Published in final edited form as:

Int J Cardiol. 2013 October 3; 168(3): 2201–2206. doi:10.1016/j.ijcard.2013.01.222.

Differential Regulation of TNF Receptor 1 and Receptor 2 in Adiponectin Expression following Myocardial Ischemia

Yajing Wang, MD, PhD^{1,2,*,\$}, Jianli Zhao, MD^{3,\$}, Yanqing Zhang, MD³, Wayne Bond Lau, MD², Li-Yuan Jiao, MS³, Baojiang Liu, MD³, Yuexing Yuan, PhD², Xiaoliang Wang, MD, PhD², Ling Tao, MD, PhD⁴, Erhe Gao, MD, PhD⁵, Walter J. Koch, PhD⁵, and Xin-Liang Ma, MD, PhD^{2,*}

¹Department of Physiology, Shanxi Medical University, Taiyuan, China, 0310003

²Department of Emergency Medicine, Thomas Jefferson University, Philadelphia, PA 19107

³Department of Anesthesiology, First Affiliated Hospital, Shanxi Medical University, Taiyuan, China, 0310003

⁴Department of Cardiology, Fourth Military Medical University, Xian, China, 710032

⁵Center for Translational Medicine, Temple University Medical School, Philadelphia, PA 19107

Abstract

Background—In vitro experiments demonstrate that adiponectin, a cardioprotective cytokine, is inhibited by tumor necrosis factor- α (TNF). However, the role of TNF in post-myocardial infarction (post-MI) adiponectin reduction remains unclear. More importantly, the TNF receptor type (TNFR1 or TNFR2) responsible for TNF-mediated suppression of adiponectin production is unknown. The current study determined the role of TNF in post-myocardial infarction (post-MI) adiponectin reduction, and identified the receptor type responsible for TNF-mediated suppression of adiponectin production.

Methods and Results—Adult male wild type (WT) and three knockout variety (TNF^{-/-}, TNFR1^{-/-}, and TNFR2^{-/-}) mice were subjected to MI via coronary artery occlusion. Histological and biochemical analyses were performed 3 and 7 days post-MI. In WT mice, MI significantly increased plasma TNF, reduced adipocyte adiponectin mRNA, and decreased plasma adiponectin levels. TNF deletion had no significant effect upon basal adiponectin level, and partially restored adiponectin expression/production post-MI ($P < 0.01$ vs. WT). Basal adiponectin levels were significantly increased in TNFR1^{-/-} ($P < 0.05$ vs. WT), and unchanged in TNFR2^{-/-} mice. Importantly, suppressed adiponectin expression/production by MI or TNF administration was markedly decreased by TNFR1 deletion ($P < 0.01$ vs. WT), but exacerbated by TNFR2 deletion ($P < 0.05$ vs. WT). Mechanistically, TNFR1 knockout significantly inhibited, whereas TNFR2 knockout further enhanced TNF-induced mRNA and protein expression of ATF3, a transcriptional factor known to significantly inhibit adiponectin expression.

© 2012 Elsevier Ireland Ltd. All rights reserved

*Address proofs to: Xin L Ma, MD., PhD., Department of Emergency Medicine, 1020 Sansom Street, Philadelphia, PA 19107 Tel: (215)955-4994; Fax: (215)923-6225; Xin.Ma@Jefferson.edu. †Yajing Wang, MD, PhD Department of Physiology Shanxi Medical University Taiyuan, China, 0310003 Yajing.Wang@jefferson.edu.

‡The first two authors contributed equally to this work

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conclusion—Our study demonstrates TNF α overproduction is responsible for reduced adiponectin expression/production following MI. Furthermore, we show TNFR1/TNFR2 exert opposite effects upon adiponectin expression/production via differential regulation of ATF3.

Keywords

Adiponectin; Diabetes; Tumor Necrosis Factor; Receptor

Introduction

Cardiac disease remains a leading cause of mortality worldwide. Although improved reperfusion strategies have led to declined death rates after acute myocardial infarction (MI), both incidence and prevalence of post-MI heart failure have continually increased in recent years(1). Defining the molecular mechanisms underlying the transition from adaptive to maladaptive remodeling in the post-MI heart, and identifying novel therapeutic strategies blocking/reversing this transition, are therefore in great need.

