Palmitoleate Reverses High Fat-induced Proinflammatory Macrophage Polarization via AMP-activated Protein Kinase (AMPK)*

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Background: Elevated saturated fats during obesity activate proinflammatory pathways in macrophages, contributing to insulin resistance.

Results: The monounsaturated fatty acid cis-palmitoleate antagonizes saturated fat-induced proinflammatory macrophage polarization through an AMPK-dependent mechanism.

Conclusion: Palmitoleate is a lipid mediator that confers an anti-inflammatory macrophage phenotype.

Significance: Understanding lipid-mediated macrophage polarization is critical to develop nutritional or cell-based strategies to combat insulin resistance.

A rise in tissue-embedded macrophages displaying “M1-like” proinflammatory polarization is a hallmark of metabolic inflammation during a high fat diet or obesity. Here we show that bone marrow-derived macrophages (BMDM) from high fat-fed mice retain a memory of their dietary environment in vivo (displaying the elevated proinflammatory genes Cxcl1, Il6, Tnf, Nos2) despite 7-day differentiation and proliferation ex vivo. Notably, 6-h incubation with palmitoleate (PO) reversed the proinflammatory gene expression and cytokine secretion seen in BMDM from high fat-fed mice. BMDM from low fat-fed mice exposed to palmitate (PA) for 18 h ex vivo also showed elevated expression of proinflammatory genes (Cxcl1, Il6, Tnf, Nos2, and Il12b) associated with M1 polarization. Conversely, PO treatment increased anti-inflammatory genes (Mrc1, Tgfb1, Il10, Mgl2) and oxidative metabolism, characteristic of M2 macrophages. Therefore, saturated and unsaturated fatty acids bring about opposite macrophage polarization states. Coincubation of BMDM with both fatty acids counteracted the PA-induced Nos2 expression in a PO dose-dependent fashion. PO also prevented PA-induced IcosB degradation, RelA nuclear translocation, NO production, and cytokine secretion. Mechanistically, PO exerted its anti-inflammatory function through AMP-activated protein kinase as AMP kinase knockout or inhibition by Compound C offset the PO-dependent prevention of PA-induced inflammation. These results demonstrate a nutritional memory of BMDM ex vivo, highlight the plasticity of BMDM polarization in response to saturated and unsaturated fatty acids, and identify the potential to reverse diet- and saturated fat-induced M1-like polarization by administering palmitoleate. These findings could have applicability to reverse obesity-linked inflammation in metabolically relevant tissues.

Insulin resistance is a major contributor to the development of type 2 diabetes, a multiorgan disease affecting more than 300 million people worldwide (1). Overnutrition and obesity lead to a systemic low-grade inflammation (2) characterized by elevated numbers of immune cells, predominantly macrophages, in metabolic tissues such as skeletal muscle, adipose, and liver (3–9). Tissue macrophage infiltration and proliferation have been associated with the appearance of peripheral insulin resistance (2, 4). Although both proinflammatory “M1-like” and anti-inflammatory “M2-like” macrophage counts rise in metabolically relevant tissues, there is a shift in their overall balance in favor of proinflammatory polarized macrophages (5, 6, 10). Conversely, the anti-inflammatory phenotype of muscle macrophages tracks with the insulin sensitivity index of the individual (11). To date, the role and plasticity of the M2-like macrophages within metabolic tissues during obesity remains poorly understood. Moreover, it is unknown whether the phenotype of resident and infiltrating macrophages is acquired within the metabolic tissues or whether high fat feeding also affects cells residing in the bone marrow.

In vitro, macrophages exposed to saturated fatty acids display increased proinflammatory gene expression and cytokine secretion (e.g. TNFα, IL6, and CXCL1/KC) (12–14). Conversely, polyunsaturated fatty acids suppress these inflammatory effects on monocytes/macrophages (15–20). A recent

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study ascribed this protection to activation of AMP-activated protein kinase (AMPK)\(^6\) (21), and, indeed, AMPK activation in vitro and in vivo correlates with macrophage skewing toward an M2 phenotype (22–24).

