Omega-3 Fatty Acids Prevent Inflammation and Metabolic Disorder through Inhibition of NLRP3 Inflammasome Activation

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SUMMARY

Omega-3 fatty acids (ω-3 FAs) have potential anti-inflammatory activity in a variety of inflammatory human diseases, but the mechanisms remain poorly understood. Here we show that stimulation of macrophages with ω-3 FAs, including eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and other family members, abolished NLRP3 inflammasome activation and inhibited subsequent caspase-1 activation and IL-1β secretion. In addition, G protein-coupled receptor 120 (GPR120) and GPR40 and their downstream scaffold protein β-arrestin-2 were shown to be involved in inflammasome inhibition by ω-3 FAs. Importantly, ω-3 FAs also prevented NLRP3 inflammasome-dependent inflammation and metabolic disorder in a high-fat-diet-induced type 2 diabetes model. Our results reveal a mechanism through which ω-3 FAs repress inflammation and prevent inflammation-driven diseases and suggest the potential clinical use of ω-3 FAs in gout, autoinflammatory syndromes, or other NLRP3 inflammasome-driven inflammatory diseases.

INTRODUCTION

Omega-3 fatty acids (ω-3 FAs) are essential to human health, and deficiencies can lead to chronic diseases (Zhang and Spite, 2012). In particular, increasing evidences from both human and animal studies have demonstrated that ω-3 FAs, primarily eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), can suppress inflammation and have a beneficial role in a variety of inflammatory human diseases, including diabetes, atherosclerosis, asthma, and arthritis (Fritsche, 2006; Zhang and Spite, 2012). In addition, studies using Fat1 transgenic mice, which have abundant endogenous ω-3 FAs, strongly support the idea that ω-3 FAs are protective in inflammatory pathologies (Hudert et al., 2006; Kang et al., 2004). Although the use of ω-3 FAs for treatment of inflammatory disorders in the clinic is promising, the anti-inflammatory mechanisms of ω-3 FAs are poorly understood.

The inflammasome is a cytosolic protein complex composed of nucleotide-binding domain and leucine-rich repeat containing proteins (NLRs) or AIM2, ASC and caspase-1, and is a central regulator of innate immunity and inflammation (Martinon et al., 2009). The inflammasome is assembled in response to pathogen infection or “danger” signals and promotes the maturation and release of several proinflammatory cytokines, including interleukin-1β (IL-1β), IL-18, and IL-33. Inflammasome is therefore involved in the pathogenesis of several inflammatory disorders, including diabetes, atherosclerosis, and arthritis (Davis et al., 2011; Schroder et al., 2010; Strowig et al., 2012), suggesting that the activation of NLRP3 inflammasome needs to be tightly controlled. Type I interferon has been shown to block NLRP3 inflammasome activation via Stat1-dependent manner, suggesting that success of IFN-β treatment for multiple sclerosis might indeed rely on suppressing inflammasome-mediated inflammation (Guarda et al., 2011). Recently, nitric oxide has been identified as another critical negative regulator of the NLRP3 inflammasome activation and can protect mice against LPS-induced septic shock (Mao et al., 2013; Mishra et al., 2013). However, the mechanism involved in inflammasome regulation is still unclear.

ω-3 FAs have been shown to inhibit the production of proinflammatory cytokines, including IL-1β (Endres et al., 1989; Oh et al., 2010; Robinson et al., 1996), and this prompted us to investigate whether ω-3 FAs exert their anti-inflammatory activity via inhibition of inflammasome activation. In this study, we demonstrate that ω-3 FAs suppressed inflammation via inhibition of inflammasome activation. We found that ω-3 FAs inhibited NLRP3 and NLRP1b-dependent caspase-1 activation and IL-1β secretion. Furthermore, ω-3 FAs prevented high-fat-diet (HFD)-induced metabolic disorder through inhibition of NLRP3 inflammasome activation in vivo, suggesting that the inflammasome is an important target for ω-3 FAs to exert their anti-inflammatory activity.
RESULTS

ω-3 FAs Suppress Caspase-1 Activation and IL-1β Secretion

To assess the effect of ω-3 FAs on inflammasome activation and IL-1β secretion, we first examined whether DHA could inhibit caspase-1 cleavage and IL-1β secretion. We indeed observed that pretreatment of LPS-primed bone-marrow-derived macrophages (BMDMs) with DHA blocked caspase-1 activation and IL-1β maturation in a dose-dependent manner (Figures 1A and 1B). Similarly, DHA also inhibited the nigericin-induced secretion of IL-18, another inflammasome-dependent cytokine (Figure 1C).