Adiponectin is a protein cytokine secreted from adipocytes with 3 major recognized biologic functions, including an insulin sensitization/metabolic regulatory function, an anti-inflammatory/vascular protective function, and an anti-oxidative /cardioprotective function(2–4). Numerous epidemiological studies have correlated decreased adiponectin levels with increased cardiovascular disease risk in obesity and diabetes(5–7). Moreover, several recent studies confirm plasma adiponectin is significantly reduced following acute MI, and such adiponectin deficiency is predictive of future adverse cardiac events(8) and associated with increased oxidative stress and inferior cardiac function recovery(9). However, the molecular mechanisms responsible for post-MI adiponectin reduction remain unidentified, and therapeutic targets against MI suppression of adiponectin are currently lacking.

TNF α is a well-recognized pro-oxidative cytokine belonging to the same structural family (C1q/TNF superfamily) as adiponectin. Despite their conformational similarities, these two cytokines exert opposite biological effects, and reciprocally regulate each other's expression and production. Early studies demonstrate that TNF α levels are significantly increased in adiponectin knockout mice(10). Recent studies further establish adiponectin inhibits TNF α -production via multiple mechanisms, including inhibiting NF- κ B (nuclear factor kappa-light-chain-enhancer of activated B cells, a protein complex involved with inflammation and infection) activation, promoting anti-oxidative cytokine production, and activating the cyclooxygenase-2/prostaglandin E2 pathway(11;12). Conversely, TNF α markedly inhibits adiponectin mRNA expression(13), increased plasma TNF α is associated with low plasma adiponectin levels(14), and anti-TNF α treatment increases plasma adiponectin (15). However, heretofore, definitive evidence demonstrating TNF α overproduction is causatively related to adiponectin deficiency under pathological conditions (such as diabetes and MI) remains lacking. Moreover, increasing evidence suggests that TNF α causes distinctive, or even opposite, effects via activation of its two receptors (TNFR1 and TNFR2) in many biological systems(16). However, the TNF receptor subtype whose activation is responsible for TNF α -mediated suppression of adiponectin expression/production has not been investigated. Such identification will advance our knowledge of adiponectin regulation in pathologic states, and may reveal novel therapeutic strategies combating MI injury and post-MI remodeling.

Therefore, the aims of the present study were to determine whether TNF α overproduction after MI is causatively related to adiponectin suppression, and if so, to identify the TNF

receptor subtype responsible for TNF α -mediated suppression of adiponectin expression/production.

Materials and Methods

Animals

Wild type (WT, C57BL/6), TNF α gene knockout (TNF α ^{-/-}), TNF receptor 1 knockout (TNFR1^{-/-}), and TNF receptor 2 knockout (TNFR2^{-/-}) mice were purchased from Jackson Laboratory (Bar Harbor, ME). Mice were confirmed by specific primer genotyping. All experiments were performed in adherence with the National Institutes of Health *Guidelines on the Use of Laboratory Animals* and were approved by the Thomas Jefferson University Committee on Animal Care.

Experimental Protocols

Male adult mice were anesthetized with 2% isoflurane, and myocardial infarction (MI) was produced by temporarily exteriorizing the heart via left thoracic incision, and placing a 6–0 silk suture slipknot around the left anterior descending coronary artery(17). Sham-operated control mice (sham MI) underwent the same surgical procedures except that the suture placed under the left coronary artery was not tied. 4 animals (1 WT, 1 TNF α ^{-/-}, 2 TNFR2^{-/-}) died within 3 days after MI, and these animals were excluded from data analysis. After 3 days of MI or sham MI, 6 animals in each group were sacrificed (3 days post-MI). Blood was drawn for ELISA determination of TNF α and adiponectin, with epididymal fat pad removal for determination of adipose tissue adiponectin mRNA expression. All remaining animals were observed for an additional 4 days. 7 days after MI, all animals were sacrificed. Plasma TNF α and adiponectin levels, and adipocyte adiponectin mRNA expression were determined as described below.

Determination of Plasma TNF- α and Adiponectin Concentrations

Plasma TNF α concentration was determined via mouse TNF α ELISA kit (BioLegend, San Diego, CA) per manufacturer's instructions. Total plasma adiponectin level was determined via ELISA (Enzyme Linked Immunosorbent Assay) kit (ALPCO Diagnostics, Salem, NH) per manufacturer's instructions.

