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Fractalkine (CX3CL1) Is Involved in the Early Activation of Hypothalamic Inflammation in Experimental Obesity

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Hypothalamic inflammation is a common feature of experimental obesity. Dietary fats are important triggers of this process, inducing the activation of toll-like receptor-4 (TLR4) signaling and endoplasmic reticulum stress. Microglia cells, which are the cellular components of the innate immune system in the brain, are expected to play a role in the early activation of diet-induced hypothalamic inflammation. Here, we use bone marrow transplants to generate mice chimeras that express a functional TLR4 in the entire body except in bone marrow–derived cells or only in bone marrow–derived cells. We show that a functional TLR4 in bone marrow–derived cells is required for the complete expression of the diet-induced obese phenotype and for the perpetuation of inflammation in the hypothalamus. In an obesity-prone mouse strain, the chemokine CX3CL1 (fractalkine) is rapidly induced in the neurons of the hypothalamus after the introduction of a high-fat diet. The inhibition of hypothalamic fractalkine reduces diet-induced hypothalamic inflammation and the recruitment of bone marrow–derived monocytic cells to the hypothalamus; in addition, this inhibition reduces obesity and protects against diet-induced glucose intolerance. Thus, fractalkine is an important player in the early induction of diet-induced hypothalamic inflammation, and its inhibition impairs the induction of the obese and glucose intolerance phenotypes.

A complex network of neurons that are responsive to hormonal, neural, and nutritional cues tightly regulates body adiposity (1,2). Cells of the medium-basal hypothalamus act as first-order neurons in this system, and many genetic, pharmacological, and environmental approaches that lead to the damage or loss of some of these neurons can affect body energy homeostasis (1,2). In many experimental models, hypothalamic dysfunction that results from local inflammation plays a central role in the pathogenesis of obesity (3,4). In addition, recent studies using neuroimaging have provided strong evidence for the existence of inflammation and dysfunction in the hypothalamus of obese humans (5,6).

Dietary long-chain saturated fatty acids are the main triggers of hypothalamic inflammation in obesity (7). These fatty acids act through toll-like receptor 4 (TLR4) (7) and induce endoplasmic reticulum stress (4,7), leading to the activation of intracellular inflammatory signaling pathways through Jun NH_2-terminal kinase (JNK), nuclear factor-κB, and protein kinase C-θ (PKC-θ) (3,4,8). The increased hypothalamic expression of inflammatory cytokines is detectable as early as 1 day after the introduction of a fat-rich diet to rodents (6). Upon prolonged high-fat feeding, signals of cellular damage become evident, such as gliosis (6), apoptosis (9), and defects in the potential for neurogenic recovery (10). Thus, diet-induced hypothalamic inflammation follows a classical path of the inflammatory response, which is detectable a few hours after the exposure to the threatening stimulus, and progresses with a wide array of damaging/recovering outcomes.

An important missing link between the exposure to dietary fats and the induction and perpetuation of

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OBESITY STUDIES

inflammation in the hypothalamus is cells first sensing the presence of the fatty acids and cells effectively generating and maintaining the inflammation. In at least one study, hypothalamic neurons were unable to respond to palmitate-producing cytokines (11). Thus, glial cells, particularly microglia, may be an important requirement of the system. In other inflammatory conditions of the central nervous system, the activation of microglia plays an important role in the progression of disease (12). Both resident and bone marrow–derived monocytic cells can, to a variable degree, exert the inflammatory and immunosuppressive actions that are frequently observed in chronic neuroinflammatory conditions (12). The recruitment and activation of microglia to the site of damage depends on the expression of chemokines and their receptors (13). Fractalkine (CX3CL1) is one of the most important chemokines that are involved in the regulation of neuroinflammatory conditions (13,14). In this context, fractalkine is produced and released by neurons, acting as a mediator in their talk with glial cells (13,14).

In the current study, we first show that bone marrow–derived cells are involved in the activation of diet-induced inflammation of the hypothalamus in obesity. Next, we show that saturated fatty acids induce an early expression of fractalkine in hypothalamic neurons and that the inhibition of this chemokine reduces diet-induced hypothalamic inflammation and body mass gain in an obesity-prone strain of mice.

RESEARCH DESIGN AND METHODS

Experimental Animals

Six-week-old (4-week-old for mice that underwent bone marrow transplantation) male Swiss, Balb-c, C57BL/6J, TNFRp55$^{-/-}$, TNFRp55$^{+/?}$, C3H/HeJ, C3H/Hepas, IL10$^{-/-}$, or IL10$^{+/?}$ mice were used in this study, which was approved by the ethics committee of the University of Campinas. Swiss mice are an outbreed related to the obesity- and diabetes-prone AKR; Balb-c mice are inbred and partially protected from metabolic diseases; C57BL/6J is wild-type control for TNFRp55$^{-/-}$ and IL10$^{-/-}$; TNFRp55$^{-/-}$ is a knockout for the p55 tumor necrosis factor (TNF)α receptor, and C3H/HeJ is a loss-of-function mutant for the TLR4 (HeJ/Hepas is its wild-type control) (both are partially protected from diabetes and obesity due to a reduced inflammatory response when fed a high-fat diet [HFD]); finally, the IL10$^{-/-}$ mouse is a knockout for interleukin (IL)10 and is prone to diabetes and obesity due to increased inflammation in response to dietary fats. In some experiments, mice were randomly selected for feeding on chow or an HFD (35% fat, measured in grams; 5.2 kcal/g) for 2–8 weeks. In some experiments, mice were stereotaxically instrumented using a Stoelting stereotaxic apparatus set on the coordinates anteroposterior, 0.0 mm; lateral, 0.0 mm; and depth, 4.8 mm—or bilaterally, to the arcuate nucleus, according to the following coordinates: anteroposterior, −1.7 mm; lateral, 0.3 mm; and depth, 5.6 mm. Single doses of a small interfering RNA (SI) against fractalkine (sense: GCC GCG UUC UUC CAU U; antisense: ACA AAU GGA AGA ACG C) or a scrambled control (SC) (sense: CAG GCU ACU UGG AGU G; antisense: AUA CAC UCC AAG UAG C) were delivered through the cannula, which was immediately withdrawn. The adequacy of the cannulation procedure was ascertained by randomly selecting some animals for methylene blue injection and posterior anatomical evaluation and by the capacity of the SI to inhibit the expression of fractalkine only near the injection site.

Cell Culture

The neuron cell line, mHypoA 2/29 CLU189, was cultivated in DMEM containing 25 mmol/L glucose and 10% FBS to reach 70% confluence. The cells were exposed to either palmitate or TNFα according to doses and times as described in RESULTS. After incubation, the cells were harvested for RNA preparation for real-time PCR. The macrophage cell line, Raw 264.7, was cultivated in DMEM containing 11 mmol/L glucose and 10% FBS. The cells were exposed to either palmitate or TNFα according to doses and times as described in RESULTS.

Bone Marrow Transplantation

During the preparation for radiation, mice were treated with a daily dose of sulfametoxazole (4.0 mg/kg) plus trimethoprim (0.8 mg/day) for 4 days. A sublethal dose of radiation (8 Gy) was used, and after 2 h, the mice received an intravenous (tail vein) injection of a bone marrow cell preparation that contained 5.0×10^6 cells. The details of the protocol have previously been published (15).

Hyperinsulinemic-Euglycemic Clamp

Glucose consumption was assessed after a 12-h fast using a method previously described (16).

DEXA

Mice were anesthetized, and the body fat mass and lean mass contents were measured using a DEXA system (Discovery Wi QDR Series; Hologic Apex Software, Hologic Inc.).

Immunoblotting

Liver specimens were homogenized and samples containing 50–125 μg protein were used in immunoblot experiments as previously described (9). Ser^{473} phospho-Akt was detected in the membranes using specific antibodies (pAKT sc7985-R; Santa Cruz Biotechnology, Santa Cruz, CA). Loading was evaluated by reblotting the membranes with an anti-Akt antibody (sc8312; Santa Cruz Biotechnology).

Flow Cytometry

Hypothalamus was dissected on ice-cold phosphate buffer and chopped in very small fragments that were transferred to a tube and centrifuged gently for 30 s. The anatomical limits for the dissection of hypothalamus were as follows: rostral, optic chiasm; caudal, mammillary bodies; lateral, optic tracts; and superior, apex of the

hypothalamic third ventricle. Trypsin (250 mg/mL) was added, and incubation was carried out at 37°C for 10 min under gently shaking conditions. DMEM and FCS were added to the suspension, which was thereafter filtered through a 100 μm nylon mesh. Cells were recovered after centrifugation and washed three times in ice-cold phosphate buffer. After counting, cells were incubated with specific antibodies and analyzed using a Becton-Dickinson FACSCalibur Cytometer (San Jose, CA) with the CellQuest Pro software (Becton-Dickinson) (17). For each measurement, cells were obtained from the hypothalamus of one mouse. Cell suspensions were used to determine DNA fragmentation and membrane integrity. The method was based on propidium iodide staining and fluorescence, which was measured using a flow cytometer. First, 50 μL isotonic solution containing propidium iodide (8.0 μg/mL in PBS) was used to homogenize 200 μL cell suspension. Fluorescence was measured using the FL2 channel (orange-red fluorescence; 585/42 nm).

Real-Time PCR

TNFα, IL1β, MCP1, CCR2, CX3CL1, CX3CR1, CD11b, CD36, CD163, and TLR4 mRNAs were measured in hypothalamus or cell culture by real-time PCR (ABI Prism 7500 detection system; Applied Biosystems, Grand Island, NY). The anatomical limits for the dissection of hypothalamus were as follows: rostral, optic chiasm; caudal, mammillary bodies; lateral, optic tracts; and superior, apex of the hypothalamic third ventricle. The intron-skipping primers were obtained from Applied Biosystems: TNFα, Mm00443258_m1; IL1β, Mm00434228_m1; MCP-1, Mm99999056_m1; CCR2, Mm00438270_m1; CX3CL1, Mm00436454_m1; CX3CR1, Mm00438354_m1; CD11b, Mm00434455_m1; CD36, Mm01135198_m1; CD163, Mm00474091_m1; and TLR4, Mm00445273_m1. GAPDH (cat. no. 4352339E, Applied Biosystems) was used as endogenous control. Real-time data were analyzed using the Sequence Detector System 1.7 (Applied Biosystems).