It has been reported that DHA can inhibit LPS-induced NF-κB activation and tumor necrosis factor-α (TNF-α) production (Oh et al., 2010), we then examined whether DHA had an impact on LPS-induced priming for inflammasome activation. As shown in Figure S1, when BMDMs were stimulated with DHA after 3 hr LPS treatment, DHA had no effect on LPS-induced TNF-α production or pro-IL-1β expression (Figures S1A–S1C), suggesting that DHA didn’t affect LPS-induced priming at this condition. In contrast, when BMDMs were stimulated with DHA for 3 hr before LPS treatment, DHA inhibited LPS-induced TNF-α production and pro-IL-1β expression (Figures S1A–S1C), although the dose was higher than the dose needed for inflammasome inhibition. These results suggest that DHA can affect both LPS-induced priming and inflammasome activation. In order to clarify the mechanism underlying DHA-induced inflammasome inhibition, we stimulated BMDMs with DHA after 3 hr LPS treatment in the later experiments. The observed inhibitory effects of DHA on IL-1β secretion were also confirmed in human THP-1 macrophages (Figure 1D). Furthermore, we found that ω-3 FAs didn’t affect nigericin-induced cell death (Figure S1D), suggesting that the effect of ω-3 FAs on IL-1β release is not due to the inhibition of cell death. We also examined the role of other ω-3 FAs, such as EPA and α-linolenic acid (ALA), and found that both of EPA and ALA suppressed nigericin-induced IL-1β secretion but had no effect on TNF-α production (Figures 1E and 1F; Figure S1E). In contrast with ω-3 FAs, ω-6 FAs—including osbond acid (OBA), dihomo-gamma-linolenic acid (DGLA), and adrenic acid (ADA)—and ω-9 FAs—including oleic acid (OA)—failed to block IL-1β secretion in BMDMs (Figure 1E), suggesting that only certain ω-3 FAs inhibit nigericin-induced NLRP3 inflammasome activation. Taken together, these results indicate that ω-3 FAs have the potential to inhibit caspase-1 activation and IL-1β secretion.

ω-3 FAs Inhibit NLRP3 or NLRP1b Inflammasome Activation

In addition to nigericin, NLRP3 inflammasome can be activated by both pathogen-associated molecular patterns (PAMPs) and...
danger-associated molecular patterns (DAMPs), including R837, aluminum salts (Alum), ATP and monosodium urate crystals (MSU) (Davis et al., 2011). To determine whether \( \omega-3 \) FAs only affect nigericin-induced NLRP3 inflammasome activation, we examined other NLRP3 agonists. As shown in Figure 2A, we found that pretreatment with DHA inhibited caspase-1 cleavage and IL-1\( \beta \) secretion triggered by all examined agonists, including MSU, Alum, R837, and ATP, similar to nigericin. These results suggest that \( \omega-3 \) FAs are potent and broad inhibitors of NLRP3 inflammasome activation. To date, four inflammasomes, including the NLRP3, the NLRC4, the NLRP1b, and the AIM2 inflammasome, have been extensively studied (Davis et al., 2011). We investigated whether the inhibitory effect of \( \omega-3 \) FAs was specific to the NLRP3 inflammasome or more general. We found that DHA, but not DGLA or OA, blocked caspase-1 activation, IL-1\( \beta \), or IL-18 secretion induced by anthrax Lethal Toxin (Lethal Toxin), an identified NLRP1b activator (Figures 2B–2D). In contrast, DHA had minimal effect on NLRC4 or AIM2 inflammasome activation, which were triggered by \textit{Salmonella typhimurium} (\textit{Salmonella}) infection or poly (dA:dT) transfection, respectively (Figures 2E and 2F). Taken together, these results demonstrate that \( \omega-3 \) FAs can specifically inhibit NLRP3 and NLRP1b inflammasome activation and subsequent IL-1\( \beta \) production.