Quantitative PCR (*Polymerase Chain Reaction*) Analysis of Adiponectin Expression in Mouse Adipose Tissues

Total RNA was isolated from epididymal adipose tissue via RNeasy Lipid Tissue Midi Kit (Qiagen, Valencia, CA, USA). Standard PCR protocol was utilized with Applied Biosystems Prism 7900 Sequence Detection System. cDNA from WT mice epididymal fat pad were synthesized from 2 μ g of total RNA and 200 ng of Oilgo dT primer utilizing the Superscript III RNase H-Reverse Transcriptase protocol (Invitrogen, Carlsbad, CA, USA). For quantitative PCR, samples were analyzed in triplicate in 15 μ l reaction volumes (10 ng of cDNA, 450 nmol of primer, 7.5 μ l of Master Mix and water) per standard protocol provided by SyBR α Green PCR Master Mix (Applied Biosystems).

Statistical Analysis

All values in the text and figures are presented as means $\pm$ SEM of n independent experiments. Data (except Western blot density) were subjected to one or two-way (where appropriate) ANOVA (Analysis of Variance) followed by Bonferoni correction for *post-hoc* test. Western blot densities were analyzed with the Kruskal-Wallis test followed by Dunn's *post-hoc* test. Probabilities of 0.05 or less were considered statistically significant.

Results

Negative Correlation between Plasma TNF α and Adiponectin Levels in MI Mice

Plasma TNF levels were significantly elevated 3 days post-MI (1.97-fold, $**P<0.01$), and remained significantly greater than control 7 days post-MI ($*P<0.05$, Figure 1A). Conversely, approximately 50% reduction in adipocyte adiponectin mRNA expression (Figure 1B) and plasma adiponectin levels (Figure 1C) was observed day 3 post-MI ($**P<0.01$). Both adipocyte adiponectin expression and plasma adiponectin levels partially recovered day 7 post-MI, but remained significantly less than control ($*P<0.05$). Most importantly, a strong negative correlation ($P<0.01$) between plasma TNF and adiponectin was observed in MI animals (Figure 1D), suggesting a relationship between increased plasma TNF and reduced adiponectin following MI.

TNF α Gene Deletion Partially Restored Adipocyte Adiponectin mRNA Expression and Plasma Adiponectin Levels

Considerable *in vitro* evidence exists that TNF inhibits adiponectin expression and production at both the transcriptional and post-transcriptional levels. To definitively determine the cause-effect relationship between increased TNF and reduced plasma adiponectin following MI, TNF knockout (TNF $^{-/-}$) mice were subjected to MI, and adiponectin expression/production was determined. Under basal control conditions, adipocyte adiponectin mRNA expression (Figure 2A) and plasma adiponectin concentration (Figure 2B) in TNF $^{-/-}$ mice was slightly greater than WT (not statistically significant). However, 3 days post-MI, adipocyte adiponectin mRNA (Figure 2A) and plasma adiponectin (Figure 2B) levels were significantly greater in TNF $^{-/-}$ mice than WT ($^{**}P<0.01$), demonstrating that TNF overproduction following MI contributes to adiponectin inhibition during this pathologic condition. The protective effect of TNF deletion against post-MI adiponectin reduction is partial, as adiponectin expression/production remained significantly decreased 3 days post-MI compared to respective control ($**P<0.01$). Surprisingly, a time-dependent improvement in adipocyte adiponectin mRNA expression and plasma adiponectin levels (7 days post-MI) observed in WT mice was not observed in TNF $^{-/-}$ mice. No significant difference in adiponectin expression/production between WT and TNF $^{-/-}$ mice 7 days post-MI was observed (Figure 2).

TNFR1 and TNFR2 Gene Knockout had Opposite Effect upon Adiponectin Expression/Production in MI Mice

Several possibilities exist explaining the partial rescuing effect of TNF deletion upon adiponectin expression/production following MI. One possibility involves cytokines in addition to TNF suppressing adiponectin expression/production post-MI. Another possibility is that TNF may elicit different or opposite effects upon adiponectin expression/production via two different receptor activations (as previously demonstrated in other biological systems), with the net effect observed in the TNF $^{-/-}$ state. To directly investigate such possibilities, TNFR1 and TNFR2 gene deleted animals were subjected to MI, with determination of consequent adiponectin expression/production. Under basal control conditions, no significant difference in plasma TNF concentration was observed between groups, and TNFR1 deletion had no significant effect upon post-MI TNF overproduction. However, in TNFR2 $^{-/-}$ mice, plasma TNF level was greater than the two other groups 3 days post-MI ($^{*}P<0.05$). At day 7 post-MI, plasma TNF remained significantly elevated in TNFR2 $^{-/-}$ animals compared to respective basal controls ($*P<0.05$), whereas a return to basal levels was observed in the two other groups (Figure 3A).