Immunofluorescence Staining

For histological evaluation, hypothalamic tissue samples were fixed in paraformaldehyde (4% final concentration in PBS) and processed routinely for embedding in a paraffin block. Five-micrometer paraffin sections were processed for immunofluorescence staining using the following primary antibodies: F4/80 (sc25830), CD11b (sc28664), CD11c (sc26693), Iba1 (sc28530), GFP (sc5385), AgRP (sc18634), and POMC (sc20148) (all from Santa Cruz Biotechnology, Santa Cruz, CA); fractalkine (CX3CL1) (cat. no. ab25088; Abcam, Cambridge, MA); and the secondary antibodies conjugated to FITC or rhodamine (cat. nos. sc2777 and sc2092, respectively; Santa Cruz Biotechnology). In some experiments, the neuron cell line mHypoA 2/29 CLU189 was cultivated in glass slides for further fixation in 4% paraformaldehyde and preparation for indirect immunofluorescence staining with anti-AgRP and anti-fractalkine antibodies.

Statistical Analysis

Results are presented as means ± SE. Levene test for the homogeneity of variances was initially used to check the fit of data to the assumptions for parametric ANOVA. All results were analyzed by *t* test or one-way ANOVA and complemented by the Tukey test to determine the significance of individual differences. The level of significance was set at $P < 0.05$.

RESULTS

TLR4-Expressing Bone Marrow–Derived Monocytic Cells Are Required for the Induction of the Obese Phenotype

A deficiency of TLR4 protects against diet-induced hypothalamic inflammation and obesity (7). In the central nervous system, TLR4 is predominantly expressed in microglia cells (18), which potentially place these cells in a pivotal position as triggers of hypothalamic inflammation in obesity. Here, we performed bone marrow transplants to generate chimeras that expressed functional TLR4 in all cells of the body except those derived from the bone marrow and chimeras that expressed functional TLR4 only in bone marrow–derived cells. Supplementary Fig. 1*A* depicts the protocol that was used to generate the chimeras and controls, and Supplementary Fig. 1*B* depicts PCR-amplified, BstOI-digested fragments that were generated from transcripts of TLR4-deficient and functional TLR4–expressing cells, which were used to monitor the efficiency of the transplants at 6 weeks of age. Four weeks on an HFD lead to the activation of microglia only in mice that express a functional TLR4 (Fig. 1*A*). However, chimeras that only express a functional TLR4 (and GFP) in bone marrow–derived cells present a hypothalamic infiltration of active microglia cells upon high-fat feeding (Fig. 1*B*). Chimeras that only express nonfunctional TLR4 in bone marrow–derived cells are protected from diet-induced obesity and hypothalamic inflammation (Fig. 1*C–G* and Supplementary Fig. 1*C*), whereas chimeras that only express functional TLR4 in bone marrow–derived cells adopt the obese phenotype with a deposition of fat in the visceral territory and increased expression of inflammatory cytokines in the hypothalamus (Fig. 1*C–G* and Supplementary Fig. 1*C*).

Fractalkine Is Induced in Hypothalamic Neurons of Obesity-Prone but Not of Obesity-Protected Mice

For investigation of chemokines and other inflammatory factors that are potentially involved in the hypothalamic recruitment of bone marrow–derived monocytic cells during the induction of obesity, we compared an obesity-prone strain, the Swiss mouse, and an obesity-protected strain, the Balb-c mouse. Supplementary Fig. 2*A–L* depicts the main metabolic and endocrine differences between the two strains. In contrast to experimental models of obesity (3,6,7), Balb-c mice present only a mild and transient increase in the expressions of hypothalamic TNFα (Fig. 2*A*) and IL1β (Fig. 2*B*). While obesity-prone Swiss mice present an early (1 day) activation of several markers

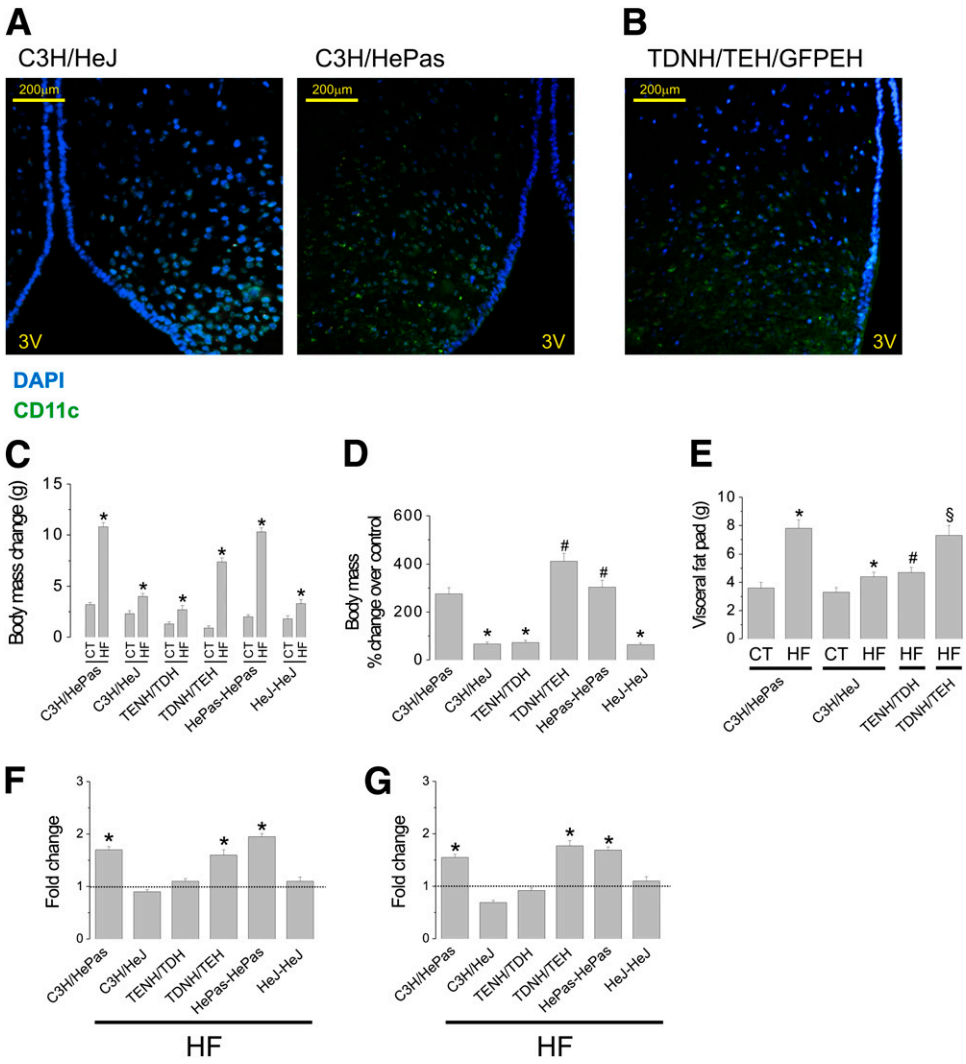

Figure 1—*A*: Cd11c indirect immunofluorescence staining of paraformaldehyde-fixed, paraffin-embedded, 5.0-μm hypothalamic sections (at approximately Bregma −1.7) that were obtained from C3H/HeJ and C3H/HePas, which were fed an HFD for 4 weeks; nuclei were counterstained with DAPI. *B*: Detection of GFP-expressing cells in paraformaldehyde-fixed, paraffin-embedded, 5.0-μm hypothalamic sections (at approximately Bregma −1.7) that were obtained from C3H/HeJ that were transplanted with bone marrow from a whole-body GFP-expressing transgenic mouse and fed an HFD for 4 weeks. *C–G*: Mice that were submitted to the transplantation protocols were fed regular chow (CT) or an HFD for 4 weeks and were evaluated for total body mass change (*C*), body mass change relative to the control (*D*), visceral fat pad (*E*), and determination of TNFα (*F*) and IL1β (*G*) expression in the hypothalamus. In *F* and *G*, at the end of the respective experimental periods, hypothalami were obtained for RNA extraction. The cDNA that was produced from total RNA was used in real-time PCR assays. The results are expressed as the fold change compared with the level of respective gene expression in the hypothalamus of lean (chow-fed), strain-specific controls. In all experiments, $n = 6$. *C*: *$P < 0.05$ compared with the respective CT. *D*: *$P < 0.05$ compared with C3H/HePas, #$P < 0.05$ compared with C3H/HeJ. *E*: *$P < 0.05$ compared with the respective CT, #$P < 0.05$ compared with C3H/HePas HF, §$P < 0.05$ compared with C3H/HeJ HF. *F* and *G*: *$P < 0.05$ compared with the respective controls fed on chow. TDNH/TEH, TLR4-deficient in nonhematopoietic tissue/TLR4-expressing hematopoietic tissue; TENH/TDH, TLR4-expressing hematopoietic tissue/TLR4-deficient nonhematopoietic tissue; TDNH/TEH/GFPEH, TLR4 deficient in nonhematopoietic tissue/TLR4-expressing hematopoietic tissue/GFP-expressing hematopoietic tissue. HF, high-fat diet.

of inflammation in the hypothalamus, which include two chemokines, MCP1 and fractalkine (Fig. 2*C–J*), Balb-c mice present a delayed activation of only some of these markers, which are MCP1 (Fig. 2*C*) and its receptor, CCR2 (Fig. 2*D*), CD11b (Fig. 2*G*), CD36 (Fig. 2*H*), and CD163 (Fig. 2*J*). Importantly, Balb-c mice present no activation of the expression of fractalkine (Fig. 2*E*) or its receptor, CX3CR1 (Fig. 2*F*). Because fractalkine is an important chemokine that is involved in neural inflammation (13,14) we decided to focus on its potential role in the induction of diet-induced hypothalamic inflammation in obesity and as a factor involved in the recruitment of bone marrow–derived monocytic cells to the hypothalamus.

