Intracellular ATP in balance of pro- and anti-inflammatory cytokines in adipose tissue with and without tissue expansion

Jong Han Lee¹, Yan Zhang², Zhiyun Zhao¹, Xin Ye¹, Xiaoying Zhang³, Hui Wang⁴, and Jianping Ye¹

¹Antioxidant and Gene Regulation Laboratory, Pennington Biomedical Research Center, Louisiana State University System, Baton Rouge, LA 70808, USA
²Third hospital, Beijing University, Beijing 100191, People’s Republic of China
³Shanghai Sixth People’s Hospital East, Shnaghai, People’s Republic of China
⁴Collaborative Innovation Center of Molecular Diagnosis and Laboratory Medicine in Henan Province, School of Laboratory Medicine, Xinxiang Medical University, Xinxiang 453003, People’s Republic of China

Abstract

OBJECTIVES—To understand the mechanism of white fat expansion in the presence of inflammation, we examined the balance of pro- and anti-inflammatory cytokines in epididymal fat during weight gain in DIO mice.

METHODS—The pro- and anti-inflammatory cytokines were examined in white fat of diet-induced obese mice and lean mice. The mechanism of gene expression was investigated with a focus on intracellular ATP (iATP). ATP activity was tested in cellular and non-cellular systems in activation of serine kinases (IKKβ, JNK and ERK).

RESULTS—The pro- (TNF-α, IL-1β, IL-6, MCP-1, IFN-γ and OPN) and the anti-inflammatory cytokines (IL-10, IL-1Ra, IL-13, sTNFR2, PEDF and adiponectin) were increased at the same time during the weight gain. The balance was observed even in the absence of tissue expansion upon feeding in lean and obese mice. The iATP levels were positively associated with the cytokine elevation in the adipose tissue. In macrophages, induction of iATP with lauric acid stimulated the expression. Inhibition of iATP with β-oxidation inhibitor (Etomoxir) or mitochondrial uncoupler (2,4-dinitrophenol, DNP) suppressed the expression. ATP exhibited an activity in the activation of inflammatory kinases (IKKβ, JNK and ERK) in the living cells and cell lysate. The kinase activation was blocked in the cells by ATP inhibition.
CONCLUSIONS—The data suggest that the pro- and anti-inflammatory cytokines are dynamically balanced in the white adipose tissue by iATP.

Keywords
Adipose tissue; ATP; IKKβ; JNK; ERK

INTRODUCTION
White adipose tissue is the primary site of chronic inflammation in obesity as indicated by the expression of proinflammatory cytokines (type 1 cytokine) and infiltration of various immune cells. It is generally believed that the adipose inflammation is detrimental to the adipose tissue function, insulin sensitivity and glucose metabolism. This view leads to the assumption that inhibition of the inflammation will improve insulin sensitivity. Although the assumption is supported by a huge body of literature, anti-inflammatory therapies fail to improve insulin sensitivity in patients in most clinical trials. In addition, more evidence supports that the adipose inflammation has favorite activities in the regulation of metabolism, such as induction of energy expenditure and stimulation of adipose tissue remodeling in obesity. The beneficial activities are further supported by the report that inflammation is required for adipose tissue growth and function in several transgenic models, which represents a new support to the “positive effect” of adipose inflammation. There is an ongoing debate on the beneficial and detrimental effects of adipose inflammation. One of the key points in the debate is that what controls the switch of the “positive” and “negative” effects in obesity? To address the issue, we propose that the switch is controlled by the balance of pro- (type 1 cytokines) and anti-inflammatory cytokines (type 2 cytokines) in the white fat. The possibility is supported by the interplay of two types of cytokines in the adipose tissue, and the favorite activities of anti-inflammatory cytokines in the regulation of metabolism. However, characteristic of the balance remains be revealed in physiological conditions.