Most importantly, deletion of the TNF receptor subtypes yielded distinct, even opposite, effects upon adiponectin expression/production. Specifically, TNFR1 deletion significantly increased adipocyte adiponectin mRNA expression and plasma adiponectin levels before MI ($^{\$}P<0.05$), whereas TNFR2 deletion had no significant effect upon adiponectin expression/production during basal control conditions (Figure 3B, 4A). Strikingly, compared to WT, adipocyte adiponectin mRNA expression and plasma adiponectin levels were significantly greater ($^{\$}P<0.01$) in TNFR1 $^{-/-}$ mice at days 3 and 7 post-MI. In contrast, both adiponectin expression and adiponectin concentration further decreased ($^{\$}P<0.05$) in TNFR2 $^{-/-}$ mice at both days 3 and 7 post-MI (Figure 3B, 4A). These results cannot be attributed to restricted expression of TNFR2 as reported for immune systems(18), because many investigators have reported abundant expression of both TNFR1 and TNFR2 in adipocytes(19–21).

Administration of TNF α Suppressed Adiponectin Production in WT mice, and TNFR1/TNFR2 Knockout Had Opposite Effects upon TNF α Regulation of Adiponectin Production

The results presented above strongly suggest that excess TNF produced after MI may differentially regulate adiponectin expression/production via TNFR1/TNFR2 activation. To obtain more direct evidence supporting this notion, recombinant TNF was administered via intraperitoneal infusion to WT, TNFR1 $^{-/-}$, and TNFR2 $^{-/-}$ mice. To mimic the MI condition, a pilot experiment was performed, and plasma TNF profile was determined after TNF administration in different doses. Based upon pilot study data, a TNF dose 40 ng/g was selected because it increased plasma TNF in WT, TNFR1 $^{-/-}$, and TNFR2 $^{-/-}$ mice (no difference between groups) to a level seen in WT mice at day 3 post-MI (data not shown). Significantly reduced plasma adiponectin levels were observed in WT mice 3 days after TNF administration ($^{**}P<0.01$, Figure 4B). Most interestingly, plasma adiponectin was significantly increased, not decreased, in TNFR1 $^{-/-}$ mice 3 days after TNF administration ($^{*}P<0.05$). A very significant difference ($^{\$}P<0.01$) in plasma APN was observed between WT and TNFR1 $^{-/-}$ mice (Figure 4B). TNF had a markedly stronger inhibitory effect upon adiponectin production in TNFR2 $^{-/-}$ mice. 3 days after TNF administration, plasma APN levels were significantly lower ($^{\$}P<0.05$) in TNFR2 $^{-/-}$ mice than WT mice (Figure 4B). 7 days after TNF administration, plasma APN returned to control levels in all 3 groups.

Opposing Effect of TNFR1 and TNFR2 on TNF-initiated ATF3 Expression

In a final attempt to determine the intracellular signaling mechanisms responsible for differential regulation of adiponectin by TNFR1 and TNFR2, multiple molecules currently known to regulate adiponectin expression were screened. Among 7 transcriptional factors investigated, ATF3 (Activating transcription factor 3), a transcriptional factor known to significantly inhibit adiponectin expression(22), was identified as a molecule whose expression was differentially regulated by TNFR1 and TNFR2 activation. The mRNA levels of ATF3 were significantly upregulated 1 day after TNF administration, and remained elevated throughout the 3-day observation period (Figure 5A). No significant difference in ATF3 mRNA level or protein level was observed between WT and TNFR knockout mice before TNF challenge (Figure 5B and 5C, vehicle group). However, the opposing effect of TNF-induced ATF3 expression was observed in TNFR1 and TNFR2 knockout adipose tissue. Specifically, TNFR1 knockout significantly inhibited, whereas TNFR2 knockout further enhanced, TNF-induced ATF3 mRNA (Figure 5B) and protein (Figure 5C) expression.