Fractalkine Is Expressed in Hypothalamic Neurons

Obesity-prone Swiss mice that were fed for 4 weeks on an HFD exhibit the preferential expression of fractalkine in neurons of the hypothalamus (Fig. 3*A–D*); however, some

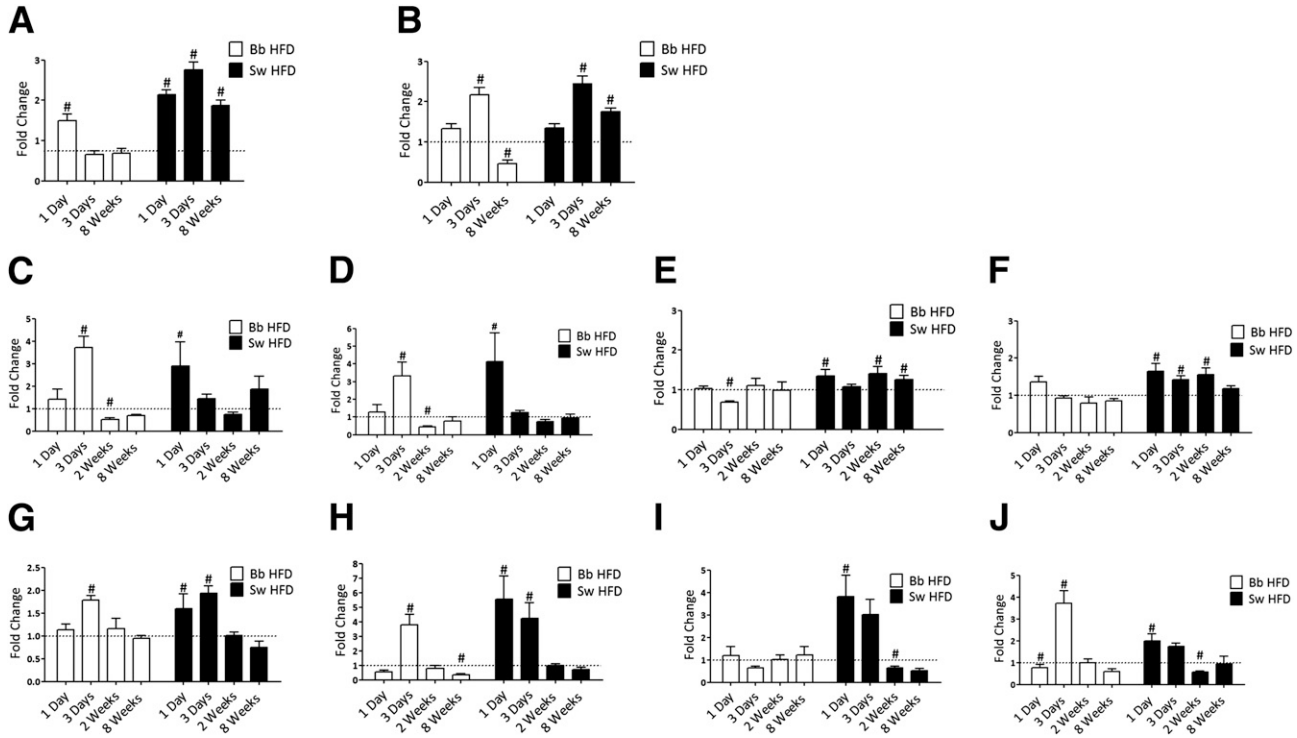

Figure 2—Six-week-old male Balb-c (Bb) or Swiss (Sw) mice were fed an HFD for 1 day to 8 weeks, as depicted in the panels. At the end of the respective experimental periods, hypothalami were obtained for RNA extraction. The cDNA that was produced from total RNA was used in real-time PCR assays to determine the expression of TNFα (*A*), IL1β (*B*), MCP1 (*C*), CCR2 (*D*), fractalkine (CX3CL1) (*E*), fractalkine receptor (CX3CR1) (*F*), CD11b (*G*), CD36 (*H*), MRC1 (*I*), and CD163 (*J*). The results are expressed as the fold change compared with the level of respective gene expression in the hypothalamus of lean (chow-fed), strain-specific controls. In all experiments, $n = 6$; #$P < 0.05$ compared with the respective lean control.

few cells expressing CD11b (Fig. 3*A*) or F4/80 (Fig. 3*B*) seem to express small amounts of fractalkine as well. In addition, both palmitate and TNFα induce a dose- and time-dependent increase in fractalkine in a hypothalamic neuronal cell line (Fig. 3*E*, *I*, *K*, and *L*). This increase in fractalkine is accompanied by the increased expression of MCP1 (Fig. 3*F* and *J*). Remarkably, whereas TNFα is capable of inducing the expression of IL1β but not of TLR4, palmitate produces an increase in TLR4 but not in IL1β (Fig. 3*G* and *H*). Finally, immunofluorescence staining reveals that palmitate produces changes not only in the expression of fractalkine but also in the morphology of the cells and in the distribution of AgRP granules, which become more concentrated in the perinuclear area (Fig. 3*K* and *L*). In contrast to neurons, a monocyte-macrophage cell line does not express fractalkine even after a stimulus with palmitate (Fig. 3*M*). CX3CR1, the fractalkine receptor, is expressed by the monocyte-macrophage cell line but undergoes no change after palmitate stimulus (Fig. 3*M*). Conversely, both TLR4 and TNFα are induced in response to palmitate in the same monocyte-macrophage cell line (Fig. 3*N* and *O*).

TNFR1 Is Required for the Diet-Induced Expression of Fractalkine in the Hypothalamus

Because fractalkine is expressed only in neurons, which produce no inflammatory response when exposed to fatty acids (11), we hypothesized that cytokines, particularly TNFα, which is produced by microglia in response to fatty acids, would mediate the spreading of inflammation from cells of the innate immune system toward the neurons. For testing of this hypothesis, TNFR1 knockout mice were fed for 8 weeks on an HFD, and inflammatory markers were evaluated in the hypothalamus. As previously reported (19), TNFR1 knockout mice are protected from diet-induced obesity (Fig. 4*A*). Although hypothalamic TNFα is increased in mice that are fed the HFD (Fig. 4*B*), this increase was accompanied by no changes in the expression of other markers of inflammation, such as IL6 (Fig. 4*C*), IL1β (Fig. 4*D*), and IL10 (Fig. 4*E*); additionally, no changes in the expression of either fractalkine (Fig. 4*F*) or its receptor were observed (Fig. 4*G*). Conversely, when the inflammation-prone IL10 knockout mice were fed an HFD for 8 weeks, body mass increased significantly (Fig. 4*H*), and the expression of inflammatory cytokines (Fig. 4*I*–*K*) and fractalkine (Fig. 4*L*) and its receptor was stimulated (Fig. 4*M*). Additional controls for these experiments are presented in Supplementary Fig. 3.

Inhibition of Hypothalamic Fractalkine Reduces Diet-Induced Hypothalamic Inflammation and Corrects Glucose Intolerance

Using an SI approach, we obtained up to a 70% reduction in the expression of fractalkine in the AgRP-expressing