In the late stage of obesity, the inflammation is a result of tissue stress responses to several factors, such as hypoxia, ER stress, and endotoxin (LPS), etc. However, the pro-inflammatory cytokines is elevated in the white fat of mice at the first week on a high fat diet (HFD), which is not associated with the stress conditions above. The molecular mechanism remains unknown for the early inflammatory response. To explore the mechanism, we tested the role of adenosine triphosphate (ATP) in the cytosol. The extracellular ATP (eATP) regulates inflammation through interaction with the adenosine receptors in the cell membrane, which have been documented in both immune and non-immune cells. The current knowledge about the ATP in inflammation is from eATP. In obesity, eATP is not required for the adipose inflammation as suggested by the phenotype of P2X7 receptor knockout mice. iATP is elevated in the liver of obese mice. It is not known if the elevation occurs in the fat tissue. iATP may have a signaling activity as suggested by its impact on AMPK through the AMP/ATP ratio. There is no direct evidence for iATP in the control of inflammatory response. We propose that iATP may play a role in the induction of early inflammation in DIO mice.
In this study, expression of pro- and anti-inflammatory cytokines was measured in epididymal fat with and without weight gain to understand the cytokine balance. The study was conducted in both lean and DIO mice. iATP was examined in those conditions to test its role in the regulation of cytokine expression. An impact of iATP was tested in the activation of several serine kinases (IKKβ, JNK and ERK). The results suggest that the balance is maintained in the white fat by iATP through activation of the serine kinases.

MATERIALS AND METHODS

Reagents

Lauric acid (L-9755) and ATP (A6559) were obtained from Sigma (St. Louis, MO). Mouse serum adipokine immunoassay kit (#MADPK-71K-01) was obtained from Multiplex™ MAP. All antibodies used in this study were from as follows: rabbit monoclonal antibodies to p-IKK (Ser176/180) (#2697), IKKβ (#8943) and AMPK (#2532) were from Cell Signaling Technology (Danvers, MA 01923). Antibodies to JNK (sc-827), p-JNK (sc-6254), p-ERK (sc-7383), ERK (sc-514302), p-AMPK (sc-101630), and p-cJUN (sc-822) were from Santa Cruz Biotechnology (Dallas, TX 75220), and antibody to p65 (PC137) was from Oncogene Science, Inc. (Uniondale, NY, 11553). Epigallocatechin gallate (50299), resveratrol (R5010), curcumin (C7727), quercetin (1592409), etomoxir (E1095) and 2,4-dinitrophenol (D198501) were purchased from Sigma.

Diet-induced obese (DIO) mice

All animal experiments were performed according to the animal protocols approved by the Institutional Animal Care and Use Committee (IACUC) at the Pennington Biomedical Research Center. Male C57BL/6J mice were purchased at 4 weeks (wks) of age from the Jackson Laboratory (Bar Harbor, ME) and housed in groups of 4 mice/cage at 12:12h light-dark cycle under room temperatures of 22–24 °C. The mice had free access to water and diet. Chow (Cat. No. 5001, containing 11% calories in fat, Labdiet, St. Louis, MO) and HFD diet (58% kcal in fat, D12331; Research Diets, New Brunswick, NJ) were used. HFD feeding was started at 8 wks of age for 10 wks. Tissues were collected in the morning under fed (non-fasted) or fasting (16 h) conditions.

Measurement of insulin

Blood were collected from each mouse weekly during the 10 wks study and the plasma was prepared after centrifugation at 4,000 rpm for 20 min. The plasma was stored at −80 °C until use. The insulin was measured using a Mouse serum adipokine immunoassay kit according to protocol provided by the manufacturer (Cat. #MMHMAG-44K, EMD Millipore Corporation, Temecula, California).

Nuclear magnetic resonance (NMR)

Body composition was measured using a quantitative Brucker model mq10 NMR analyzer. Shortly, the individual mice placed in small tube were inserted into NMR and then monitored about the fat and lean mass of each mouse within 1 min.
Blood glucose

The blood glucose test was conducted as described in our previous study.²⁶

Cell culture

Raw 264.7 macrophages (ATCC TIB-71) were purchased from American Type Culture Collection (Manassas, VA). The cells were cultured in DMEM culture medium supplemented with 10% FBS and 50 mg/l gentamicin.