A cluster of recent studies has revealed that, in vivo, adipose tissue secretes cis-palmitoleate (C16:1 n-7), a monounsaturated fatty acid that, in turn, protects against hepatic steatosis and improves whole-body insulin sensitivity (25–27). Moreover, the circulating levels of palmitoleate in humans strongly correlate with insulin action (28). Notably, however, the direct actions of palmitoleate on immune cells remain unknown.

Here we show that BMDM from high fat-fed mice exhibit increased markers of inflammation and, interestingly, that this phenotype is reversed by ex vivo incubation with palmitoleate. We next investigate the differential effects of palmitoleate alone and in combination with the saturated fatty acid palmitate (C16:0) on BMDM phenotypic skewing. The results indicate that palmitoleate confers an anti-inflammatory M2-like polarization to macrophages ex vivo that can counteract the inflammatory state brought upon by palmitate. Finally, we find that these anti-inflammatory effects are, in part, mediated by AMPK signaling.

**Experimental Procedures**

**Animal Studies**—Rodent studies were approved by The Hospital for Sick Children Animal Care Committee. Male C57BL/6 mice (The Jackson Laboratory, Bar Harbor, ME, stock no. 000664) were fed a low fat (10% by kilocalories; catalog no. D12450B, Research Diets, New Brunswick, NJ) or high fat defined diet (60% by kilocalories; catalog no. D12492, Research Diets, New Brunswick, NJ) for 18 weeks, starting from 9 weeks of age. Cages were maintained at 21–22 °C on a 12-h light cycle. Mice were euthanized via cervical dislocation following a 4-h fast.

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**Isolation and Culture of BMDM**—Bone marrow was extracted from the femur and tibia of male chow-fed (catalog no. 5P07 Prolab RMH 1000, LabDiet, St. Louis, MO) 6- to 8-week-old C57BL/6 (Charles River Laboratories, St. Constant, QC, Canada) or C57BL/6 mice (The Jackson Laboratory) by centrifuging bones at 15,000 × g for 10 s. AMPKβ1 knockout (Prkab1\(^{-/-}\)) mice (generated by Dr. Bruce E. Kemp, St. Vincent's Institute of Medical Research, Melbourne, Australia) and appropriate wild-type controls were made available by Dr. Gregory R. Steinberg (McMaster University, Hamilton, ON, Canada) and have been described previously (29). Extracted cells were cultured in RPMI medium (Wisent, Saint-Bruno, QC, Canada) supplemented with 10% heat-inactivated fetal bovine serum, 1 × nonessential amino acids (Wisent), 1 mM sodium pyruvate (Wisent), 275 μM 2-mercaptoethanol (Life Technologies), and 1 × antibiotic-antimycotic (Wisent). L929 fibroblasts were grown to confluence in low glucose DMEM (Wisent) supplemented with 10% fetal bovine serum and 1 × antibiotic-antimycotic (Wisent) and then washed with PBS and serum-starved for 10 days. Medium was then collected and filtered-sterilized, aliquoted, and stored at −20 °C as L929 conditioned medium. BMDM were seeded at 1 × 10⁶ cells/ml and differentiated using 10% L929 conditioned medium for 7 days at 37 °C, 5% CO₂.

**Reagents**—Palmitate (PA), cis-palmitoleate (PO), cis-oleate (catalog nos. P9767, P9417, and O1008, Sigma-Aldrich, St. Louis, MO), and palmitelaidic acid (trans-PO, catalog no. 9001798, Cayman Chemical, Ann Arbor, MI) stock solutions (200 mM) were prepared in 50% ethanol by heating at 50 °C. Fatty acid-free, low-endotoxin BSA (catalog no. A8806, Sigma-Aldrich) was dissolved in serum-free Eagle’s medium (Wisent) to 10.5%. Fatty acid stocks were diluted 25 x in the BSA solution and conjugated under agitation at 40 °C for 2 h. These solutions (lipid:BSA ratio, 5:1) were aliquoted and stored at −20 °C and then diluted further in cell culture medium. Compound C (catalog no. 171260, Millipore Canada, Etobicoke, ON, Canada) was dissolved in DMSO to a stock concentration of 10 mM before use in cell culture.