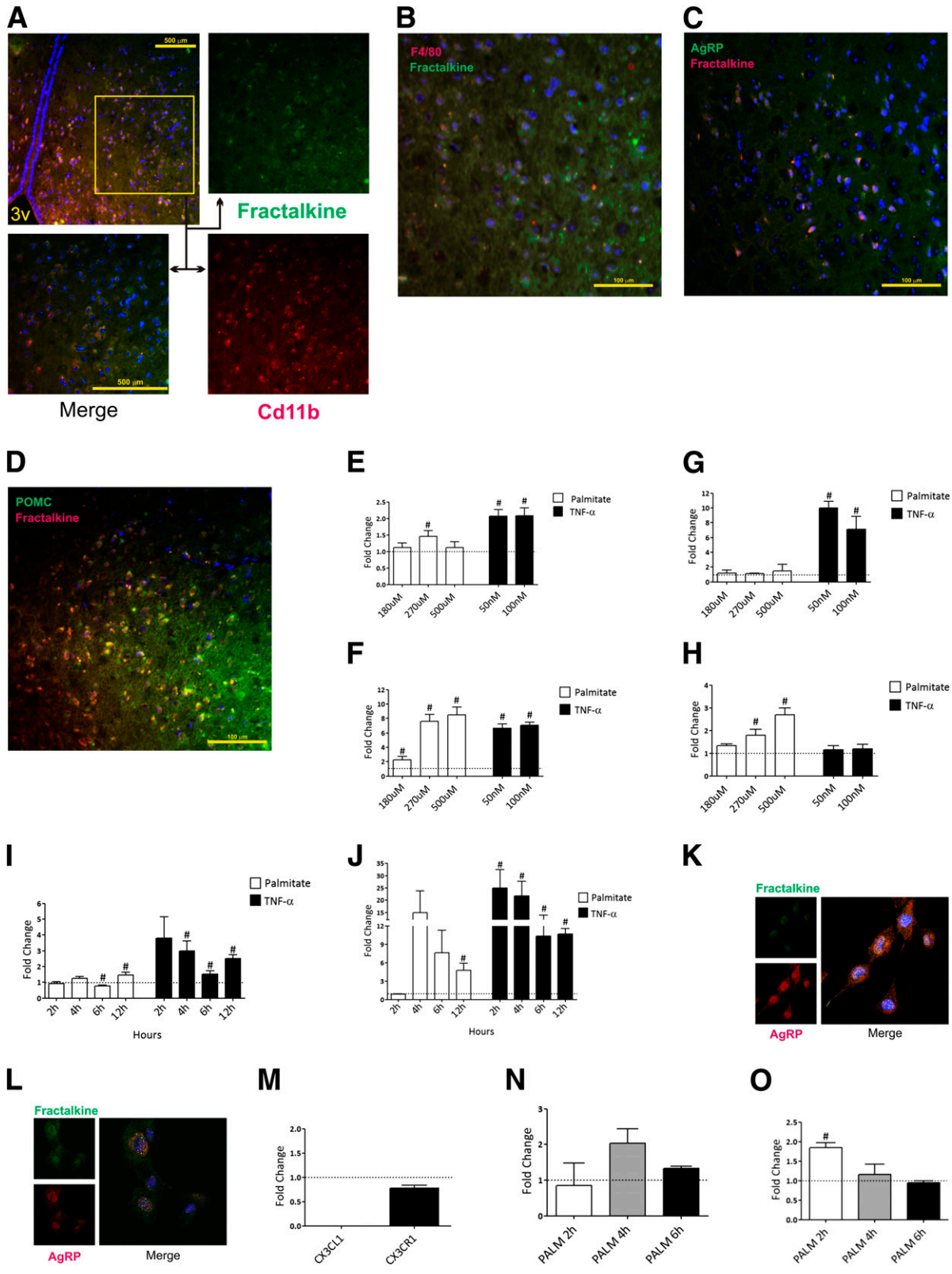

Figure 3—Six-week-old male Swiss mice were fed an HFD for 4 weeks and then were used in the following experiments: fractalkine compared with CD11b (*A*), fractalkine compared with F4/80 (*B*), fractalkine compared with AgRP (*C*), and fractalkine compared with POMC (*D*) indirect immunofluorescence staining of paraformaldehyde-fixed, paraffin-embedded, 5.0-μm hypothalamic sections (at approximately Bregma −1.7). In *E–H*, the AgRP-expressing mHypoA 2/29 CLU189 neuron cell line was treated for 4 h with the concentrations of palmitate or TNFα, as depicted in the panels; at the end of the experimental periods, cells were harvested, RNA was extracted, and the resulting cDNA was used in real-time PCR experiments to determine the expression of fractalkine (CX3CL1) (*E*), MCP1 (*F*), IL1β (*G*), and TLR4 (*H*). *I* and *J*: The AgRP-expressing mHypoA 2/29 CLU189 neuron cell line was exposed to 270 μmol/L palmitate or 50 nmol/L TNFα for the time

mHypoA 2/29 CLU189 neuron cell line (Fig. 5*A*). A protocol was conducted according to Fig. 5*B*; thus, a single dose of the SI was injected on the same day that the HFD was introduced; some mice were used in experiments after 2 days, while the remaining mice were monitored for 2 weeks before additional experiments were performed. The inhibition of hypothalamic fractalkine resulted in the reduction of the diet-induced activation of several inflammatory markers in the hypothalamus of Swiss mice that were fed the HFD for 2 days (Fig. 5*C*–*N*); this was not accompanied by significant changes in caloric intake and body mass (not shown). After 2 weeks, the inhibition of hypothalamic fractalkine (Fig. 6*A*) was still insufficient to produce significant changes in body mass (Fig. 6*B* and *C*); however, there was a reduction of body fat mass (Fig. 6*D*) but not lean mass (Fig. 6*E*). In addition, a glucose tolerance test (Fig. 6*F*) and an insulin tolerance test (Fig. 6*G*) revealed a beneficial effect of inhibiting hypothalamic fractalkine, which was confirmed by a reduction in the glucose area under the curve (Fig. 6*F*) and in the constant for glucose decay (Fig. 6*G*). The levels of fractalkine receptor (Fig. 6*H*) and TNFα (Fig. 6*I*) transcripts were no longer lower; however, IL1β was still significantly reduced (Fig. 6*J*).

Medium-Basal Hypothalamic Inhibition of Fractalkine Is Sufficient to Recapitulate the Phenotype Induced by Intracerebroventricular Injection of Fractalkine SI

Some mice were randomly selected for a bilateral medium-basal hypothalamic injection of SI or SC against fractalkine according to the protocol illustrated in Fig. 6*K*. This protocol resulted in the inhibition of fractalkine expression in the medium-basal hypothalamus (Fig. 6*L*) but not in the frontal cortex (Fig. 6*M*). There was a trend for body mass reduction (not shown), and glucose tolerance was improved as determined by the evaluation of glucose area under the curve during a glucose tolerance test (Fig. 6*N*).

Inhibition of Fractalkine Reduces the Diet-Induced Activation of Inflammatory Cells in the Hypothalamus

Using a protocol similar to the one depicted in Fig. 5*B*, we inhibited the expression of fractalkine in the hypothalamus of wild-type mice that were previously submitted to a bone marrow transplant from GFP (Fig. 7*A*–*E*). Two weeks after the intracerebroventricular injection of the SI and the introduction of the HFD, the number of cells that expressed fractalkine was greatly reduced, and there were virtually no GFP-expressing cells in the arcuate nucleus (Fig. 7*A*–*C*) compared with the controls that were treated with the SC. Moreover, there were only few Iba1-expressing cells in the hypothalamus of SI-treated mice (Fig. 7*D* and *E*). In additional experiments, Swiss mice were treated with SI against fractalkine, and after 2 weeks on the HFD, the phenotype of the microglia/macrophage population in the hypothalamus was evaluated by flow cytometry. As depicted in Fig. 7*F*, the inhibition of fractalkine resulted in an increased number of cells that expressed the anti-inflammatory marker CD206.

Inhibition of Hypothalamic Fractalkine Reduces Diet-Induced Adiposity

In the 2-week protocol, as depicted in Fig. 5*B*, no significant change in body mass was detected; however, a trend, as shown in Fig. 6*B*, prompted us to extend the protocol. Therefore, we repeated the intracerebroventricular treatments with either SI against fractalkine or its scrambled control and monitored the mice for 6 weeks (Fig. 8*A*). The extended time resulted in significant changes in body mass (Fig. 8*B* and *C* and Supplementary Fig. 4) and adiposity (Fig. 8*D*) without any significant modification in caloric intake (Fig. 8*E*).

DISCUSSION

Body mass stability is defended by a complex and integrated neuronal circuitry that controls caloric intake and energy expenditure (1). Neurons of the hypothalamus are an important part of this circuitry because they are capable of sensing the signals that reflect the amount of energy that is stored in the body and of integrating this information with effector neurons of other brain regions (1). At least parts of these signals are delivered by leptin and insulin, and resistance to these hormones plays an important role in obesity (1,2,20–22). For most people, and for experimental animals, changes in the defended set point of adiposity are rapidly followed by adaptations that gradually lead to a return to the original body mass (20,23). However, environmental and genetic factors can act in concert to disrupt this stability (1,24). Dietary fats, particularly long-chain saturated fatty acids, can trigger hypothalamic inflammation through the activation of TLR4 signaling and through the induction of endoplasmic

depicted in the panels; at the end of the experimental periods, cells were harvested, RNA was extracted, and the resulting cDNA was used in real-time PCR experiments to determine the expression of fractalkine (CX3CL1) (*I*) and MCP1 (*J*). The expression and distribution of fractalkine and AgRP in steady-state– (control) (*K*) or palmitate- (270 μmol/L for 4 h) (*L*) treated mHypoA 2/29 CLU189 cells were determined by indirect immunofluorescence staining. *M*: The monocyte cell line RAW 264.7 was treated for 4 h with 270 μmol/L palmitate or vehicle; at the end of the experimental period, cells were harvested and RNA was extracted, and the resulting cDNA was used in real-time PCR experiments to determine the expression of fractalkine (CX3CL1) and its receptor (CX3CR1). *N* and *O*: RAW 264.7 cells were treated with 270 μmol/L palmitate for 2–6 h, as depicted in the panels; cells were harvested and RNA was extracted, and the resulting cDNA was used in real-time PCR experiments to determine the expression of TLR4 (*N*) and TNFα (*O*). The results in the real-time experiments are expressed as the fold change compared with the level of respective gene expression in vehicle-treated cells. In all experiments, $n = 6$; #$P <$ 0.05 compared with vehicle-treated cells.