Discussion

We made several important observations in the present study. Although strong evidence exists that TNF inhibits adiponectin production in cultured cells, and clinical observations demonstrate plasma adiponectin is negatively associated with plasma TNF in MI patients,

definitive evidence validating TNF α overproduction is causatively related to reduced plasma adiponectin post-MI is lacking. Utilizing a TNF α knockout mouse model, we have obtained definitive evidence demonstrating TNF α overproduction contributes to post-MI hypoadiponectinemia. However, we report the inhibitory effect of MI (simulated via a pathologically relevant model) upon adiponectin expression/production to be less than observed in various in vitro cell culture studies (91–97% reduction in APN mRNA expression after 1–3 days of TNF α incubation)(13). Because TNF α inhibits the expression and secretion of adiponectin in dose-dependent manner(23), this discrepancy is most likely due to the different TNF α levels investigated. In most in vitro cell culture studies, high TNF α concentrations (in the ng/ml range) were typically utilized, with nearly complete adiponectin expression inhibition observed. Although MI induced a very significant increase in plasma TNF α compared to control, the maximal plasma TNF α level in MI animals remained in the pg/ml range (approximately 80–100 pg/ml). In the current study, we demonstrated recombinant TNF α , administered at a dose eliciting pathologically relevant plasma TNF α elevation, inhibited adiponectin expression/production to a comparable extent caused by MI (at day 3).

Emerging evidence indicates that activation of TNFR2 by TNF α exerts opposite biologic effects as activation of TNFR1. For example, TNFR1 $^{-/-}$ reduces apoptosis, attenuates hypertrophy, improves contractile function, promotes angiogenesis, and improves survival(24–27). In contrast, TNFR2 $^{-/-}$ further exaggerates MI injury(24;26–28). More recent studies demonstrate the opposite effects of TNFR1/TNFR2 activation upon MI injury are due to opposing regulatory effects upon NF- κ B. Specifically, TNFR1 deletion diminishes MI-induced NF- κ B activation, whereas TNFR2 deletion further augments MI-induced NF- κ B activation(27).

Although it is well-recognized that TNF α inhibits adiponectin expression/activity at multiple levels (including transcriptional, posttranslational, and APN receptor level antagonisms(29),(30)), the TNF receptor subtype responsible for TNF α -mediated inhibition of adiponectin has never been previously investigated. In the current study, we demonstrate that expression/production of adiponectin, an anti-diabetic, vascular protective and cardiac protective adipokine, is inhibited by TNF α via its type-1 receptor, but stimulated via its type-2 receptor. This result provides clear evidence that although adipocyte TNFR1 is expressed with 10-fold preponderance over TNFR2 (data not shown), and TNFR1 principally inhibits adiponectin expression/production (adiponectin expression/production is significantly increased in TNF α $^{-/-}$ mice), TNFR1-dependent APN suppression remains under the yoke of TNFR2, which acts as a critical limiting factor.

We have obtained several lines of evidence supporting knockout of TNF receptor 1 exerts better protection than TNF α deletion in promoting/preserving adiponectin expression/production. Firstly, basal adipocyte adiponectin mRNA expression and plasma adiponectin levels were both significantly increased in TNFR1 $^{-/-}$ mice, but not in TNF α $^{-/-}$ mice. Secondly, 3 days post-MI, adiponectin expression/production is better protected by TNFR1 deletion than TNF α deletion. Thirdly, in TNF α $^{-/-}$ mice, adiponectin expression/production remained stagnant 7 days post-MI, similar to levels observed 3 days post-MI, without significant difference between TNF α $^{-/-}$ and WT mice at this time point. In contrast, in TNFR1 $^{-/-}$ mice, adiponectin expression/production continually recovers 7 days after MI, and plasma adiponectin levels are significantly greater than WT throughout this period. This result suggests that other cytokines capable of activating TNFR1 may play a significant role at a later time point after MI.

In summary, the current study provided direct evidence that TNF α overproduction post-MI inhibits cardioprotective adiponectin expression/production via TNFR1 activation. In

contrast, TNFR2 activation stimulates adiponectin expression/production in a cardioprotective manner. These experimental results suggest that selective, targeted inhibition of TNFR1 function may represent a new approach restoring adiponectin expression/production in patients with obesity/diabetes/MI, being potentially superior to strategies suppressing TNF activity in general.

Acknowledgments

This research was supported by grants from the American Diabetes Association (1-11-JF56) and the Natural Science Foundation of China (30900592, 81170199) (to YJW), and NIH (HL-63828, HL-096686) and American Diabetes Association (7-11-BS-93) (to XLM).