Western blot

Cells were harvested by trypsinization in 0.05% trypsin-0.02% EDTA and used in preparation of whole cell lysate in lysis buffer (1% Triton X-100, 50 mM KCl, 125 μM dithiothreitol, 25mM HEPES, pH 7.8, 10 μg/ml leupeptin, 20 μg/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride, and 1 mM sodiumorthovanadate) with sonication. After vigorously vortex, the supernatant was collected as the whole cell lysate after centrifugation at 12,000 rpm, 4 °C for 10 mins. Western blotting was conducted using the whole cell lysate after quantification of protein concentration using the BCA Protein Assay Kit (71285, EMD Millipore Corporation). The blot membrane was stripped with stripper buffer (GM6001, GM Biosciences, Inc., Rockville, MD 20847-2501) when different signals were blotted. The intensity of the individual protein was quantified using NIH Image software (Image J).

qRT-PCR

mRNA expression was examined in total RNA prepared with the Trizol methods (Sigma). The TaqMan probes were used in qRT-PCR to determine mRNA of tumor necrosis factor alpha (TNF-α, Mm00443258_ml), interleukin 6 (IL-6, Mm00446190_ml), interleukin 1 beta (IL-1β, Mm00434228_ml), monocyte chemoattractant protein 1 (MCP1, Mm00441242_ml), interferon gamma (IFN-γ, Mm01168134_ml), osteopontin (OPN, Mm00436767_ml), F4/80 (Mm00802530_ml), inducible nitric oxide synthase (iNOS, Mm00440485_ml), interleukin 13 (IL-13, Mm00434204_ml), interleukin 10 (IL-10, Mm01288386_ml), interleukin 1 receptor antagonist (IL-1Ra, Mm00446185_ml), soluble TNF receptor 2 (sTNFR2, Mm00441889_ml), pigment epithelium-derived factor (PEDF, Mm00441270_ml) and adiponectin (ACDC, Mm00456425_ml) with the 7900 HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA). The expression was normalized to mouse ribosome 18S rRNA.

ATP assay

ATP was determined in the whole cell lysate after treatment of cells with 200 μM of lauric acid. Anti-inflammation agents such as EGCG (Epigallocatechin gallate), Res (Resveratrol), Cur (Curcumin) and Que (Quercetin) were used at the final concentration of 100 μM to inhibit the inflammatory response. Etomoxir (ET, 50 μM) that blocks β-oxidation of fatty acid and 2,4-dinitrophenol (DNP, 100 μM) that uncouples oxidative phosphorylation in mitochondria were used as ATP inhibitors. All of the chemical inhibitors were dissolved in DMSO and the final concentration of DMSO was below 0.05% in the cell culture. Intracellular ATP was determined using the ATP determination Kit (A22066; Thermo Fisher Scientific, Waltham, MA USA 02451) according to the instruction by the manufacturer.
Induction of kinase phosphorylation by ATP

The whole cells lysate was kept in −80 °C overnight to decrease the endogenous ATP. The lysate was incubated with exogenous ATP at 37 °C for different times. ATP was added into the cell lysate at different concentrations as indicated in the figure legend. The phosphorylation status of IKKβ, JNK, c-JUN and ERK were determined in Western blot to access their activation status.

Statistical analysis

All data were analyzed by Student’s t test or one way ANOVA using SPSS software (version 11.0 for Windows, SPSS Inc., Chicago, IL). A statistical significance was considered at P < 0.05. The results are presented as the mean values ± SEM of three individual experiments.