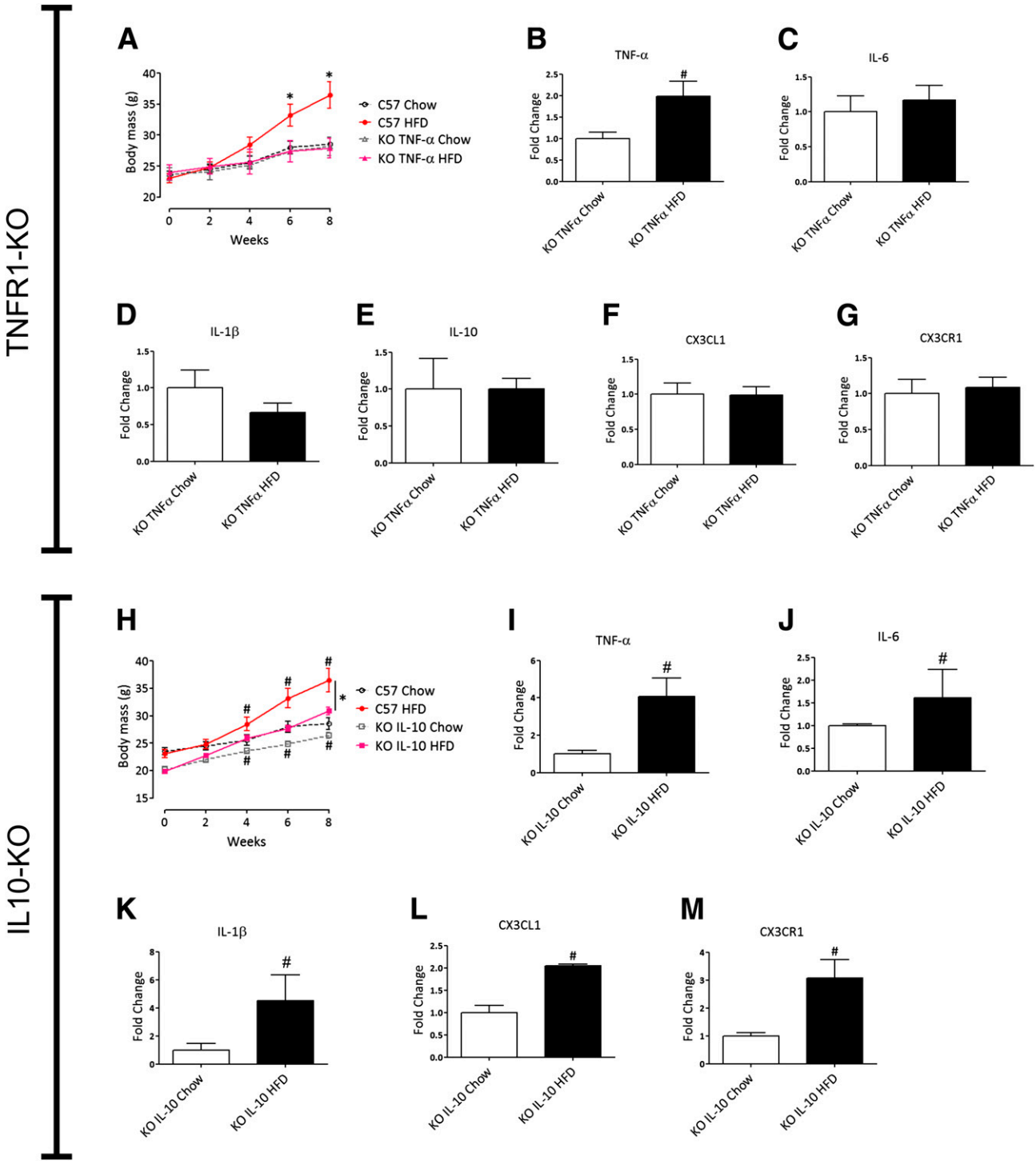

Figure 4—Six-week-old male TNFRp55$^{-/-}$ (TNFR1-KO) (*A–G*) and IL10$^{-/-}$ (IL10-KO) (*H–M*) mice and the respective control C57BL/6J mice were randomly assigned to either chow or high-fat diet (HFD) for 8 weeks. Body mass was determined every second week (*A* and *H*). At the end of the experimental period, hypothalami were obtained for RNA extraction. The cDNA produced from total RNA was used in real-time PCR assays to determine the expression of TNFα (*B* and *I*), IL6 (*C* and *J*), IL1β (*D* and *K*), IL10 (*E*), fractalkine (CX3CL1) (*F* and *L*), and fractalkine receptor (CX3CR1) (*G* and *M*). The results are expressed as the fold change compared with the level of respective gene expression in the hypothalamus of lean (chow-fed), strain-specific controls. In all experiments, $n = 6$. #$P < 0.05$ compared with respective lean control; *$P < 0.05$ compared with wild-type on the same diet.

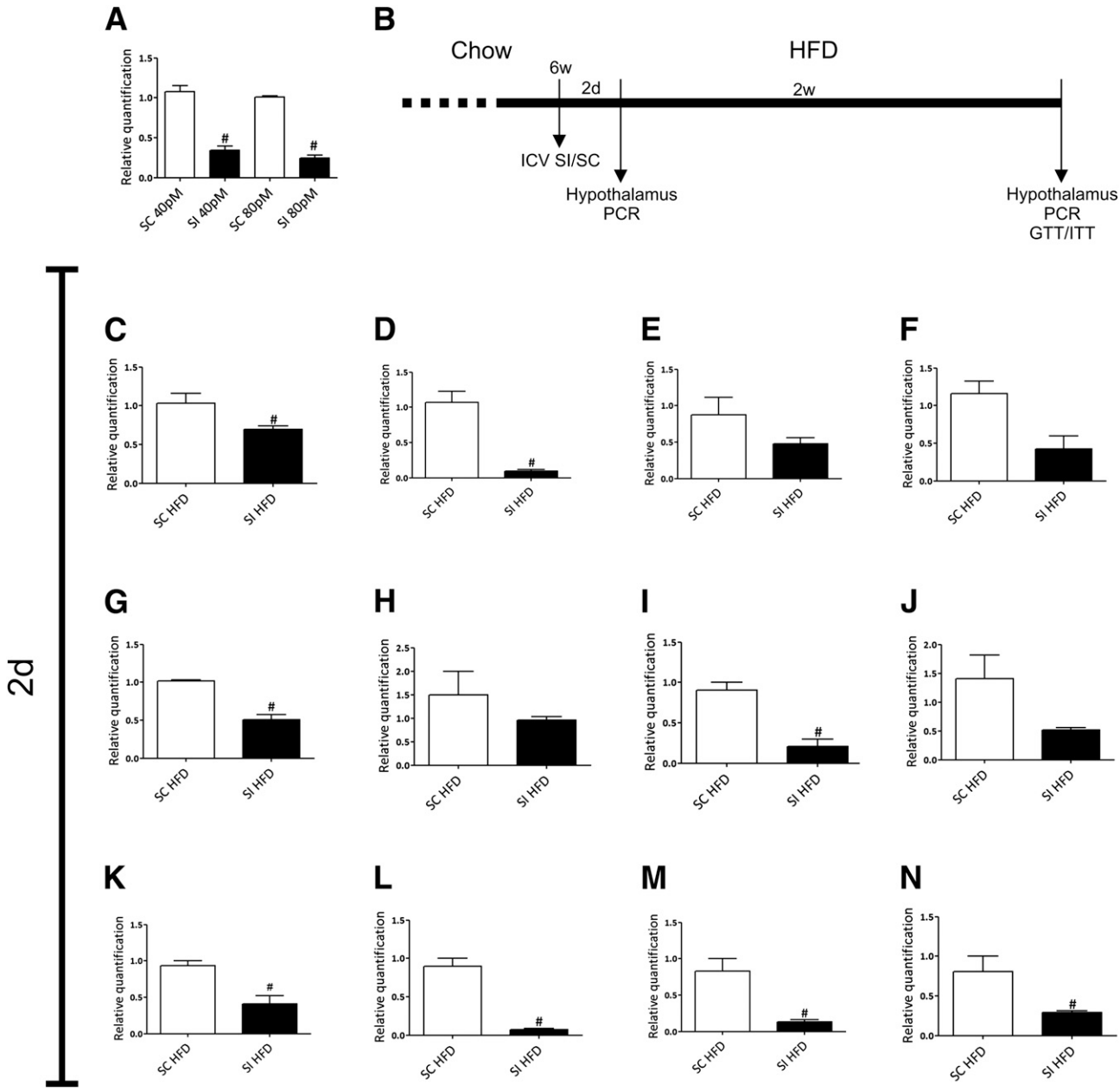

Figure 5—The AgRP-expressing mHypoA 2/29 CLU189 neuron cell line was exposed to an SI that was designed to inhibit the expression of fractalkine or its respective SC at the concentrations depicted in *A*; at the end of the experimental period, cells were harvested and RNA was extracted, and the resulting cDNA was used in real-time PCR experiments to determine the expression of fractalkine (CX3CL1). *B*: The protocol that was used to treat male Swiss mice with a single dose (2.0 μL; 40 pmol/L i.c.v.) of SI or SC against fractalkine. After SI or SC injections, mice were assigned an HFD. Some mice were randomly selected for evaluation of hypothalamic gene expression 2 days (2d) after treatment (*C*–*N*); the remaining mice were evaluated 2 weeks (2w) after treatment (Fig. 6). *C*–*N*: Hypothalami were obtained for RNA extraction. The cDNA that was produced from total RNA was used in real-time PCR assays to determine the expression of fractalkine (CX3CL1) (*C*), MCP1 (*D*), CD11b (*E*), CD36 (*F*), MRC1 (*G*), CD163 (*H*), MCP3 (*I*), F4/80 (*J*), TNFα (*K*), IL6 (*L*), IL1β (*M*), and IL10 (*N*). In real-time PCR experiments, the results are expressed as the relative gene expression. In all experiments, $n = 6$. #$P < 0.05$ compared with SC. GTT, glucose tolerance test; ITT, insulin tolerance test.

reticulum stress (4,7). Prolonged exposure to this inflamed milieu results in the loss of neurons and in the impaired capacity of neuronal regeneration (9,10). Therefore, it is currently accepted that hypothalamic dysfunction that results from prolonged inflammation is an important mechanism that leads to obesity (24).

A recent study has shown that hypothalamic inflammation is rapidly induced after the introduction of a fat-rich diet (6). However, upon continuous and prolonged exposure to dietary fats, the expression of inflammatory markers undergoes oscillation, fading away during the second and third weeks and returning and remaining