References

- (1). Roger VL, Go AS, Lloyd-Jones DM, Adams RJ, Berry JD, Brown TM, Carnethon MR, Dai S, de SG, Ford ES, Fox CS, Fullerton HJ, et al. Heart disease and stroke statistics--2011 update: a report from the American Heart Association. *Circulation*. 2011; 123:e18–e209. [PubMed: 21160056]
- (2). Wang ZV, Scherer PE. Adiponectin, Cardiovascular Function, and Hypertension. *Hypertension*. 2008; 51:8–14. [PubMed: 17998473]
- (3). Smith CC, Yellon DM. Adipocytokines, cardiovascular pathophysiology and myocardial protection. *Pharmacol Ther*. 2011; 129:206–219. [PubMed: 20920528]
- (4). Goldstein BJ, Scalia RG, Ma XL. Protective vascular and myocardial effects of adiponectin. *Nat Clin Pract Cardiovasc Med*. 2009; 6:27–35. [PubMed: 19029992]
- (5). Kumada M, Kihara S, Sumitsuji S, Kawamoto T, Matsumoto S, Ouchi N, Arita Y, Okamoto Y, Shimomura I, Hiraoka H, Nakamura T, Funahashi T, et al. Association of hypoadiponectinemia with coronary artery disease in men. *Arterioscler Thromb Vasc Biol*. 2003; 23:85–89. [PubMed: 12524229]
- (6). Pischon T, Girman CJ, Hotamisligil GS, Rifai N, Hu FB, Rimm EB. Plasma adiponectin levels and risk of myocardial infarction in men. *JAMA*. 2004; 291:1730–1737. [PubMed: 15082700]
- (7). Frystyk J, Berne C, Berglund L, Jensenik K, Flyvbjerg A, Zethelius B. Serum adiponectin is a predictor of coronary heart disease: a population-based 10-year follow-up study in elderly men. *J Clin Endocrinol Metab*. 2007; 92:571–576. [PubMed: 17119002]
- (8). Kojima S, Funahashi T, Otsuka F, Maruyoshi H, Yamashita T, Kajiura I, Shimomura H, Miyao Y, Fujimoto K, Sugiyama S, Sakamoto T, Yoshimura M, et al. Future adverse cardiac events can be predicted by persistently low plasma adiponectin concentrations in men and marked reductions of adiponectin in women after acute myocardial infarction. *Atherosclerosis*. 2006; 194:204–213. [PubMed: 16970953]
- (9). Shibata R, Numaguchi Y, Matsushita K, Sone T, Kubota R, Ohashi T, Ishii M, Kihara S, Walsh K, Ouchi N, Murohara T. Usefulness of adiponectin to predict myocardial salvage following successful reperfusion in patients with acute myocardial infarction. *Am J Cardiol*. 2008; 101:1712–1715. [PubMed: 18549845]
- (10). Yamauchi T, Hara K, Kubota N, Terauchi Y, Tobe K, Froguel P, Nagai R, Kadowaki T. Dual roles of adiponectin/Acrp30 in vivo as an anti-diabetic and anti-atherogenic adipokine. *Curr Drug Targets Immune Endocr Metabol Disord*. 2003; 3:243–254. [PubMed: 14683455]
- (11). Higuchi A, Ohashi K, Kihara S, Walsh K, Ouchi N. Adiponectin Suppresses Pathological Microvessel Formation in Retina Through Modulation of Tumor Necrosis Factor- α Expression. *Circ Res*. 2009; 104:1058–1065. [PubMed: 19342600]
- (12). Shibata R, Sato K, Pimentel DR, Takemura Y, Kihara S, Ohashi K, Funahashi T, Ouchi N, Walsh K. Adiponectin protects against myocardial ischemia-reperfusion injury through AMPK- and COX-2-dependent mechanisms. *Nat Med*. 2005; 11:1096–1103. [PubMed: 16155579]
- (13). Hector J, Schwarzlöh B, Goehring J, Strate TG, Hess UF, Deuretzbacher G, Hansen-Algenstaedt N, Beil FU, Algenstaedt P. TNF- α alters visfatin and adiponectin levels in human fat. *Horm Metab Res*. 2007; 39:250–255. [PubMed: 17447161]