**Gene Expression Analysis by qPCR**—RNA was extracted using TRIzol (Life Technologies), and cDNA was synthesized by reverse transcription using the SuperScript VILO cDNA kit (Life Technologies) according to the instructions of the manufacturer. qPCR reactions were run using 10 ng of cDNA and predesigned TaqMan probes (Life Technologies) on a StepOne Plus real-time PCR system (Life Technologies) using the following parameters: one cycle of 95 °C for 20 s, followed by 40 cycles at 95 °C for 1 s and 60 °C for 20 s. Gene expression was normalized to that of the housekeeping genes Abt1 and/or Hprt.

**Nitric Oxide Measurement**—Inducible nitric oxide synthase (iNOS) activity was assessed by the measurement of the NO breakdown product (NO\(_2\)⁻) into cell culture medium using the Griess reagent system. 1 mM sulfanilamide (catalog no. A13001, Alfa Aesar, Ward Hill, MA), 1 N HCl, and 7 mM naphthylethylenediamine (catalog no. 5230-16, Ricca Chemical Co., Arlington, TX) were added to cell culture samples before absorbance was read at 540 nm in a microplate reader.

**ELISA**—Following experimentation, cell culture supernatants were centrifuged at 15,000 × g for 10 min at 4 °C, aliquoted, and stored at −80 °C until analysis. Secreted IL6 and TNFα were analyzed using ELISA MAX Deluxe kits (BioLegend, San Diego, CA) according to the instructions of the manufacturer. Cell culture supernatants were analyzed for CXCL1/KC (keratinocyte chemotactrant) using Mouse KC ELISA (RayBiotech, Norcross, GA).

**Immunoblotting**—Cells were collected in lysis buffer (20 mM Tris, 138 mM NaCl, 2.7 mM KCl, 1 mM MgCl\(_2\), 5 mM EDTA, 1 mM Na\(_2\)VO\(_4\), 20 mM NaF, 1 mM dithiothreitol, 5% glycerol, 1% Nonidet P-40, and 1% protease inhibitor mixture (Sigma-Aldrich)) on ice. Lysates were centrifuged at 15,000 × g for 10 min, and protein concentration was determined in supernatants via bicinchoninic acid assay (Thermo Fisher Scientific, Wilmington, MA). 20 μg of protein was run on a 10% SDS-PAGE gel and transferred to a PVDF membrane (Bio-Rad). Membranes were incubated with antibodies against IκBα (catalog no. 9242, Cell Signaling Technology, Danvers, MA), phospho-AMPKα (Thr-172, catalog no. 2531, Cell Signaling Technology), AMPKα (F6, catalog no. 2793, Cell Signaling Technology).

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\(^6\)The abbreviations used are: AMPK, AMP-activated protein kinase; BMDM, bone marrow–derived macrophages; PA, palmitate; PO, palmitoleate; qPCR, quantitative PCR; iNOS, inducible nitric oxide synthase; OCR, oxygen consumption rate; ANOVA, analysis of variance.
Palmitoleate Reverses Inflammatory Macrophage Polarization