\( \omega-3 \) FAs Inhibit Inflammasome Activation Independent of Enzymatic Products

Next, we investigated the mechanisms underlying the inhibitory activity of \( \omega-3 \) FAs on NLRP3 inflammasome activation. In recent years, some enzymatic oxygenated products generated from \( \omega-3 \) FAs have been shown to exert anti-inflammatory activity and contribute functionally to the resolution of inflammation (Arita et al., 2007; Serhan et al., 2002; Spite et al., 2009). We thus asked whether \( \omega-3 \) FAs inhibited NLRP3 inflammasome activation via such modified products. DHA can be converted to several bioactive compounds, including Protectin D1 (PD1), Resolvin D1 (RvD1) and aspirin-triggered Resolvin D1 (AT-RvD1), by lipoxygenases (AlOX5 and AlOX15) and aspirin-acetylated COX-2 (Serhan et al., 2000; Zhang and Spite, 2012). As shown in Figure 3, PD1, RvD1 or AT-RvD1 pretreatment had no effect on nigericin-induced caspase-1 activation and IL-1\( \beta \) or IL-18 secretion (Figures 3A–3C). Furthermore, we also found that \textit{Alox5} and \textit{Alox15} double deficiency or inhibition of COX-2 activity by celecoxib had no impact on the inhibitory activity of DHA on NLRP3 or NLRP1b inflammasome activation (Figures 3D and 3E; Figure S2). These results suggest that these enzyme modified enzymatic products are not involved in \( \omega-3 \) FA-mediated inflammasome inhibition.

Involvement of GPR40, GPR120, and \( \beta \)-Arrestin-2 in the Inhibition of NLRP3 Inflammasome Activation Induced by \( \omega-3 \) FAs

It has been previously reported that long-chain FAs can activate G protein-coupled receptor 120 (GPR120) and GPR40. As GPR120 is involved in the anti-inflammatory effects of \( \omega-3 \) FAs (Cintra et al., 2012; Hirasawa et al., 2005; Itoh et al., 2003; Liou et al., 2011), we examined whether \( \omega-3 \) FAs inhibited NLRP3
inflammasome activation via GPR120 or GPR40. First, we found that pretreatment with GW9508, a small-molecular agonist of GPR120 and GPR40 (Briscoe et al., 2006), markedly repressed caspase-1 activation and IL-1β or IL-18 secretion induced by nigericin or Lethal Toxin, similar to DHA (Figure 4A; Figure S3A, B). To further confirm the role of GPR120 and GPR40 in the inhibitory role of ω-3 FAs in inflammasome activation, we established THP-1 cells stably expressing shRNA targeting the mRNA encoding either GPR40, GPR120, or both of them (Figure S3C). We found that, although single deficiency of either GPR40 or GPR120 only partially impaired the inhibitory effect of DHA on NLRP3 inflammasome activation (Figures 4B and 4D; Figure S3D), double deficiency of GPR40 and GPR120 significantly relieved DHA-mediated inflammasome inhibition (Figures 4C–4E; Figure S3E). Similar results were observed when THP-1 cells were pretreated with EPA (Figure 4F). These data suggest that ω-3 FAs can signal through GPR40 and GPR120 to inhibit NLRP3 inflammasome activation and may explain the enhanced inflammation observed in adipose tissue of Gpr120−/−C0/C0 mice (Ichimura et al., 2012).