- (14). Kern PA, Di Gregorio GB, Lu T, Rassouli N, Ranganathan G. Adiponectin expression from human adipose tissue: relation to obesity, insulin resistance, and tumor necrosis factor- α expression. *Diabetes*. 2003; 52:1779–1785. [PubMed: 12829646]
- (15). Komai N, Morita Y, Sakuta T, Kuwabara A, Kashihara N. Anti-tumor necrosis factor therapy increases serum adiponectin levels with the improvement of endothelial dysfunction in patients with rheumatoid arthritis. *Mod Rheumatol*. 2007; 17:385–390. [PubMed: 17929130]
- (16). Thommesen L, Laegreid A. Distinct differences between TNF receptor 1- and TNF receptor 2-mediated activation of NF κ B. *J Biochem Mol Biol*. 2005; 38:281–289. [PubMed: 15943902]
- (17). Wang Y, Tao L, Yuan Y, Lau WB, Li R, Lopez BL, Christopher TA, Tian R, Ma XL. Cardioprotective effect of adiponectin is partially mediated by its AMPK-independent antinflammatory action. *Am J Physiol Endocrinol Metab*. 2009; 297:E384–E391. [PubMed: 19470831]
- (18). Faustman D, Davis M. TNF receptor 2 pathway: drug target for autoimmune diseases. *Nat Rev Drug Discov*. 2010; 9:482–493. [PubMed: 20489699]
- (19). Tesz GJ, Guilherme A, Guntur KV, Hubbard AC, Tang X, Chawla A, Czech MP. Tumor necrosis factor α (TNF α) stimulates Map4k4 expression through TNF α receptor 1 signaling to c-Jun and activating transcription factor 2. *J Biol Chem*. 2007; 282:19302–19312. [PubMed: 17500068]
- (20). Good M, Newell FM, Haupt LM, Whitehead JP, Hutley LJ, Prins JB. TNF and TNF receptor expression and insulin sensitivity in human omental and subcutaneous adipose tissue--influence of BMI and adipose distribution. *Diab Vasc Dis Res*. 2006; 3:26–33. [PubMed: 16784178]
- (21). Sethi JK, Xu H, Uysal KT, Wiesbrock SM, Scheja L, Hotamisligil GS. Characterisation of receptor-specific TNF α functions in adipocyte cell lines lacking type 1 and 2 TNF receptors. *FEBS Lett*. 2000; 469:77–82. [PubMed: 10708760]
- (22). Su H, Lau WB, Ma XL. Hypoadiponectinaemia in diabetes mellitus type 2: molecular mechanisms and clinical significance. *Clin Exp Pharmacol Physiol*. 2011; 38:897–904. [PubMed: 21916932]
- (23). Maeda N, Takahashi M, Funahashi T, Kihara S, Nishizawa H, Kishida K, Nagaretani H, Matsuda M, Komuro R, Ouchi N, Kuriyama H, Hotta K, et al. PPAR γ Ligands Increase Expression and Plasma Concentrations of Adiponectin, an Adipose-Derived Protein. *Diabetes*. 2001; 50:2094–2099. [PubMed: 11522676]
- (24). Higuchi Y, McTiernan CF, Frye CB, McGowan BS, Chan TO, Feldman AM. Tumor necrosis factor receptors 1 and 2 differentially regulate survival, cardiac dysfunction, and remodeling in transgenic mice with tumor necrosis factor- α -induced cardiomyopathy. *Circulation*. 2004; 109:1892–1897. [PubMed: 15051641]
- (25). Monden Y, Kubota T, Inoue T, Tsutsumi T, Kawano S, Ide T, Tsutsui H, Sunagawa K. Tumor necrosis factor- α is toxic via receptor 1 and protective via receptor 2 in a murine model of myocardial infarction. *Am J Physiol Heart Circ Physiol*. 2007; 293:H743–H753. [PubMed: 17416608]
- (26). Kishore R, Tkebuchava T, Sasi SP, Silver M, Gilbert HY, Yoon YS, Park HY, Thorne T, Losordo DW, Goukassian DA. Tumor necrosis factor- α signaling via TNFR1/p55 is deleterious whereas TNFR2/p75 signaling is protective in adult infarct myocardium. *Adv Exp Med Biol*. 2011; 691:433–448. [PubMed: 21153348]
- (27). Hamid T, Gu Y, Ortines RV, Bhattacharya C, Wang G, Xuan YT, Prabhu SD. Divergent Tumor Necrosis Factor Receptor-Related Remodeling Responses in Heart Failure: Role of Nuclear Factor- κ B and Inflammatory Activation. *Circulation*. 2009; 119:1386–1397. [PubMed: 19255345]
- (28). Goukassian DA, Qin G, Dolan C, Murayama T, Silver M, Curry C, Eaton E, Luedemann C, Ma H, Asahara T, Zak V, Mehta S, et al. Tumor necrosis factor- α receptor p75 is required in ischemia-induced neovascularization. *Circulation*. 2007; 115:752–762. [PubMed: 17261656]
- (29). Liu M, Zhou L, Xu A, Lam KSL, Wetzel MD, Xiang R, Zhang J, Xin X, Dong LQ, Liu F. A disulfide-bond A oxidoreductase-like protein (DsbA-L) regulates adiponectin multimerization. *PNAS*. 2008; 105:18302–18307. [PubMed: 19011089]