Saturated and Unsaturated Fatty Acids Differentially Regulate Macrophage Polarization—On the basis of the observation that palmitoleate counteracted high fat-induced macrophage inflammation, we aimed to dissect the mechanisms underlying the effect of fats using isolated BMDM from regular chow-fed (6–8% kcal from fat) mice. We compared the action of palmitoleate vis a vis that of palmitate, a same chain length saturated fatty acid of known inflammatory effects. Palmitate is one of the most abundant dietary saturated fatty acids; its plasma concentration approaches 0.4 mM during obesity, with total circulating free fatty acids reaching 1 mM (31, 32). In cell culture, a 0.5 mM palmitate concentration is widely used to recreate a high saturated fat environment (33–35). BMDM from mice were treated with BSA-conjugated PA, BSA-conjugated PO, or BSA alone as vehicle control, and macrophage polarization was assessed by expression of proinflammatory and anti-inflammatory genes through qPCR. BMDM exposed to 0.5 mM PA for 18 h showed elevated expression of proinflammatory genes (Ccl3, Cxcl1, Il6, Nos2, and Tnf) associated with M1 macrophage polarization relative to the PO-treated BMDM (Fig. 2). Conversely, anti-inflammatory M2 genes (Mrc1, Tgfβ1, Il10, Arg1, Chi3l3, and Mgl2) rose with PO treatment relative to the PA-treated group. These results demonstrate that saturated and unsaturated fatty acids lead to distinct macrophage polarization states, with PA producing an M1-like phenotype and PO producing an M2-like phenotype.

Palmitoleate Selectively Prevents Palmitate-induced Macrophage Inflammation—Given that PO both increased anti-inflammatory markers in macrophages and reversed the proinflammatory signature seen in macrophages generated from high fat-fed mice, we next tested whether it could offset the inflammatory markers in macrophages generated from high fat-fed mice. BMDM from high fat-fed mice displayed higher levels of proinflammatory gene expression (Nos2, Il6, Cxcl1, and Tnf) compared with macrophages from low fat-fed mice (Fig. 1).

Palmitoleate is a monounsaturated fatty acid produced by adipose tissue that has been correlated with the relief of insulin resistance in liver and skeletal muscle. We therefore explored whether palmitoleate could counteract the BMDM proinflammatory cascade caused by chronic high fat feeding. Notably, ex vivo incubation of BMDM with palmitoleate for 6 h reversed the proinflammatory gene expression (Fig. 1, A–D) and cytokine secretion (Fig. 1, B and D) seen in BMDM derived from high fat-fed mice. These findings highlight the plasticity of macrophage polarization and their capability to regulate their functional phenotype in response to lipid mediators such as palmitoleate.

Macrophages from High Fat-fed Mice Display Inflammation, Which Can Be Reversed by Palmitoleate—A hallmark feature of metabolic inflammation is a shift of tissue macrophages toward an M1-like polarization during a high fat diet or obesity. However, it is not known whether BMDM from high fat-fed mice retain a memory of their dietary in vivo environment. This is an important question because the generation of BMDM from naïve bone marrow cells requires 7 days of incubation with growth factors ex vivo. To explore the possibility of this “dietary memory,” we generated BMDM from C57BL/6J mice fed either a high fat (60% kcal from fat) or low fat (10% kcal from fat) diet for 18 weeks. Strikingly, BMDM from high fat-fed mice displayed higher levels of proinflammatory gene expression (Nos2, Il6, Cxcl1, and Tnf) compared with macrophages from low fat-fed mice (Fig. 1).

Statistical Tests—Data are expressed as mean ± S.E. An unpaired Student’s t test or one-way ANOVA with Bonferroni or Tukey post test was used to detect differences between means in datasets containing multiple groups. A two-way ANOVA with Bonferroni or Tukey post test was used to detect differences in datasets containing two variables. Statistical significance was set at p < 0.05. Graphs were prepared and data were analyzed using GraphPad Prism version 6 (GraphPad Software, La Jolla, CA).

Results

Macrophages from High Fat-fed Mice Display Inflammation, Which Can Be Reversed by Palmitoleate—A hallmark feature of metabolic inflammation is a shift of tissue macrophages toward an M1-like polarization during a high fat diet or obesity. However, it is not known whether BMDM from high fat-fed mice
estingly, this anti-inflammatory effect was specific to the cis form of palmitoleate because neither trans-palmitoleate nor the monounsaturated fatty acid oleate reduced PA-induced Nos2 expression (Fig. 3B). Therefore, the anti-inflammatory effects of PO on macrophages appear to be a selective property not shared by other monounsaturated fatty acids, including the abundant dietary fatty acid oleate.