β-arrestin-2 (ARRB2) is a downstream scaffold protein of GPR120 (Oh et al., 2010), so to further investigate the molecular mechanisms mediating the inhibitory effect of ω-3 FAs, we investigated the role of ARRB2. We found that deficiency of ARRB2 in THP-1 cells abrogated the inhibitory effects of DHA and EPA on NLRP3 or NLRP1b inflammasome activation (Figure 5A; Figure S4A–S4C). We further used Arrb2−/− mice to confirm the results observed in THP-1 cells and found that deletion of Arrb2 only partially inhibited the activity of DHA (Figures 5B and 5C), suggesting that another mechanism might be involved in the inhibition of NLRP3 inflammasome activation induced by DHA in BMDMs. We next tested the possibility that ARRB2 interacts with NLRP3 in HEK293T cells. Overexpressed ARRB2 bound full-length NLRP3 and the leucine-rich repeat (LRR) and NACHT regions of NLRP3 (Figure 5D). We further found that ARRB2 interacted with NLRP1, but not with NLRC4 or AIM2. This might explain why DHA can inhibit NLRP3 or NLRP1b inflammasome activation but has no effect on NLRC4 or AIM2 inflammasome activation (Figure 5E). Importantly, DHA and EPA treatments induced the endogenous interaction between NLRP3 and ARRB2 in THP-1 cells via GPR120 and GPR40, whereas DGLA did not (Figure 5F; Figure S4D). These results indicate that ARRB2 acts downstream of GPR120 and GPR40 to inhibit inflammasome activation via binding with NLRP3 or NLRP1.

ω-3 FAs Prevent HFD-Induced Insulin Resistance through Inhibition of NLRP3 Inflammasome Activation

Type 2 diabetes (T2D) is a leading cause of morbidity and mortality worldwide, and increasing evidence suggests chronic
inflammation as an important pathogenetic factor in the development of insulin resistance and T2D (Donath and Shoelson, 2011). As we and others have shown that the NLRP3 inflammasome is implicated in the development of insulin resistance in T2D (Masters et al., 2010; Vandanmagsar et al., 2011; Wen et al., 2011; Zhou et al., 2010), we investigated whether ω-3 FAs can improve insulin sensitivity via inhibition of inflammasome activation. We fed mice with HFD or normal diet (ND) for 10 weeks. HFD-treated wild-type (WT) mice exhibited a robust elevation in plasma insulin accompanied by increased plasma glucose in fasted states (Figures 6A and 6B; Figures S5A and S5B), indicating the emergence of insulin resistance. However, HFD-induced elevations of plasma glucose and insulin were ameliorated by DHA supplementation, but not by DGLA or OA supplementation, suggesting that DHA has a beneficial effect on HFD-induced insulin resistance (Figures 6A and 6B; Figures S5A and S5B). To confirm these results, we performed glucose tolerance test (GTT) and insulin tolerance test (ITT) in HFD-treated mice with or without DHA supplementation. GTT showed that DHA supplemented mice were more glucose tolerant than WT mice (Figure 6C). ITT showed that insulin sensitivity, as measured by the reduction in plasma glucose after insulin administration, was markedly improved by DHA in HFD-treated mice (Figure 6E). Furthermore, phosphorylation of the major marker for insulin signaling, Akt (on Ser473), was enhanced both in liver and adipose tissues in DHA-treated mice as compared to WT mice (Figures S5C and S5D). In contrast with WT mice, the beneficial effects of DHA, including reduction of fasted glucose concentrations, improved glucose tolerance and better insulin sensitivity, were all abrogated in \( Nlrp3 \)/−/− mice, suggesting that the beneficial effect of ω-3 FAs on metabolic disorder depends on NLRP3 inflammasome inhibition (Figures 6A–6F).

To further confirm that DHA prevents HFD-induced metabolic disorder through inhibition of NLRP3 inflammasome activation, we tested whether DHA supplementation inhibited metabolic stress-induced inflammasome activation in vivo. Consistent with above results, liver and adipose tissue from HFD-treated mice showed higher caspase-1 activation and IL-1β or IL-18 production as compared to ND-treated mice (Figures 7A–7D, 7I). Importantly, DHA administration or \( Nlrp3 \)/−/− deficiency blocked HFD-induced IL-1β or IL-18 production and caspase-1 activation in these tissues (Figures 7A–7D, 7I), indicating that DHA supplementation can suppress metabolic stress-induced inflammasome activation in HFD-treated mice. Moreover, DHA also suppressed the upregulation of proinflammatory cytokines, such as TNF-α and MCP-1, in WT mice, but not in \( Nlrp3 \)/−/− mice (Figures 7E–7H), suggesting that NLRP3 inflammasome is a key target through which ω-3 FAs exert their broad anti-inflammatory activity in vivo. Taken together, these results indicate that ω-3 FAs can exert beneficial effect on HFD-induced insulin resistance by blocking metabolic stress-induced NLRP3 inflammasome activation.
**DISCUSSION**

