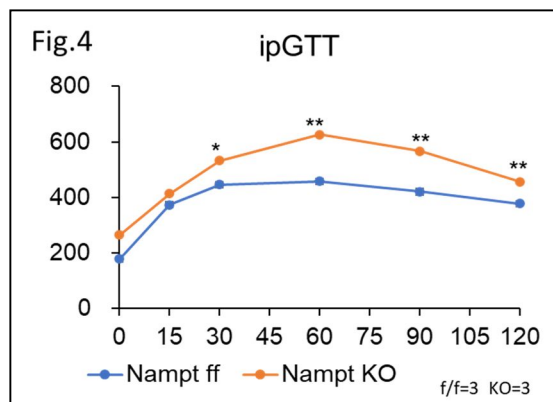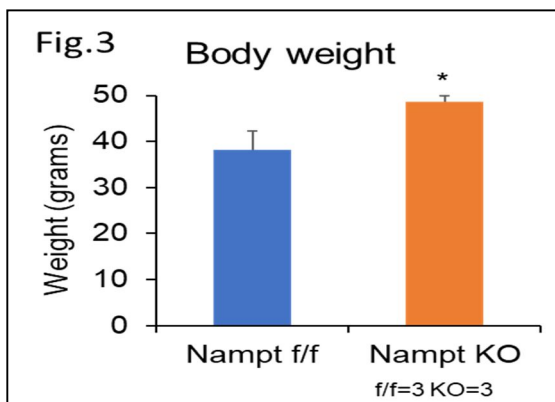
NC fed iTT — Nampt f/f, Nampt KO (f/f=3 KO=3)
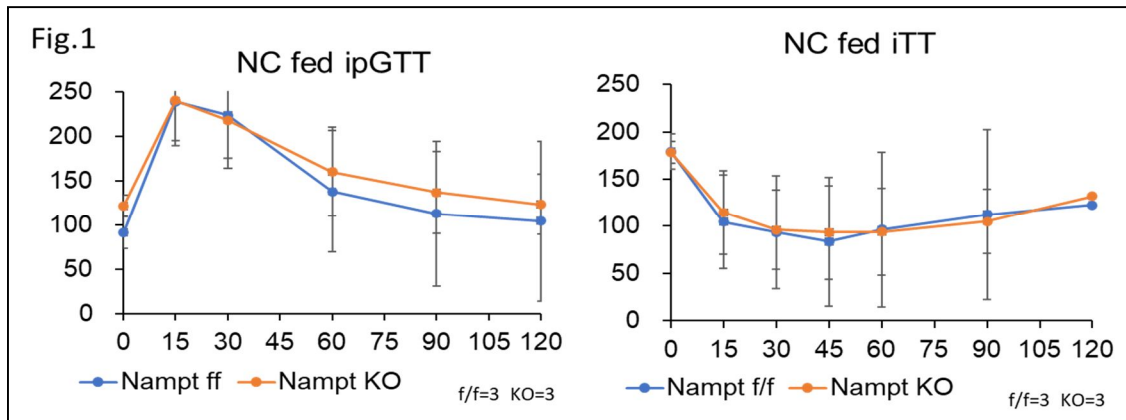
Then these mice were put on HFD. In consistent with my hypothesis, Nampt-KO mice gain more body weight compared to the control mice (fig.2). This data strongly suggested that CD206+ macrophages regulated diet-induced obesity or weight gain via Nampt-derived NAD+. However, the underlying molecular mechanism is unknown.

After 8 weeks of HFD-feeding, I found that Nampt-KO gain more body weight compared to their control mice (fig.3). I have performed ipGTT. My data showed that Nampt-KO mice worsen glucose metabolism compared to their control of floxed mice (Fig 4). My data showed that metabolism in CD206+ macrophages derived Nampt regulates the glucose metabolism in obese mice. However, how CD206+ macrophages phenotypically behave in Nampt-KO mice is to be under investigation.

Fig.2 Body weight — Nampt f/f, Nampt KO (f/f=3 KO=3)

Fig.3 Body weight — Nampt f/f, Nampt KO (f/f=3 KO=3)

Fig.4 ipGTT — Nampt ff, Nampt KO (f/f=3 KO=3)

Next, I will perform iTT of these mice and will harvest the tissue after sacrificing the mice. I will perform immunohistochemistry, RNA extraction, western blotting, flow cytometry, and the qPCR of FACS-sorted CD206+ macrophages to elucidate the underlying mechanism. I will also perform single-cell RNA sequencing.

Up till now, the data suggests that by targeting CD206+ derived Nampt in the adipose tissue, adipose tissue metabolism can be improved. This novel mechanism will open a novel strategy for the treatment of obesity-related type 2 diabetes. This data suggests that obesity-related decline in NAD+ and Nampt deficiency deteriorate systemic glucose tolerance and insulin sensitivity, while NAD+ boosting therapy restores this metabolic disturbance. Next, I am to utilize another transgenic mouse that lack the Mrc1 receptor, a receptor expressed in most M2 macrophages. This will help to elucidate underlying mechanisms in more detail.

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