- (30). Kupchak BR, Garitaonandia I, Villa NY, Smith JL, Lyons TJ. Antagonism of human adiponectin receptors and their membrane progesterone receptor paralogs by TNFalpha and a ceramidase inhibitor. *Biochemistry*. 2009; 48:5504–5506. [PubMed: 19453184]

Figure 1.

MI significantly increased plasma TNF-α (A), inhibited adipocyte adiponectin mRNA expression (B), and decreased plasma adiponectin levels (C). A significant negative correlation between plasma TNF-α and plasma adiponectin levels in MI animals was observed (D, $n=17$). $N=6$ for control group (no MI); $N=6$ for MI@3days; $N=11$ for MI@7 days (plasma TNF-α and adiponectin) or $N=9$ for MI@7 days (adipocyte APN mRNA). * $P<0.05$, ** $P<0.01$ vs. sham MI control. Control concentrations of plasma TNF-α and adiponectin were measured in 6 sham MI animals and 17 MI animals prior to coronary artery occlusion. These data were not used in correlation analysis.

Figure 2.

TNF gene deletion significantly attenuated MI inhibition of adipocyte adiponectin mRNA expression (A) and enhanced plasma adiponectin levels (B) 3 days post-MI, but not 7 days post-MI. N=6–11/group. *P<0.05, **P<0.01 vs. own sham MI control; \$\$P<0.01 vs. WT at the same time point.

Figure 3.

(A) TNFR1 gene deletion had no significant effect upon MI-induced TNF α overproduction, but TNFR2 gene deletion further augmented plasma TNF α concentration following MI. (B) TNFR1 gene deletion significantly increased adipocyte adiponectin mRNA expression under basal control conditions, but TNFR2 gene deletion had no significant effect upon basal control adiponectin expression. More importantly, TNFR1 gene deletion markedly attenuated, whereas TNFR2 gene deletion further exaggerated MI suppression of adiponectin expression. N=6–11/group. *P<0.05, **P<0.01 vs. own sham MI control; \$P<0.05, \$\$P<0.01 vs. WT at the same time point.

Figure 4.

(A) TNFR1 gene deletion significantly increased plasma adiponectin levels under basal control conditions, but TNFR2 gene deletion had no significant effect upon basal control adiponectin production. More importantly, TNFR1 gene deletion markedly attenuated, whereas TNFR2 gene deletion further exaggerated MI suppression of adiponectin production. (B) 3 days post-TNF α administration, gene deletion of TNFR1 (increases) and TNFR2 (reduces) had opposite effects upon plasma adiponectin levels. N=6–11/group. *P<0.05, **P<0.01 vs. own sham MI control; \$P<0.05, \$\$P<0.01 vs. WT at the same time point.

Figure 5.

Time course of adipocyte ATF3 mRNA expression after TNF α injection in WT mice (A). Results were normalized against mean value of day 0. Effect of TNFR1 and TNFR2 knockout upon TNF α -induced adipocyte ATF3 mRNA expression (B). Results were normalized against mean value of WT mice treated with vehicle. Effect of TNFR1 and TNFR2 knockout upon TNF α -induced adipocyte ATF3 protein expression (C). Results were normalized against GAPDH. N=6-7 animals/group. **P<0.01 vs. vehicle group in the same strain; \$P<0.05, \$\$P<0.01 vs. WT mouse treated with TNF α .