RESULTS

Association of pro- and anti-inflammatory cytokines in adipose tissue

An increase in pro- and anti-inflammatory cytokines has been widely reported in the adipose tissue of obese models. However, the increases have not been examined simultaneously during adipose tissue expansion. To address the issue, the expression was monitored in the epididymal fat pads of DIO mice in the 10 wk study. Six representative pro-inflammatory cytokines (TNF-α, IL-6, MCP-1, IL-1β, IFN-γ and OPN) were examined together with iNOS and F4/80 (macrophage markers). Four of them (TNF-α, IL-6, MCP-1 and IL-1β) were elevated in the 1st week on HFD (Fig. 1, A–D), and the expression was further increased thereafter along the HFD feeding. An increase in IFN-γ and OPN were detected in the second week (Fig. 1, E and F). Interestingly, iNOS expression was decreased in the tissue (Fig. 1G). An increase in macrophage infiltration was detected in the second week by F4/80 (Fig. 1H). Six representative anti-inflammatory cytokines (IL-10, IL-1Ra, IL-13, sTNFR2, PEDF, and adiponectin) were examined in the same condition. A similar pattern of increase was observed for most of the cytokines except adiponectin (Fig. 2). Adiponectin was not significantly altered by the weight gain. These data suggest that expression of pro- and anti-inflammatory genes are increased together during the fat tissue expansion in DIO mice. The activities of pro-inflammatory cytokines are balanced by the anti-inflammatory cytokines to favor adipose tissue expansion, which explains adipose tissue growth in the presence of pro-inflammatory cytokines.

Cytokine expression without weight gain

The gene expression was associated with weight gain in DIO mice. It is not known the expression is a result of tissue expansion or energy surplus in the obese condition. To address this issue, fasting and fed conditions were used as energy deficient and surplus models. The cytokines were determined in the two conditions in both lean and obese mice. A low level of expression was observed in the fasting condition in both lean and obese mice (Fig. 3). A high level of expression was observed in the fed condition (Fig. 3). The increase was detected in TNFα, MCP-1, IL-1β (Fig. 3, A, B and D) and F4/80 (Fig. 3E).

Interestingly, IL-6 expression was not significantly altered by the feeding (Fig. 3C). The anti-inflammatory cytokines (IL-10 and IL-13) exhibited a similar pattern of increase to the
pro-inflammatory cytokines (Fig. 3. F and G). Adiponectin expression was not changed by feeding or obesity in the fat tissue (Fig. 3H). The feeding effect was observed in some of the anti-inflammatory cytokines (IL-10 and IL-13), but not in others including IL-1Ra, sTNFR2 and PEDF (data not shown). The data suggest that the balance of pro- and anti-inflammatory cytokines is regulated by energy supply in the fat tissue regardless of fat tissue expansion.

**ATP in control of gene expression**

Feeding is associated with an increase in energy metabolism as indicated by an increase in oxygen consumption. Oxygen promotes ATP production in mitochondria through stimulation of oxidative phosphorylation, which may increase the intracellular ATP abundance. This possibility was examined by monitoring ATP in the adipose tissue of lean and obese mice. The ATP level was significantly elevated in the fed condition in both lean and obese mice (Fig. 4A). The basal level of ATP was significantly higher in the obese mice over the lean mice, which is positively associated with the cytokine expression in the obese condition. The data suggest that ATP may regulate the cytokine expression.

Macrophage is the major source of pro-inflammatory cytokines in the adipose tissue. To test the ATP activity, cytokine expression was investigated in mouse macrophage cell line Raw cells, in which intracellular ATP was up- and down-regulated in multiple conditions. ATP abundance was induced in the cultured cells with lauric acid treatment in a time-dependent study (Fig. 4B). The abundance was significantly increased at 4 h, and further elevated at 8 h of the treatment. Expression of TNF-α and IL-10 was examined to determine the ATP activity. Both cytokines were induced by lauric acid in the macrophages (Fig. 4C). The induction was suppressed by several chemicals with anti-inflammatory activities, such as epigallocatechin gallate (EGCG), resveratrol (Res), curcumin (Cur) and quercetin (Que) (Fig. 4, D and E). The suppression was observed with a reduction in the intracellular ATP (Fig. 4F), suggesting that ATP elevation is required for the induction of TNF-α and IL-10 expression by lauric acid.

**Inhibition of cytokine expression by ATP inhibitors**

To confirm the role of ATP, the cytokine expression was examined after suppression of ATP production in macrophages with two additional ATP inhibitors. If ATP elevation is required for the cytokine induction, the expression should be blocked by inhibitors. Etomoxir is an inhibitor of β-oxidation of fatty acids and DNP is an uncoupler of the oxidative phosphorylation. The induction of ATP was blocked by the two inhibitors as expected (Fig. 5A), in which lauric acid was unable to induce the intracellular ATP. Under the condition, induction of TNF-α and IL-10 was also blocked in the macrophages (Fig. 5, C and D). These data suggest that ATP elevation is required for the inflammatory response of macrophages.