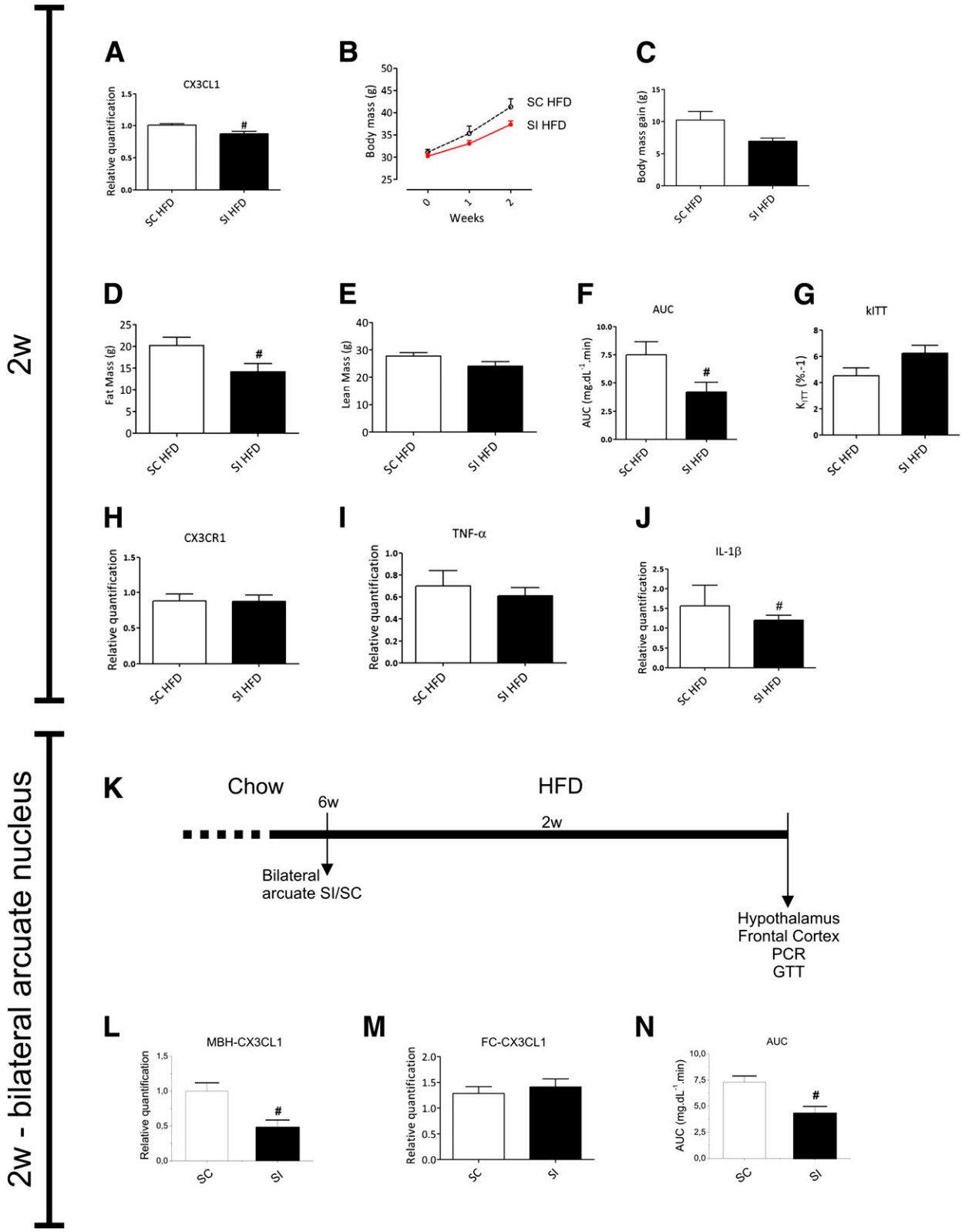

Figure 6—Mice handled with the same protocol as depicted in Fig. 5*B* were evaluated 2 weeks (2w) after treatment. *A*: Hypothalami were obtained for RNA extraction. The cDNA that was produced from total RNA was used in real-time PCR assays to determine the expression of fractalkine (CX3CL1) (*A*). Body mass was determined weekly (*B*); body mass gain was determined at the end of the experimental period (*C*), as was body mass composition by densitometric scanning: fat mass (*D*) and lean mass (*E*). At the end of the experimental period, randomly selected mice were submitted to either a glucose tolerance test, where the area under the glucose curve (AUC) was calculated (*F*), or an insulin tolerance test, where the constant for glucose decay (kITT) was calculated (*G*). *H–J*: Hypothalami were obtained for RNA extraction. The cDNA that was produced from total RNA was used in real-time PCR assays to determine the expression of fractalkine

elevated thereafter (6). This oscillatory pattern of inflammatory activity has been described in other contexts and is the result of a balance between pro- and anti-inflammatory factors that are allied to the recruitment of new cells to the affected anatomical site (25,26). In the current study, we initially asked whether bone marrow–derived cells would be recruited to the hypothalamus during the induction of diet-induced obesity. To test this hypothesis, we took advantage of the paradigm that a functional TLR4 is necessary for the complete induction of diet-induced hypothalamic inflammation (7). Using bone marrow transplantation, we produced chimeras that expressed functional TLR4 only in bone marrow–derived cells or only in non–bone marrow–derived cells. Taken together, the results of these experiments showed that bone marrow–derived cells with a functional TLR4 are important for the full induction of diet-induced hypothalamic inflammation and the obese phenotype.

In other inflammatory conditions of the central nervous system, the recruitment of bone marrow–derived cells is known to play important roles in the progression of these diseases. For instance, in experimental Parkinson disease, bone marrow–derived microglia can express the inducible form of nitric oxide synthase, which is an important effector of neurodegenerative damage (27). However, cells that migrate from the bone marrow do not only deliver damaging effects; a recent study has shown that, in another model of experimental Parkinson disease, bone marrow–derived cells can act protectively by delivering neurotrophic factors to the site of injury (28).

Alzheimer disease is yet another neurodegenerative condition that is associated with inflammation (29). Interestingly, epidemiological studies indicate an increased risk for the development of Alzheimer disease in patients with obesity and type 2 diabetes (30), and a recent study has provided a molecular basis for this association showing that amyloid β peptide oligomers can activate TNFα/JNK signaling, which leads to the inhibitory Ser phosphorylation of brain insulin receptor substrate 1 (31). This is very similar to the mechanisms linking inflammation to insulin resistance in peripheral tissues that are classic targets for insulin action (32,33). As in Parkinson disease, Alzheimer disease is characterized by the recruitment of bone marrow–derived microglia that can exert both pro- and anti-inflammatory roles (29).

In the second part of this study, we evaluated the potential role of fractalkine as a chemokine that is involved in the recruitment of bone marrow–derived cells to the hypothalamus during the induction of diet-induced obesity. Fractalkine signals through the CX3CR1 selective receptor to recruit bone marrow–derived cells to the site of inflammation (34). In this study, fractalkine was chosen because it is expressed in neurons (35) and is involved in the modulation of inflammatory activity in distinct neurological conditions (36).

Initially, in working with mouse strains with different predispositions to obesity, we demonstrated that upon high-fat feeding, fractalkine was the inflammatory marker with the most remarkable difference between the strains. In fact, even after prolonged exposure to dietary fats, Balb-c mice, which are resistant to diet-induced obesity and diabetes, did not present an increased expression of this chemokine in the hypothalamus. In the obesity-prone Swiss mouse, the introduction of dietary fat was rapidly followed by an increase in the expression of fractalkine in hypothalamic neurons. No stimulus for fractalkine expression was detected in other brain regions (not shown), which is in accordance with previous reports that showed the anatomical selectivity of the brain inflammatory process in obesity (3,6,7,9). In cell culture experiments, the expression of fractalkine was limited to neurons, whereas the monocyte cell line expresses only the fractalkine receptor. We used a cell line expressing AgRP, mHypoA 2/29 CLU189; the main purpose of the experiment was to determine whether a neuron cell line would express fractalkine in response to an inflammatory cytokine and/or fatty acids. The combination of results suggests that monocytes are more responsive to palmitate expressing TNFα, whereas the neuron cell line is more responsive to TNFα producing fractalkine and other inflammatory markers. This observation is in accordance with a previous study (11) and suggests that neurons are not the primary targets for dietary fats, which are affected only after glial cells are engaged, as previously proposed (6).

Next, we inhibited fractalkine. In the obesity-prone Swiss mouse, the inhibition of hypothalamic fractalkine resulted in the reduction of the inflammatory activity and in the recruitment of bone marrow–derived cells to the hypothalamus. These effects were accompanied by a reduction of adiposity and a reduction of glucose intolerance. In several studies that explored the inflammatory mechanisms that lead to an anomalous function of the hypothalamus in obesity, the pharmacological and/or genetic inhibition of distinct components of the inflammatory machinery resulted in the reduction of the obese phenotype. This result was obtained by targeting proteins that

receptor (CX3CR1) (*H*), TNFα (*I*), and IL1β (*J*). *K*: The protocol that was used to treat male Swiss mice with a bilateral intracerebral injection in the arcuate nucleus (2.0 μL, 40 pmol/L, each side) of SI or SC against fractalkine; after SI or SC injections, mice were assigned an HFD for 2 weeks (2w), and gene expression of fractalkine (CX3CL1) was determined by real-time PCR in the medium-basal hypothalamus (MBH) (*L*) and in the frontal cortex (FC) (*M*); some mice were randomly selected for a glucose tolerance test, where the area under the glucose curve (AUC) was calculated (*N*). In real-time PCR experiments, the results are expressed as the relative gene expression. In all experiments, $n = 6$: #$P < 0.05$ compared with SC.