ω-3 FAs are attractive candidates for prevention of many inflammation-driven human diseases because of their anti-inflammatory properties. The first evidence of the important role of ω-3 FAs in inflammation was derived from epidemiological observations of the low incidence of inflammatory disorders, such as psoriasis, diabetes, and multiple sclerosis in Greenland Eskimos compared with gender and age-matched people living in Denmark (Dyerberg and Bang, 1979). In the last years, both human clinical studies and animal experiments have demonstrated that ω-3 FAs have anti-inflammatory activity and beneficial roles in a variety of inflammatory human diseases, including diabetes, atherosclerosis, asthma, and arthritis. Thus, understanding the underlying anti-inflammatory mechanisms is helpful for an appropriate use of ω-3 FAs in the clinic. We show here that the inflammasome was a key target for ω-3 FAs to suppress inflammation and exert their anti-inflammatory activity in inflammatory disorders. ω-3 FAs suppressed both LPS-induced priming and inflammasome activation (caspase-1 cleavage) in macrophages. The inhibitory effect of ω-3 FAs on inflammasome activation was specific to NLRP3 or NLRP1b inflammasome, because ω-3 FAs did not inhibit NLRC4 or AIM2 inflammasome activation. Mechanistically, we found that ω-3 FAs repressed NLRP3 inflammasome activation independently of their enzyme modified products, including PD1, RvD1, and AT-RvD1. Furthermore, our data suggest that ω-3 FAs inhibited NLRP3 inflammasome activation via binding to NLRP3, at least partially. Importantly, ω-3 FAs supplementation could suppress HFD-induced NLRP3 inflammasome.
activation and prevent NLRP3 inflammasome-dependent insulin resistance in vivo. Taken together, our results demonstrate a previously unrecognized mechanism through which \( \omega-3 \) FAs repress inflammation and prevent inflammation-driven diseases.

T2D is a leading cause of morbidity and mortality worldwide and its etiology remains unclear. Oxidative stress, endoplasmic reticulum stress, amyloid deposition in the pancreas, lipotoxicity, and glucoxicity are the most popular mechanisms being used to explain insulin resistance and islet \( \beta \)-cell dysfunction in T2D (Harding and Ron, 2002; Prentki and Nolan, 2006; Robertson et al., 2004; Weir and Bonner-Weir, 2004), although it has been difficult to determine which one is the most important. It is noteworthy that each of these cellular stresses is also thought to elicit or associate with inflammatory response (Hotamisligil and Erbay, 2008; Masters et al., 2010). Indeed, increasing evidence suggests chronic inflammation as an important pathogenetic factor in the development of insulin resistance and T2D (Donath and Shoelson, 2011). Proinflammatory cytokines, such as TNF-\( \alpha \) and IL-1\( \beta \), are significantly elevated in T2D (Herder et al., 2009; Spranger et al., 2003). \( \omega-3 \) FAs have been reported to enhance insulin resistance and prevent inflammation-driven diseases. Here we present evidence that \( \omega-3 \) FAs may also exert beneficial effects on inflammation-driven diseases.

Figure 6. \( \omega-3 \) FAs Prevent NLRP3-Dependent Insulin Resistance-Induced by HFD

WT or Nlrp3\(-/-\) mice were fed with normal diet (ND) or high fat diet (HFD) for 10 weeks with or without treatment with DHA. Fasting serum glucose (A), fasting serum insulin (B), glucose tolerance (C and D), and insulin tolerance (E and F) were tested as indicated. Values are means ± SEM of 6–9 mice per group. *p < 0.05, **p < 0.01 (nonparametric Mann Whitney test). Data are representative of three independent experiments. See also Figure S5.
as inhibitors for NLRP3 inflammasome activation and suggest the potential clinic use of \( \omega-3 \) FAs in NLRP3 inflammasome-driven diseases. We also demonstrate that \( \omega-3 \) FAs inhibit NLRP3 inflammasome activation via GPR40 and GPR120-dependent pathway, at least partially, suggesting that GPR40 and GPR120 downstream signaling pathway are involved in the signaling network that controls NLRP3 inflammasome activation, although the precise mechanisms need to be further investigated. The role of GPR40 and GPR120 signaling in NLRP3 inflammasome inhibition may also explain the enhanced inflammation observed in adipose tissue of \( \text{Gpr}120^{-/}\) mice (Ichimura et al., 2012).