**Activation of IKKβ, JNK and ERK by ATP**

Cytokine expression is controlled at the transcriptional level, which involves activation of the transcription factors, such as NF-κB and AP-1. The activation is dependent on the upstream serine kinases, such as IKKβ, JNK and ERK in the cytoplasm. To understand the mechanism of ATP action, activities of those kinases were examined. An increase in their
activities was observed in adipose tissue of DIO mice as indicated by the phosphorylation status (Fig. 6A). The increase was observed with ATP elevation as indicated by the decreased AMPK phosphorylation (Fig. 6A). In vitro, IKKβ and JNK (c-JUN phosphorylation) were activated in cells by lauric acids (Fig. 6B). The activation was blocked by the ATP inhibitors (Fig. 6C). Serine kinases use ATP as a substrate in the phosphorylation of target proteins. Substrate elevation should be able to activate the kinases. To test the possibility, the kinase activities were examined in the whole cell lysate upon ATP elevation through addition of exogenous ATP. Activation was observed in both IKKβ and JNK in the system (Fig. 6, D and E). ERK, another serine kinase associated with inflammation response, was also activated by ATP in the system (Fig. 6D). These data suggest that ATP may directly induce activation of IKKβ, JNK and ERK to promote transcriptional expression of the inflammatory cytokines, which provides a new mechanism for the induction of inflammatory genes by fatty acids.

DISCUSSION

In this study, a balance of pro- and anti-inflammatory genes was demonstrated in the white fat tissue regardless of tissue expansion. Expression of pro-inflammatory cytokines was accompanied by the anti-inflammatory cytokines in the tissue in both lean and obese mice. The balance was dynamic and affected by feeding in the two conditions. The profile of pro-inflammatory cytokines was relatively consistent regardless of fat expansion. However, the profile of anti-inflammatory cytokines was not. Expression of IL-1Ra, sTNFR2 and PEDF were induced by fat expansion, but not by feeding. The association of those anti-inflammatory cytokine with fat expansion suggests that the increased activity of anti-inflammatory cytokines is more pronounced during fat expansion to override the increased activity of pro-inflammatory cytokines. This balance provides a restriction of the pro-inflammatory activities in the inhibition of fat tissue expansion in favor of tissue expansion. The possibility is in line with the observations on the dynamic changes of type 1 and type 2 macrophages during adipose tissue expansion9,27.

Our observation suggests a role of iATP in the control of inflammation gene expression. The resting metabolic rate is decreased by fasting and calorie restriction8. The decrease correlates to the reduction in pro- and anti-inflammatory cytokines in this study, which suggests a role of energy supply in the control of the gene expression. This possibility was tested with a focus on intracellular ATP in the regulation of gene expressions in current study. The role of ATP was examined in multiple systems including adipose tissue, macrophages, and whole cell lysate. Induction of ATP abundance led to the stimulation of gene expression, and inhibition of ATP led to the suppression of gene expression. The inhibition was observed with chemical inhibitors such as Etomoxir, DNP, EGCG (Epigallocatechin gallate), Res (resveratrol), Cur (curcumin) and Que (quercetin). This group of data suggests that iATP may induce inflammatory gene expression in the early stage of obesity before the occurrence of adipose tissue hypoxia14,28.

Activation of serine kinases (IKK, JNK and ERK) by ATP in the cytosol represents a new mechanism of inflammation response. eATP has been documented in the regulation of inflammatory response in several reviews20,21. eATP is elevated in the cellular stress
conditions due to release of iATP. eATP interacts with the purinergic receptor P2X7 to trigger secretion of IL-1β and IL-18 in the inflammasome response. However, this eATP pathway is not required for the adipose inflammation in obesity\(^2\). In addition, eATP may inhibit the inflammatory response through an interaction with the receptor P2Y and its downstream cAMP-PKA pathway. Therefore, eATP has both pro- and anti-inflammatory activities according to the difference in receptor types. iATP is at a high concentration (1–10mM) in the cytosol, which is 1000 times higher than that of eATP (1–10 nM)\(^2\). However, not much is known for iATP activity in the regulation of inflammatory response. Our data suggest that iATP may activate IKKβ, JNK and ERK in the cytosol to trigger the cytokine expression. The activity is unlikely mediated by the cell membrane receptors as the activity was observed in the cell free system of cell lysate, suggesting a receptor-independent mechanism of ATP activity. IKKβ activation is able to induce transcription of both type 1 and type 2 cytokines through the NF-kB pathway in macrophages\(^2\).