The preventive action of PO was further characterized by examining its effects on the genes most up-regulated by PA, seen in Fig. 2. In addition to Nos2, PO (0.5 mM) prevented PA-induced expression of Il6 and Cxcl1 (Fig. 4, A–C). If functional, the increase in Nos2/iNOS should lead to NO production, a key product of the immune response. We therefore analyzed nitrite levels as a reflection of NO production as well as the release of the proinflammatory cytokines IL6 and CXCL1/KC. The results paralleled those in gene expression, with PO preventing PA-induced NO production and cytokine secretion (Fig. 4, D–F).

Each of the proinflammatory genes analyzed above are under transcriptional regulation by NFκB. Therefore, we examined whether PO would inhibit activation of the NFκB pathway in BMDM. The results clearly revealed that PA induced IκBα degradation and nuclear translocation of the NFκB p65 (RelA) subunit. Addition of PO during the incubation with PA prevented both IκBα degradation and RelA nuclear translocation (Fig. 5), reversing the values to those observed in the BSA-treated control group. Collectively, these results demonstrate that PO exerts a protective, anti-inflammatory effect on macrophages by antagonizing saturated fat-induced NFκB activation, iNOS production of NO, and proinflammatory cytokine gene expression as well as the consequent cytokine secretion.

Palmitoleate Increases Fatty Acid Oxidation—A hallmark of M2 macrophages is a shift from glycolytic toward oxidative metabolism (36, 37). Because PO induced an M2-like macrophage polarization, we analyzed whether PO increased oxi-
dative respiration in macrophages. As expected, the lipids increased the OCR relative to the BSA control in both the PA and PO treatment groups. However, the OCR was greater in macrophages given PO compared with those given PA (Fig. 6, A and B). Interestingly, when coincubated with both PA and PO together, the OCR resembled the phenotype seen in the PO-treated group (Fig. 6C) despite the presence of twice as much lipid as under either the PA or PO conditions. These results indicate that, when exposed to PO, macrophages increase fatty acid oxidation and rely less on glycolysis.

**AMPK Mediates the Anti-inflammatory Effects of Palmitoleate**—Because direct AMPK activation up-regulates fatty acid oxidation in tissues (38, 39) and can antagonize saturated fatty acid-induced inflammation (23, 24), we tested whether PO would exert its anti-inflammatory function on BMDM through AMPK. After 30 min, PO elevated AMPK phosphorylation in BMDM relative to the BSA-treated control group, whereas PA decreased AMPK phosphorylation. Under coincubation with both PA and PO, AMPK phosphorylation levels were similar to those seen with PO alone (Fig. 7A), highlighting that AMPK activation is specific to the unsaturated fatty acid. By 18 h, there were no significant differences in AMPK phosphorylation between treatment groups (data not shown). Although a drop in cellular ATP levels is a signal for AMPK activation, we could not find any differences in cytosolic ATP levels between any of the treatment groups. The nucleotide levels did not change at 30 min of incubation with palmitoleate, when AMPK phosphorylation is clearly observed (Fig. 7B), nor at earlier time points (data not shown), suggesting that AMPK may be activated by PO through an ATP-independent mechanism. To evaluate whether AMPK activity was necessary for the anti-inflammatory effects of PO, we used an AMPK inhibitor, Compound C, in conjunction with fatty acid treatments of BMDM. As shown above, PO prevented the IκB degradation induced by PA. However, AMPK inhibition diminished the ability of PO to prevent PA-induced IκB degradation (Fig. 7C). Accordingly, the PO-induced prevention of PA-induced Nos2 expression (Fig. 7D) and NO production (Fig. 7E) were abolished in the presence of Compound C.
were voided when AMPK was inhibited using Compound C. Furthermore, a similar trend is seen in AMPK knockout macrophages (Fig. 7E), which lack the β1 subunit essential for assembling the AMPK heterotrimer, resulting in AMPK degradation (29). These results demonstrate that AMPK partially mediates the anti-inflammatory effects of PO on BMDM.