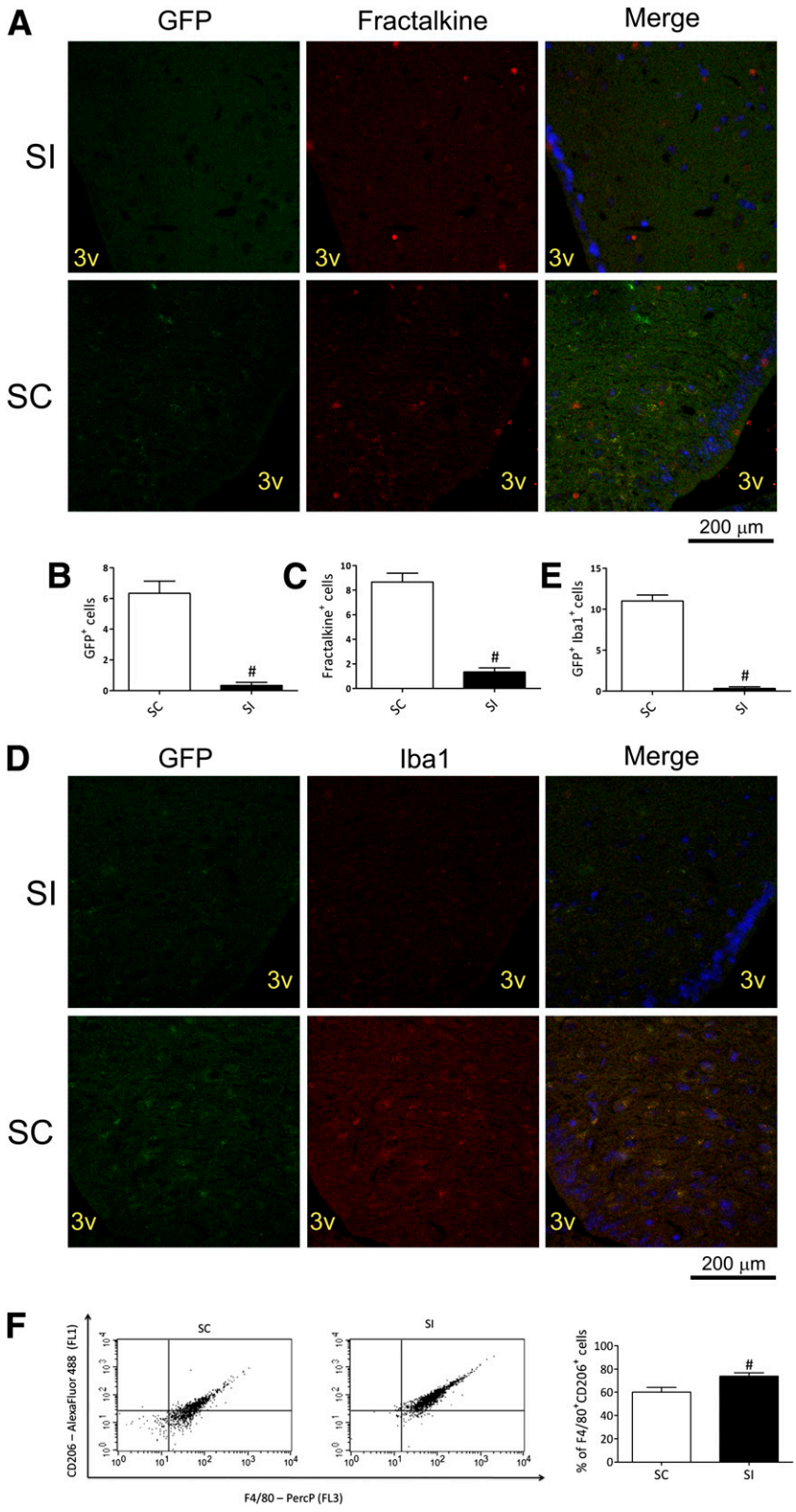

Figure 7—Male 6-week-old Swiss mice were submitted to a bone marrow transplantation from a GFP donor (*A–E*). After 2 weeks, mice were injected with a single dose (2.0 μL, 40 pmol/L i.c.v.) of SI or SC against fractalkine, and then mice were assigned an HFD for 2 weeks. *A* and *D*: Indirect immunofluorescence staining of paraformaldehyde-fixed, paraffin-embedded, 5.0-μm hypothalamic sections (at approximately Bregma −1.7) that were labeled for fractalkine (*A*) or Iba1 (*D*); nuclei were counterstained with DAPI. *B*: GFP-positive cells were counted (cells/500 μm^2 fields). *C*: Fractalkine-positive cells were counted (cells/500 μm^2 fields). *E*: GFP/Iba1 double-positive cells were counted (cells/500 μm^2 fields). *F*: Mice that were handled with the same protocol as depicted in Fig. 5*B* were used 2 weeks (2w) after treatment for flow cytometry evaluation of hypothalamic cells expressing F4/80 and CD206. In all experiments, $n = 6$. #$P < 0.05$ compared with SC.

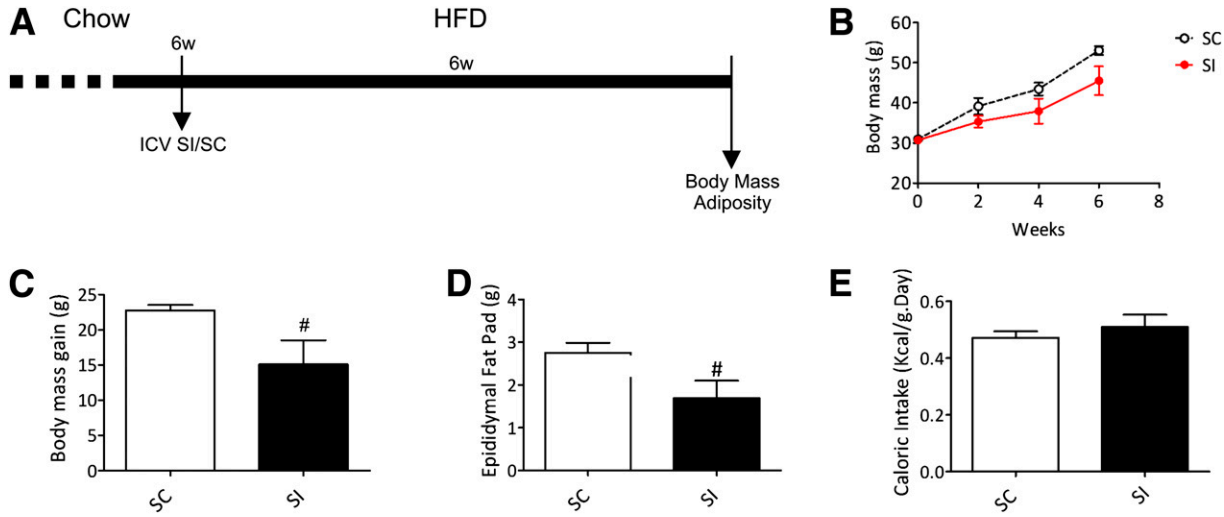

Figure 8— Male 6-week-old Swiss mice were injected with a single dose (2.0 μL, 40 pmol/L i.c.v.) of SI or SC against fractalkine; after SI or SC injections, mice were assigned an HFD for 6 weeks (6w) (*A*). Body mass was determined every second week (*B*). The variation of body mass (*C*) and the mass of epididymal fat (*D*) were determined at the end of the experimental period. The mean daily caloric intake was calculated for the entire experimental period (*E*). In all experiments, $n = 6$. #$P < 0.05$ compared with SC.

are involved in intracellular signaling, such as JNK (37), inhibitor of nuclear factor κB kinase (IKK) (4), and PKC-θ (8); a protein that is involved in the regulation of the inflammatory signal, SOCS3 (38); membrane receptors, such as TLR4 (7) and TNFR1 (19); endoplasmic reticulum stress (4,7); and at least one cytokine, TNFα (39). One important outcome resulting from the inhibition of inflammatory signaling in the hypothalamus of obese animals is the improvement of glucose intolerance. As explored recently by our group, this regulation can occur, at least in part, through parasympathetic connection with the liver (39). Thus, we suspect that the improved glucose homeostasis occurring under hypothalamic fractalkine inhibition, may, as well, be mediated at least in part by neural connection with the liver. Notably, 2 weeks after fractalkine inhibition, when glucose tolerance was improved, there were no changes in body mass; however, there was a reduction in adiposity.

All previous work exploring hypothalamic inflammation in experimental obesity has focused on cytokines or proinflammatory intracellular mechanisms. Now, we provide the first evidence for the role of a chemokine in this process. Within the multistep progression of most types of inflammatory responses, the recruitment of bone marrow–derived cells to the site of damage is an important event that warrants the equilibrium between effector and regulatory activities (40). The initial descriptions of fractalkine actions in inflammatory conditions of the brain suggested that it predominantly exerted an anti-inflammatory activity (41). However, subsequent studies provided evidence for its role in the recruitment of proinflammatory cells as well (42,43). Here, in the diet-induced inflammatory process of the hypothalamus, fractalkine is also involved in the induction of the inflammatory activity.

Two lines of evidence support the proinflammatory role of fractalkine in the hypothalamus of obese mice. First, in the context of cytokine expression, the inhibition of fractalkine resulted in the reduction of TNFα and IL1β. This observation is in line with previous studies that showed an important role for both of these proinflammatory cytokines in diet-induced hypothalamic inflammation (3,4,19). Second, in the context of cell infiltration, a reduction in the expression of fractalkine led to an inhibition of the migration of bone marrow–derived cells toward the hypothalamus and to an increased relative presence of monocytic cells of an anti-inflammatory phenotype, which expressed CD206. In other inflammatory conditions of the brain, an increased presence of CD206 cells was related to the reduction of local inflammatory activity (44).

It is noteworthy that recent studies have implicated fractalkine, directly or indirectly, in whole-body metabolic control. The TACE-TIMP3 dyad, as well as the ADAM17/TACE metalloprotease, are anomalously regulated in humans with type 2 diabetes and obesity, which can, potentially, modulate the levels of bioactive fractalkine (45–47). Moreover, fractalkine is involved in the regulation of insulin secretion by pancreatic β-cells (48). Thus, fractalkine may be an important modulator of inflammation and metabolic activity not only in the brain but also in other tissues.

A recent study has shown that even in mice that are not fed an HFD, bone marrow–derived cells can modulate food intake and energy expenditure by delivering brain-derived neurotrophic factor (BDNF) to the hypothalamus (49). The defective expression of BDNF in hematopoietic cells resulted in obesity, which was corrected by wild-type bone marrow transplantation. This observation raises the

intriguing question of whether the hypothalamus, as a sensor for peripheral signals that indicate the variations in whole-body energy stores, is continuously monitored by bone marrow–derived immune cells. If that scenario is indeed the case, it may explain why there is such an anatomical specificity in brain inflammation in obesity. We propose that fractalkine is one of the earliest regulators of this process by controlling the homing of pro- and anti-inflammatory macrophages to the hypothalamus. In this model, resident microglia are rapidly activated in response to dietary fats by producing signals that induce the expression of fractalkine by neurons. If the exposure to fats is rapidly interrupted, then the first wave of inflammation can be self-contained (6); however, upon the persistence of the dietary stimulus, a permanent wave of inflammation is warranted by the recruitment of bone marrow–derived cells.

In summary, this study shows that bone marrow–derived cells are important for the progression of the inflammatory process in the hypothalamus in diet-induced obesity and that fractalkine plays an important role in this process.