In summary, we found that the pro-inflammatory cytokines are companied by the anti-inflammatory cytokines in the white fat in the lean and obese conditions. The dynamic balance of two types of cytokines is regulated by iATP through activation of IKK, JNK and ERK. The observations provide a mechanism for the adipose tissue expansion in the presence of elevated pro-inflammatory cytokines. The role of iATP suggests a mechanism for the association of inflammatory status and energy expenditure in physiological conditions such as obesity and calorie restriction\(^8\).

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

**Acknowledgments**

This work was supported by National Institutes of Health grant R56 DK068036-11 (J.Y.), the National Natural Science Foundation of China (grant number 81370915), Program for Innovative Research Team (in Science and Technology) in University of Henan Province (grant number 15IRTSTHN025) and CNRU (NIH 1P30-DK072476) center grant to the genomic core. The qRT-PCR assay was performed in core facility supported by NIH grant (2P30DK072476). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**References**

1. Olefsky JM, Glass CK. Macrophages, inflammation, and insulin resistance. Annu Rev Physiol. 2010; 72:219–46. [PubMed: 20148674]
2. Donath MY, Shoelson SE. Type 2 diabetes as an inflammatory disease. Nature reviews Immunology. 2011; 11(2):98–107.
3. Talukdar S, Oh DY, Bandyopadhyay G, Li D, Xu J, McNelis J, et al. Neutrophils mediate insulin resistance in mice fed a high-fat diet through secreted elastase. Nat Med. 2012; 18(9):1407–12. [PubMed: 22863787]
4. Ye J, McGuinness OP. Inflammation during obesity is not all bad: Evidence from animal and human studies. Ann J Physiol Endocrinol Metab. 2013; 304(5):E466–E477. [PubMed: 23269411]
5. Ye J, Keller J. Regulation of energy metabolism by inflammation: A feedback response in obesity and calorie restriction. Aging. 2010; 2(6):361–368. [PubMed: 20606248]
6. Sun K, Kusminski CM, Scherer PE. Adipose tissue remodeling and obesity. J Clin Invest. 2011; 121(6):2094–101. [PubMed: 21633177]
7. Wernstedt Asterholm I, Tao C, Morley Thomas S, Wang Qiong A, Delgado-Lopez F, Wang Zhao V, et al. Adipocyte Inflammation Is Essential for Healthy Adipose Tissue Expansion and Remodeling. Cell Metabolism. 2014; 20(1):103–118. [PubMed: 24930973]

8. Wang H, Ye J. Regulation of energy balance by inflammation: common theme in physiology and pathology. Rev Endocr Metab Disord. 2015; 16(1):47–54. [PubMed: 25526866]

9. Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J Clin Invest. 2007; 117(1):175–184. [PubMed: 17200717]

10. Wang Y, Wang H, Hegde V, Dubuisson O, Gao Z, Dhurandhar NV, et al. Interplay of pro- and anti-inflammatory cytokines to determine lipid accretion in adipocytes. Int J Obes (Lond). 2013; 37(11):1490–8. [PubMed: 23381555]

11. Kang K, Reilly SM, Karabacak V, Gangl MR, Fitzgerald K, Hatano B, et al. Adipocyte-derived Th2 cytokines and myeloid PPARdelta regulate macrophage polarization and insulin sensitivity. Cell Metab. 2008; 7(6):485–95. [PubMed: 18522830]

12. Qiu Y, Nguyen Khoa D, Odegaard Justin I, Cui X, Tian X, Locksley Richard M, et al. Eosinophils and Type 2 Cytokine Signaling in Macrophages Orchestrate Development of Functional Beige Fat. Cell. 2014; 157(6):1292–1308. [PubMed: 24906148]