Collectively, our findings demonstrate that \( \omega-3 \) FAs suppressed inflammation by reducing NLRP3 inflammasome activation in macrophages and provide a new anti-inflammatory mechanism for \( \omega-3 \) FAs. Our results also show that the inhibitory activity of \( \omega-3 \) FAs on inflammasome activation was important for their beneficial effects in T2D. Considering that NLRP3 inflammasome is involved in the development of a broad range of inflammatory diseases, \( \omega-3 \) FAs might have potential clinic use in gout, autoinflammatory syndromes, or other NLRP3-driven diseases.

**EXPERIMENTAL PROCEDURES**

**Mice**

\( \text{Nlrp}3^{-/-} \) and \( \text{Arrb}2^{-/-} \) mice were described previously (Bohn et al., 1999; Martinon et al., 2006). \( \text{Alox}5^{-/-} \) and \( \text{Alox}15^{-/-} \) mice were from Jackson laboratory. All mice are in C57BL/6 background except that the cells used for the Lethal Toxin stimulation were from BALB/c mice. All animal experiments were approved by a local ethics committee (The Ethics Committee of University of Science and Technology of China; Service Vétérinaire Cantonal, Lausanne, Switzerland).

**Reagents**

Nigericin, MSU, ATP, PMA, GW9508, poly (dA:dT), celecoxib, insulin, and glucose were purchased from Sigma. R837 and ultrapure LPS were obtained from Invivogen. Docosahexaenoic acid, eicosapentaenoic acid, \( \alpha-\)Linolenic acid, ombon acid, dihomo-gamma-linolenic acid, adrenic acid, oleic acid, protectin D1, resolvin D1, and aspirin-triggered Resolvin D1 were from Cayman chemical. Lethal Toxin, consisting of protective antigen and lethal factor, was from List Biological Laboratories. *Salmonella* is a gift from R.V. Bruggen. Inject-Alum was from Pierce Biochemicals. Anti-Flag (M2) antibody and anti-VSV antibody were from Sigma. Anti-human cleaved IL-1\( \beta \) (D11B), anti-human caspase-1, anti-AKT, and anti-pAKT were purchased from Cell Signaling. Anti-human Pro-IL-1\( \beta \) was from Proteintech. The antibody against mouse IL-1\( \beta \) was from R&D. Anti-mouse caspase-1 (p20) and anti-NLRP3 were from Adipogen. Anti-\( \beta \)-actin was from Abmart. Anti-ARRB2, anti-GPR40, anti-ARRB2 antibody was from Service  

### Figure 7. \( \omega-3 \) FAs Suppress HFD-Induced NLRP3 Inflammasome Activation In Vivo

WT or \( \text{Nlrp}3^{-/-} \) mice were fed with ND or HFD for 10 weeks with or without treatment with DHA. Adipose tissue (WAT) (A, C, E, and F) and liver (B, D, G, and H) were isolated and cultured for 24 hr, and supernatants were analyzed by ELISA for IL-18 (A and B), IL-18 (C and D), TNF-\( \alpha \) (E and G) and MCP-1 (F and H) release. Values are means \( \pm \) SEM of 6–9 mice per group. Caspase-1 activation in WAT was analyzed by immunoblotting as indicated (I). *p < 0.05, **p < 0.01, NS p > 0.05. Data are representative of three independent experiments.
and anti-GPR120 were from Abcam. All tissue culture reagents were bought from Invitrogen.

**Generation of THP-1 Cells Expressing shRNA**

The shRNA targeting the mRNA encoding Arrb2, Gpr40, and Gpr120 were from Sigma. The protocol generating THP-1 cells stably expressing shRNA was described previously (Papin et al., 2007).