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Author Contributions. J.M. and J.C.M. performed most of the experiments and wrote the manuscript. G.F.A., M.J.S., and I.L.-C. provided facilities and expertise in some steps of the work and revised the text. L.F.N., R.F.d.M., D.R., C.S., D.G., G.S., A.H.M., and V.D.P. performed some experiments guiding J.M. in unfamiliar steps. N.T. and C.D.R. performed the evaluation of body composition using DEXA. L.A.V. designed the study, proposed experiments, obtained grants, and wrote the manuscript. L.A.V. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Morton GJ, Cummings DE, Baskin DG, Barsh GS, Schwartz MW. Central nervous system control of food intake and body weight. Nature 2006;443:289–295
2. Myers MG, Cowley MA, Münzberg H. Mechanisms of leptin action and leptin resistance. Annu Rev Physiol 2008;70:537–556
3. De Souza CT, Araujo EP, Bordin S, et al. Consumption of a fat-rich diet activates a proinflammatory response and induces insulin resistance in the hypothalamus. Endocrinology 2005;146:4192–4199
4. Zhang X, Zhang G, Zhang H, Karin M, Bai H, Cai D. Hypothalamic IKKbeta/NF-kappaB and ER stress link overnutrition to energy imbalance and obesity. Cell 2008;135:61–73
5. van de Sande-Lee S, Pereira FR, Cintra DE, et al. Partial reversibility of hypothalamic dysfunction and changes in brain activity after body mass reduction in obese subjects. Diabetes 2011;60:1699–1704
6. Thaler JP, Yi CX, Schur EA, et al. Obesity is associated with hypothalamic injury in rodents and humans. J Clin Invest 2012;122:153–162
7. Milanski M, Degasperi G, Coope A, et al. Saturated fatty acids produce an inflammatory response predominantly through the activation of TLR4 signaling in hypothalamus: implications for the pathogenesis of obesity. J Neurosci 2009;29:359–370
8. Benoit SC, Kemp CJ, Elias CF, et al. Palmitic acid mediates hypothalamic insulin resistance by altering PKC-theta subcellular localization in rodents. J Clin Invest 2009;119:2577–2589
9. Moraes JC, Coope A, Morari J, et al. High-fat diet induces apoptosis of hypothalamic neurons. PLoS ONE 2009;4:e5045
10. Li J, Tang Y, Cai D. IKKβ/NF-κB disrupts adult hypothalamic neural stem cells to mediate a neurodegenerative mechanism of dietary obesity and pre-diabetes. Nat Cell Biol 2012;14:999–1012
11. Choi SJ, Kim F, Schwartz MW, Wisse BE. Cultured hypothalamic neurons are resistant to inflammation and insulin resistance induced by saturated fatty acids. Am J Physiol Endocrinol Metab 2010;298:E1122–E1130
12. Schwartz M, Shechter R. Systemic inflammatory cells fight off neurodegenerative disease. Nat Rev Neurol 2010;6:405–410
13. Cardona AE, Li M, Liu L, Savarin C, Ransohoff RM. Chemokines in and out of the central nervous system: much more than chemotaxis and inflammation. J Leukoc Biol 2008;84:587–594
14. Cardona AE, Pioro EP, Sasse ME, et al. Control of microglial neurotoxicity by the fractalkine receptor. Nat Neurosci 2006;9:917–924
15. Simard AR, Soulet D, Gowing G, Julien JP, Rivest S. Bone marrow-derived microglia play a critical role in restricting senile plaque formation in Alzheimer's disease. Neuron 2006;49:489–502
16. Prada PO, Zecchin HG, Gasparetti AL, et al. Western diet modulates insulin signaling, c-Jun N-terminal kinase activity, and insulin receptor substrate-1ser307 phosphorylation in a tissue-specific fashion. Endocrinology 2005;146:1576–1587
17. Camacho A, Rodriguez-Cuenca S, Blount M, et al. Ablation of PGC1 beta prevents mTOR dependent endoplasmic reticulum stress response. Exp Neurol 2012;237:396–406
18. Chakravarty S, Herkenham M. Toll-like receptor 4 on nonhematopoietic cells sustains CNS inflammation during endotoxemia, independent of systemic cytokines. J Neurosci 2005;25:1788–1796
19. Romanatto T, Roman EA, Arruda AP, et al. Deletion of tumor necrosis factor-alpha receptor 1 (TNFR1) protects against diet-induced obesity by means of increased thermogenesis. J Biol Chem 2009;284:36213–36222
20. Rosenbaum M, Kissileff HR, Mayer LE, Hirsch J, Leibel RL. Energy intake in weight-reduced humans. Brain Res 2010;1350:95–102
21. Folli F, Bonfanti L, Renard E, Kahn CR, Merighi A. Insulin receptor substrate-1 (IRS-1) distribution in the rat central nervous system. J Neurosci 1994;14:6412–6422
22. Daniele G, Guardado Mendoza R, Winnier D, et al. The inflammatory status score including IL-6, TNF-α, osteopontin, fractalkine, MCP-1 and adiponectin underlies whole-body insulin resistance and hyperglycemia in type 2 diabetes mellitus. Acta Diabetol 2014;51:123–131
23. Sims EA, Goldman RF, Gluck CM, Horton ES, Kelleher PC, Rowe DW. Experimental obesity in man. Trans Assoc Am Physicians 1968;81:153–170
24. Velloso LA, Schwartz MW. Altered hypothalamic function in diet-induced obesity. Int J Obes (Lond) 2011;35:1455–1465
25. Hickman SE, El Khoury J. Mechanisms of mononuclear phagocyte recruitment in Alzheimer's disease. CNS Neurol Disord Drug Targets 2010;9:168–173
26. Aguzzi A, Barres BA, Bennett ML. Microglia: scapegoat, saboteur, or something else? Science 2013;339:156–161
27. Kokovay E, Cunningham LA. Bone marrow-derived microglia contribute to the neuroinflammatory response and express iNOS in the MPTP mouse model of Parkinson's disease. Neurobiol Dis 2005;19:471–478
28. Biju KC, Santacruz RA, Chen C, et al. Bone marrow-derived microglia-based neurturin delivery protects against dopaminergic neurodegeneration in a mouse model of Parkinson's disease. Neurosci Lett 2013;535:24–29

29. Rivest S. Regulation of innate immune responses in the brain. Nat Rev Immunol 2009;9:429–439
30. Craft S. Alzheimer disease: Insulin resistance and AD—extending the translational path. Nat Rev Neurol 2012;8:360–362
31. Bomfim TR, Forny-Germano L, Sathler LB, et al. An anti-diabetes agent protects the mouse brain from defective insulin signaling caused by Alzheimer's disease- associated Aβ oligomers. J Clin Invest 2012;122:1339–1353
32. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 1993;259:87–91
33. Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. Science 1996;271:665–668
34. Imai T, Hieshima K, Haskell C, et al. Identification and molecular characterization of fractalkine receptor CX3CR1, which mediates both leukocyte migration and adhesion. Cell 1997;91:521–530
35. Harrison JK, Jiang Y, Chen S, et al. Role for neuronally derived fractalkine in mediating interactions between neurons and CX3CR1-expressing microglia. Proc Natl Acad Sci U S A 1998;95:10896–10901
36. Cho SH, Sun B, Zhou Y, et al. CX3CR1 protein signaling modulates microglial activation and protects against plaque-independent cognitive deficits in a mouse model of Alzheimer disease. J Biol Chem 2011;286:32713–32722
37. De Souza CT, Araújo EP, Prada PO, Saad MJ, Boschero AC, Velloso LA. Short-term inhibition of peroxisome proliferator-activated receptor-gamma coactivator-1alpha expression reverses diet-induced diabetes mellitus and hepatic steatosis in mice. Diabetologia 2005;48:1860–1871
38. Kievit P, Howard JK, Badman MK, et al. Enhanced leptin sensitivity and improved glucose homeostasis in mice lacking suppressor of cytokine signaling-3 in POMC-expressing cells. Cell Metab 2006;4:123–132
39. Milanski M, Arruda AP, Coope A, et al. Inhibition of hypothalamic inflammation reverses diet-induced insulin resistance in the liver. Diabetes 2012;61:1455–1462
40. Mizuno T. The biphasic role of microglia in Alzheimer's disease. Int J Alzheimers Dis 2012;2012:737846
41. Zujovic V, Benavides J, Vigé X, Carter C, Taupin V. Fractalkine modulates TNF-alpha secretion and neurotoxicity induced by microglial activation. Glia 2000;29:305–315
42. Huang D, Shi FD, Jung S, et al. The neuronal chemokine CX3CL1/fractalkine selectively recruits NK cells that modify experimental autoimmune encephalomyelitis within the central nervous system. FASEB J 2006;20:896–905
43. Dénes A, Ferenczi S, Halász J, Környei Z, Kovács KJ. Role of CX3CR1 (fractalkine receptor) in brain damage and inflammation induced by focal cerebral ischemia in mouse. J Cereb Blood Flow Metab 2008;28:1707–1721
44. Hjorth E, Zhu M, Toro VC, et al. Omega-3 fatty acids enhance phagocytosis of Alzheimer's disease-related amyloid-β42 by human microglia and decrease inflammatory markers. J Alzheimers Dis 2013;35:697–713
45. Federici M, Hribal ML, Menghini R, et al. Timp3 deficiency in insulin receptor-haploinsufficient mice promotes diabetes and vascular inflammation via increased TNF-alpha. J Clin Invest 2005;115:3494–3505
46. Monroy A, Kamath S, Chavez AO, et al. Impaired regulation of the TNF-alpha converting enzyme/tissue inhibitor of metalloproteinase 3 proteolytic system in skeletal muscle of obese type 2 diabetic patients: a new mechanism of insulin resistance in humans. Diabetologia 2009;52:2169–2181
47. Tripathy D, Daniele G, Fiorentino TV, et al. Pioglitazone improves glucose metabolism and modulates skeletal muscle TIMP-3-TACE dyad in type 2 diabetes mellitus: a randomised, double-blind, placebo-controlled, mechanistic study. Diabetologia 2013;56:2153–2163
48. Lee YS, Morinaga H, Kim JJ, et al. The fractalkine/CX3CR1 system regulates β cell function and insulin secretion. Cell 2013;153:413–425
49. Urabe H, Kojima H, Chan L, et al. Haematopoietic cells produce BDNF and regulate appetite upon migration to the hypothalamus. Nat Commun 2013;4:1526