13. Brestoff JR, Kim BS, Saenz SA, Stine RR, Monticelli LA, Sonnenberg GF, et al. Group 2 innate lymphoid cells promote beiging of white adipose tissue and limit obesity. Nature. 2015; 519(7542):242–6. [PubMed: 25533952]

14. Ye J, Gao Z, Yin J, He H. Hypoxia is a potential risk factor for chronic inflammation and adiponectin reduction in adipose tissue of ob/ob and dietary obese mice. Am J Physiol Endocrinol Metab. 2007; 293:E1118–E1128. [PubMed: 17666485]

15. Halberg N, Khan T, Trujillo ME, Wernstedt-Asterholm I, Attie AD, Sherwani S, et al. HIF 1 alpha Induces Fibrosis and Insulin Resistance in White Adipose Tissue. Mol Cell Biol. 2009; 29(16):4467–83. [PubMed: 19546236]

16. Zhang K, Shen X, Wu J, Sakaki K, Saunders T, Rutkowski DT, et al. Endoplasmic reticulum stress activates cleavage of CREBH to induce a systemic inflammatory response. Cell. 2006; 124(3):587–99. [PubMed: 16469704]

17. Ozcan U, Yilmaz E, Ozcan L, Furuhashi M, Vaillancourt E, Smith RO, et al. Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. Science. 2006; 313(5790):1137–40. [PubMed: 16931765]

18. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, et al. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes. 2007; 56(7):1761–72. [PubMed: 17456850]

19. Lee YS, Li P, Huh JY, Hwang IJ, Lu M, Kim JJ, et al. Inflammation is necessary for long-term but not short-term high-fat diet-induced insulin resistance. Diabetes. 2011; 60(10):2474–83. [PubMed: 21911747]

20. Ham J, Rees DA. The adenosine a2b receptor: its role in inflammation. Endocr Metab Immune Disord Drug Targets. 2008; 8(4):244–54. [PubMed: 19075778]

21. Vitiello L, Gorini S, Rosano G, la Sala A. Immunoregulation through extracellular nucleotides. Blood. 2012; 120(3):511–8. [PubMed: 22661701]

22. Sun S, Xia S, Ji Y, Kersten S, Qi L. The ATP-P2X7 signaling axis is dispensable for obesity-associated inflammasome activation in adipose tissue. Diabetes. 2012; 61(6):1471–83. [PubMed: 22415881]

23. Cacicedo JM, Yagihashi N, Keaney JF Jr, Ruderman NB, Ido Y. AMPK inhibits fatty acid-induced increases in NF-kappaB transactivation in cultured human umbilical vein endothelial cells. Biochem Biophys Res Commun. 2004; 324(4):1204–9. [PubMed: 15504342]

24. Jeong HW, Hsu KC, Lee JW, Ham M, Huh JY, Shin HI, et al. Berberine suppresses proinflammatory responses through AMPK activation in macrophages. Am J Physiol Endocrinol Metab. 2009; 296(4):E955–64. [PubMed: 19208854]
26. Gao Z, Wang Z, Zhang X, Butler AA, Zuberi A, Gawronska-Kozak B, et al. Inactivation of PKC{theta} leads to increased susceptibility to obesity and dietary insulin resistance in mice. Am J Physiol Endocrinol Metab. 2007; 292(1):E84–91. [PubMed: 16896164]

27. Odegaard JI, Ricardo-Gonzalez RR, Goforth MH, Morel CR, Subramanian V, Mukundan L, et al. Macrophage-specific PPARgamma controls alternative activation and improves insulin resistance. Nature. 2007; 447(7148):1116–20. [PubMed: 17515919]

28. Yin J, Gao Z, He Q, Ye J. Role of hypoxia in obesity-induced disorders of glucose and lipid metabolism in adipose tissue. Am J Physiol Endocrinol Metab. 2009; 296:E333–E342. [PubMed: 19066318]