**Discussion**

The main observations of this study are that the omega-7 monounsaturated fatty acid cis-palmitoleate can readily antagonize or reverse proinflammatory macrophage polarization induced by a high fat diet in vivo or by palmitate in vitro and that AMPK participates in this reversal of macrophage metabolic inflammation.

**Inflammatory Status of BMDM from High Fat-fed Mice—**

Over the past decade, high fat diets have been linked to tissue macrophage inflammation contributing to peripheral insulin resistance (2, 4). *Ex vivo*, saturated fatty acids evoke a proinflammatory response in primary and cell line macrophages (12, 13), and products thereof confer insulin resistance to muscle cells in culture (14, 40). The inflammatory status of macrophages induced by a high fat diet has been shown previously in tissue-resident macrophages, including muscle and adipose tissue macrophages and liver Kupffer cells (5, 6, 8). Strikingly, we observed *ex vivo* persistence of a dietary

7F were voided when AMPK was inhibited using Compound C. Furthermore, a similar trend is seen in AMPKβ1 knockout macrophages (Fig. 7E), which lack the β1 subunit essential for assembling the AMPK heterotrimer, resulting in AMPKα degradation (29). These results demonstrate that AMPK partially mediates the anti-inflammatory effects of PO on BMDM.
memory displayed by macrophages derived from the bone marrow of high fat-fed mice, which remained following 7 days of cellular differentiation \textit{ex vivo}.

Current thinking has been that immune cells become polarized upon exposure to fatty acids in the circulation or target tissues. Our findings raise the possibility that these fatty acids may first affect precursor cells in the bone marrow, where the majority of circulating monocytes originate, potentially initiating inflammatory programs in monocytes before they reach the circulation. Future studies should investigate how fats act on the bone marrow and how the \textit{ex vivo} dietary memory is maintained, whether through shifts in bone marrow cell populations, epigenetic modifications, or other mechanisms.

Using BMDM from high fat-fed mice as a model of metabolically inflamed macrophages, our next aim was to identify novel anti-inflammatory lipid mediators. Cao \textit{et al.} (25) have highlighted \textit{cis}-palmitoleate as an adipose-derived "lipokine" with beneficial insulin-sensitizing effects on a whole-body level (25). Notably, we found that observed in macrophage cell lines and BMDM treated with diverse saturated fatty acids (41, 42). Notably, we found that coinubcation with palmitoleate inhibited each of these palmi-

![FIGURE 7. AMPK mediates the anti-inflammatory effects of palmitoleate.](image-url)

**Mechanism of Palmitoleate Counteraction of the Proinflammatory Action of Palmitate on BMDM**—To investigate the mechanism of action of palmitoleate to counteract fat-induced inflammation, we tested its effect on palmitate-challenged BMDM from chow-fed mice. Palmitate-challenged BMDM displayed activation of the NF\textalpha activation in myotubes (55).

Toll-like receptor 4 (TLR4) has been linked to saturated fat-induced inflammation in many cell types, and its inhibition or depletion protects against diet-induced inflammation and insu-

lin resistance (12, 45, 46). However, it is unlikely that the counteraction of palmitate effects by palmitoleate is simply the result of TLR4 inhibition because palmitoleate did not completely abolish BMDM inflammation evoked by the TLR4 agonist lipopolysaccharide (data not shown). Similarly, we deem it
unlikely that palmitoleate simply prevents or competes for palmitate uptake into macrophages because the disappearance of fatty acids from cell culture medium was additive upon coinubcation with both fatty acids (data not shown). Further, palmitoleate exerted anti-inflammatory effects independently of the presence of palmitate, as in the case of BMDM from chow-fed and high fat-fed mice.