**Cell Preparation and Stimulation**

Human THP-1 cells were grown in RPMI 1640 medium, supplemented with 10% FBS and 50 μM 2-mercaptoethanol. THP-1 cell were differentiated for 3 hr with 100 nM phorbol-12-myristate-13-acetate (PMA). Bone marrow macrophages were derived from tibia and femoral bone marrow cells as described elsewhere and cultured in DMEM complemented with 10% FBS, 1 mM sodium pyruvate, and 2 mM L-glutamine (Didierlaurent et al., 2006).

For inducing IL-1β, 10^6 macrophages were plated in 12-well plates overnight and the medium was changed to opti-MEM in the following morning, and then the cells were primed with ultrapure LPS (100 ng/ml) for 3 hr. After that, α-3 FAs were added into the culture for another 1 hr and then the cells were stimulated with MSU (150 μg/ml), R837 (15 μg/ml), and Alum (500 μg/ml) for 6 hr, or α-3 FAs were added into the culture for another 3 hr and then the cells were stimulated with ATP (1 mM) and nigericin (5 μM) for 45 min. For poly(dAdT) transfection, poly(dAdT) (0.5 μg/ml) was transfected by using Lipofectamine (1 μg/ml) as the manufacturer’s protocol (Invitrogen). Cell extracts and precipitated supernatants were analyzed by immunoblotting.

**ELISA**

Supernatants from cell culture or tissue culture were assayed for mouse IL-1β (R&D), human IL-1β (BD biosciences), mouse IL-18 (eBioscience), and mouse MCP-1 (R&D) according to manufacturer’s instructions.

**Tissue Culture**

Adipose or liver tissues were isolated and then washed in cold PBS supplemented with penicillin and streptomycin (HyClone). These tissues were cultured in 12-well plates in opti-MEM medium supplemented with penicillin and streptomycin. After 24 hr, supernatants were collected and stored at −20°C until analyzed.

**Metabolic Studies**

At 6 weeks of age, mice were placed on ND, HFD (D12331, Researchdiets), or HFD with α-3 FAs supplementation for 10 weeks. α-3 FAs (100 mg/kg, twice a week) were administrated by gavage for 6 weeks after mice were fed with HFD for 4 weeks. For testing fasting blood glucose or insulin, the mice were fasted for 8 hr and the samples were collected for glucose measurements by using a Glucometer (Roche) or for insulin measurements by using ELISA. For GTT, mice fasted for 8 hr and were injected intraperitoneally with glucose at a dose of 1.5 mg/g body weight. Blood samples were obtained at different time points for glucose measurements by using a Glucometer. For ITT, mice were injected with 1.5 IU/kg body weight of recombinant human insulin after 2 hr fasting, and blood glucose concentration was determined with the Glucometer.

**Salmonella Infection**

Salmonella was precultured on day 1. On day 2, BMDMs were infected for 1 hr with the Salmonella culture (1:100) and then incubated for 3 hr in the presence of gentamycin (Invitrogen).

**Transfection and Immunoprecipitation**

Constructs were transfected into HEK293T cells by using polyethyleneimine. After 18 hr, cells were collected and resuspended in lysis buffer (50 mM Tris, pH 7.8, 50 mM NaCl, 0.1% [vol/vol] Nonidet-P40, 5 mM EDTA and 10% [vol/vol] glycerol). Extracts were immunoprecipitated with anti-Flag antibody and beads and then were assessed by immunoblotting. THP-1 cells (1 × 10^6) were differentiated with PMA and then were treated with DHA and EPA for different time points. THP-1 cells were then resuspended in lysis buffer and proteins were immunoprecipitated from extracts with anti-NLRP3 antibody.

**Statistical Analyses**

All values are expressed as the mean SEM of individual samples. Samples were analyzed by using Student’s t test unless indicated in the figure legends.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes five figures and can be found with this article online at http://dx.doi.org/10.1016/j.immuni.2013.05.015.

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Immunity

Omega-3 Fatty Acids Inhibits NLRP3 Inflammasome