29. Hoesel B, Schmid JA. The complexity of NF-kappaB signaling in inflammation and cancer. Mol Cancer. 2013; 12:86. [PubMed: 23915189]
Figure 1.
Pro-inflammation in adipose tissue of DIO mice. mRNA of representative pro-inflammatory cytokines was measured weekly in epididymal fat of mice fed HFD and the data on week 1, 2 and 10 are shown. (a) TNF-α. (b) IL-6. (c) MCP1. (d) IL-1β. (e) IFN-γ. (f) OPN. (g) iNOS. (h) F4/80. The data represent fold change in means ± SEM (n=5). * p<0.05 and ** p<0.001 compared with the chow group.
Figure 2.
Anti-inflammatory cytokines in epididymal fat of DIO mice. The anti-inflammatory cytokines were measured weekly in the epididymal fat of DIO mice and the mRNA data of 1, 2 and 10 wks are shown. (a) IL-10. (b) IL-1Ra. (c) IL-13. (d) sTNFR2. (e) PEDF. (f) Adiponectin (ACDC). The data represent fold change in means ± SEM (n=5). * p<0.05 and ** p<0.001 compared with the chow group.
Figure 3.
Feeding increased inflammatory response. mRNA of representative pro- and anti-inflammatory cytokines were compared between fed and overnight fasting conditions in the epididymal fat of mice. The study was performed at 2 wks on HFD. (a) TNF-α. (b) MCP1. (c) IL-6. (d) IL-1β. (e) F4/80. (f) IL-10. (g) IL-13. (h) Adiponectin. The data represents fold change over the fed mice with means ± SEM (n=5). * p<0.05 and ** p<0.001 compared with the fasting group.
Figure 4.
Inhibition of ATP by inflammation inhibitors. (a) ATP in adipose tissue. ATP was determined in epididymal fat of fed (non-fasting) and fasting mice. The study was performed in mice at 4 wks on HFD. (b) ATP in Raw cells. ATP was determined in Raw cells after treatment with lauric acid (200 μM) at various times. (c) Expression of TNF-α and IL-10 in Raw cells treated with lauric acid. mRNA was determined at 8 h with lauric acid treatment. (d) Inhibition of TNF-α expression by inhibitors. TNF-α mRNA was examined in Raw cells after pretreatment with the inhibitors and then lauric acids for 8 h. The inhibitors were used at 100 μM. EGCG: Epigallocatechin gallate; Res: Resveratrol; Cur: Curcumin; Que: Quercetin. (e) Inhibition of IL-10 expression by the inhibitors. (f) Reduction of ATP by the inhibitors. ATP levels were determined in cell lysate after 4 h treatment of RAW cells with lauric acid in the presence of anti-inflammatory agents. The data represent the fold change relative to the control in means ± SEM (n=5). * or # p<0.05 and ** p<0.001 compared with the control.
Figure 5.
Suppression of cytokine expression by ATP inhibitors. (a) ATP inhibition by ET and DNP. ATP was examined in Raw cells after pretreatment with ET (50 μM) and DNP (100 μM) followed by lauric acid (LA) treatment for 4 h. (b) Inhibition of TNF-α expression. mRNA was determined in Raw cells after the inhibitor treatment. (c) Inhibition of IL-10 expression. The bar figure represents data of mean ± SEM (n=3). # or *, p< 0.05 compared with the controls.
Figure 6.
Activation of IKKβ, JNK and ERK by ATP. (a) Kinase activation in adipose tissue by HFD. Phosphorylation of IKKβ, JNK and AMPK were determined in epididymal fat of mice at 5 wks on HFD. (b) Kinase activation by lauric acid in Raw cells. Phosphorylation of IKKβ and c-JUN were determined in Raw cells after lauric acid treatment at different time points as indicated. (c) Suppression of IKKβ and JNK by ATP inhibitors. Phosphorylation of IKKβ and JNK was determined in Raw cell after treatment with the inhibitors. (d) Activation of IKKβ by ATP in cell lysate. Phosphorylation of IKKβ was determined in Raw cell lysate after incubation with 10 mM ATP in the test tube at 37 °C. (e) Activation of JNK and ERK by ATP. Phosphorylation of JNK and ERK were determined in Raw cell lysate after incubation with ATP at different concentration for 15 mins. The bar figure represents data of mean ± SEM (n=3). # or *, p< 0.05 compared with the controls.