**Role of AMPK in Palmitoleate Anti-inflammatory Action**— Instead, our results indicate that the anti-inflammatory effects of palmitoleate involve AMPK signaling because palmitoleate administration activated AMPK, and an AMPK inhibitor offset the anti-inflammatory input of palmitoleate on palmitate-challenged BMDM. The involvement of AMPK in the anti-inflammatory and counterinflammatory actions of palmitoleate is reminiscent of the reported AMPK activation by polyunsaturated fats (21) and the association of AMPK with anti-inflammatory M2 macrophage polarization, oxidative metabolism, and NFκB inhibition (22, 47, 48). The mechanism through which any lipid, including palmitoleate, activates AMPK is still unknown. AMPK functions as a master cellular energy sensor, becoming activated during low-energy states, and, therefore, appears as a converging signal linking nutrient sensing to inflammation in immune cells.

Although a drop in the ATP/AMP ratio is a signal for AMPK activation and some studies show that polyunsaturated fats inhibit glycolysis or can deplete cellular energy through mitochondrioucoupling (49–51), we did not detect any changes in cytosolic ATP levels under any of the treatments studied. Moreover, palmitoleate increased oxidative metabolism in BMDM, as expected from AMPK activation. We hypothesize that AMPK activation by palmitoleate occurs through a nucleotide-independent mechanism. Indeed, other signaling pathways impact AMPK activity status, and a number of upstream kinases, including Ca2+/calmodulin dependent kinase kinase B, can activate AMPK (52, 53).

It is also conceivable that palmitoleate acts through a receptor to activate signals upstream of AMPK. Oh et al. (54) identified the G protein-coupled receptor 120 (GPR120) as a sensor mediating anti-inflammatory effects of polyunsaturated fats (54). Although that study focused on omega-3 polyunsaturated fatty acids, palmitoleate appeared during their screen of fatty acids that can activate GPR120 in HEK293 cells, raising the possibility that GPR120 may act as the receptor for monounsaturated fatty acids as well. Curiously, the monounsaturated fatty acid oleate also appeared to activate GPR120 in that study. However, we did not observe any anti-inflammatory effects of oleate on BMDM, indicating that GPR120 activation may not necessarily lead to an anti-inflammatory phenotype under all circumstances. We also found that other monounsaturated fats, including trans-palmitoleate, are unable to antagonize palmitate-induced Nos2 expression, highlighting the specificity of cis-palmitoleate to offset palmitate-induced inflammation of BMDM. This result correlates with a recent observation that palmitoleate, but not oleate, can attenuate diet-induced glucose and insulin intolerance in mice (27).

Finally, considering the association between glycolytic metabolism with proinflammatory M1 macrophage polarization and of oxidative metabolism with anti-inflammatory M2 polarization (37, 39), future experiments should address whether changes in these metabolic pathways are a cause or consequence of the phenotypic skewing of cells.

Overall, our study demonstrates an AMPK-mediated anti-inflammatory effect of the monounsaturated fatty acid cis-palmitoleate in macrophages that can offset the macrophage inflammation conferred by palmitate or a prior high fat diet in vivo. These findings may provide the basis for dietary interventions or for cell-specific strategies to treat inflammatory diseases, including obesity-linked type 2 diabetes.

**Author Contributions**—K. L. C. participated in the design of the study, coordinated and carried out experiments, performed data analysis, and wrote the manuscript. N. J. P. participated in scientific discussion and assisted with the experiments shown in Fig. 1. D. M. S. helped with the experiments and data analysis shown in Figs. 2–4. S. R. C. participated in the study design and assisted with the experiments shown in Fig. 1. Z. L. provided technical assistance throughout the study and participated in the experiments shown in Figs. 2–4. M. T. and B. C. advised on the experiments for Fig. 7 and provided input in the writing of the manuscript. A. K. conceived and coordinated the study and participated in the writing of the manuscript. All authors reviewed the results and approved the final version of the manuscript.

**Acknowledgments**—We thank Dr. Philip J. Bilan for insightful discussions throughout the course of this study, Dr. Yi Sun for help with immunofluorescence, and the SPARC Biocenter at The Hospital for Sick Children for help with performing the cell metabolism analysis. We also thank Dr. Bruce Kemp and Dr. Gregory Steinberg for generating and making available AMPKβ1−/− mice.

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