Inflammation is necessary for long-term but not short-term high-fat diet-induced insulin resistance

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Abstract: OBJECTIVE Tissue inflammation is a key factor underlying insulin resistance in established obesity. Several models of immuno-compromised mice are protected from obesity-induced insulin resistance. However, it is unanswered whether inflammation triggers systemic insulin resistance or vice versa in obesity. The purpose of this study was to assess these questions. RESEARCH DESIGN AND METHODS We fed a high-fat diet (HFD) to wild-type mice and three different immuno-compromised mouse models (lymphocyte-deficient Rag1 knockout, macrophage-depleted, and hematopoietic cell-specific Jun NH(2)-terminal kinase-deficient mice) and measured the time course of changes in macrophage content, inflammatory markers, and lipid accumulation in adipose tissue, liver, and skeletal muscle along with systemic insulin sensitivity. RESULTS In wild-type mice, body weight and adipose tissue mass, as well as insulin resistance, were clearly increased by 3 days of HFD. Concurrently, in the short-term HFD period inflammation was selectively elevated in adipose tissue. Interestingly, however, all three immuno-compromised mouse models were not protected from insulin resistance induced by the short-term HFD. On the other hand, lipid content was markedly increased in liver and skeletal muscle at day 3 of HFD. CONCLUSIONS These data suggest that the initial stage of HFD-induced insulin resistance is independent of inflammation, whereas the more chronic state of insulin resistance in established obesity is largely mediated by macrophage-induced proinflammatory actions. The early-onset insulin resistance during HFD feeding is more likely related to acute tissue lipid overload.

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Supplementary Figure 1. The 3 metabolic tissues, adipose tissue, liver and muscle rapidly respond to the metabolic changes by HFD. A: mRNA levels of genes involved in regulation of energy metabolism were measured by Q-PCR. B: PPARγ and LXRs are activated by short-term HFD in liver and adipose tissue. Expressions of PPARγ, LXRα and their target genes were measured in liver and epididymal adipose tissue (WAT) from NCD- or short term HFD-treated mice by Q-PCR analysis. D0, day 0; D1, day 1; D3, day 3; D7, day 7.
Supplementary Figure 2. Blood glucose level and hepatic glucose production after clamp. n = 6 or 8. A: Blood glucose level after clamp. B: Hepatic glucose production after clamp. *P < 0.05; **P < 0.01. C-E: HFD rapidly changes circulating adipocytokine concentration. C: Plasma adiponectin concentration was decreased in 3 days of HFD. D: Plasma level of IL-6 in NCD- or HFD-treated mice. E: Plasma level of TNF-α in NCD- or HFD-treated mice. Plasma levels of adiponectin, IL-6 and TNF-α were measured using ELISA. *P < 0.05; **P < 0.01; ***P<0.001.
Supplementary Figure 3. Inflammatory gene expression is induced selectively in adipose tissue after short term HFD. A: Microarray analysis of genes increased by short-term HFD in WAT. B: Inflammatory gene expression in adipose tissue, liver, skeletal muscle, and spleen after short- and long-term HFD. mRNA levels of pro-inflammatory genes were measured using Q-PCR. C: Q-PCR analysis of gene expression changes by short-term HFD in adipocytes and SVCs. mRNA expression of inflammatory genes in adipocytes and SVCs from adipose tissue of NCD or HFD mice were normalized by that in adipocytes of NCD mice.
Supplementary Figure 4. Intraperitoneal injection of clodronate depletes Kupffer cells in liver. Mice were given clodronate injection (intraperitoneal; 100 mg/kg) 3 days before HFD (1 week), which was followed by 2nd and 3rd injections every 3 days. After 10 days from the 1st injection, Kupffer cell depletion was assessed by immunohistochemistry using anti-F4/80 antibody. Dark brown signals represent F4/80 positive Kupffer cells as indicated by arrows. Veh, vehicle; Cld, clodronate.
Supplementary Figure 5. Kupffer cell inhibition using gadolinium does not affect short-term HFD-dependent insulin resistance. Mice were treated with NCD or 60% HFD (1 week) in the presence or absence of gadolinium injection. Mice were given gadolinium injection (tail vein; 10 mg/kg) at day 0, 2 and 5 of HFD, and analyzed at day 7. A: Epididymal adipose tissue mass. Veh, vehicle; Gad, gadolinium. B: Immunohistochemistry analysis of liver using anti-F4/80 antibody. Dark brown signals represent F4/80 positive Kupffer cells as indicated by arrows. C: Oral glucose tolerance test. #, \( P < 0.05 \) NCD vs. HFD in gadolinium-treated mice; **, \( P < 0.01 \) NCD vs. HFD in vehicle-treated mice.
Supplementary Figure 6. Depletion of macrophages using clodronate improves glucose tolerance in severely obese mice. C57BL/6J mice treated with HFD for 14 weeks were given clodronate for 14 days (5 injection every 3 days), and then subjected to FACS analyses for adipose tissue CD11b positive macrophages using anti-CD11b, anti-F4/80 and anti-CD11c antibodies (A) after glucose tolerance tests (B).