Transgenic Restoration of Long-Chain n-3 Fatty Acids in Insulin Target Tissues Improves Resolution Capacity and Alleviates Obesity-Linked Inflammation and Insulin Resistance in High-Fat–Fed Mice

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OBJECTIVE—The catabasis of inflammation is an active process directed by n-3 derived pro-resolving lipid mediators. We aimed to determine whether high-fat (HF) diet-induced n-3 deficiency compromises the resolution capacity of obese mice and thereby contributes to obesity-linked inflammation and insulin resistance.

RESEARCH DESIGN AND METHODS—We used transgenic expression of the *fat-1* n-3 fatty acid desaturase from *C. elegans* to endogenously restore n-3 fatty acids in HF-fed mice. After 8 weeks on HF or chow diets, wild-type and *fat-1* transgenic mice were subjected to insulin and glucose tolerance tests and a resolution assay was performed. Metabolic tissues were then harvested for biochemical analyses.

RESULTS—We report that the n-3 docosanoid resolution mediator protectin D1 is lacking in muscle and adipose tissue of HF-fed wild-type mice. Accordingly, HF-fed wild-type mice have an impaired capacity to resolve an acute inflammatory response and display elevated adipose macrophage accrual and chemokine/cytokine expression. This is associated with insulin resistance and higher activation of iNOS and JNK in muscle and liver. These defects are reversed in HF-fed *fat-1* mice, in which the biosynthesis of this important n-3 docosanoid resolution mediator is improved. Importantly, transgenic restoration of n-3 fatty acids prevented obesity-linked inflammation and insulin resistance in HF-fed mice without altering food intake, weight gain, or adiposity.

CONCLUSIONS—We conclude that inefficient biosynthesis of n-3 resolution mediators in muscle and adipose tissue contributes to the maintenance of chronic inflammation in obesity and that these novel lipids offer exciting potential for the treatment of insulin resistance and diabetes. ***Diabetes*** **59:3066–3073, 2010**

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Obesity is linked to chronic inflammation that plays a key role in the pathogenesis of insulin resistance, leading the way to type 2 diabetes and cardiovascular disease (1,2). Efforts to understand this process have focused on identifying the many factors that may initiate and promote inflammation. We took an alternate approach with the view that pathological inflammation in obesity likely represents an impaired endogenous capacity to "switch off" or more precisely counterregulate the natural immune response to adipose tissue expansion and lipid excess.

The newly identified genus of n-3 derived lipid mediators termed resolvins and protectins have been shown to play an important role in the endogenous regulation of inflammation (3,4). Interestingly, dietary long-chain n-3 polyunsaturated fatty acid (PUFA) insufficiency has been linked to the incidence of chronic metabolic disorders, including type 2 diabetes and cardiovascular disease (5–7). It is thus conceivable that inefficient biosynthesis of n-3 resolution mediators due to low substrate availability might inherently contribute to the development of obesity-linked inflammation.

González-Périz et al. recently showed that acute administration of n-3 derived Resolvin E1 (RvE1) prevents hepatic steatosis in genetically obese mice (8). However, the other main resolution mediator Protectin D1 (PD1) remains to be investigated, and it is unknown whether high-fat (HF) feeding per se actually restricts resolution mediator biosynthesis and whether this might alter the endogenous resolution capacity of obese mice. Furthermore, it is critical to determine whether n-3 lipid mediators regulate key obesity-related inflammatory reactions such as macrophage accrual in adipose tissue or activation of inflammatory signaling molecules such as JNK and iNOS that play a role in the etiology of insulin resistance (1,2).

Unfortunately, studying the effects of dietary n-3 content in the context of HF feeding has proven to be rather complicated because incorporation of n-3 fatty acids in rodent diets often prevents weight gain (9). As a result, it is not clear whether it is the lack of weight gain or the n-3 fatty acids themselves that offer the protection from insulin resistance and type 2 diabetes and what mechanism underlies this protection. Therefore, innovative models that overcome the requirement for dietary manipulation are needed to help clarify whether or not n-3 fatty acids act directly to prevent obesity-linked insulin resistance and which mechanisms are involved.

The *fat-1* transgenic mouse has been genetically engi-

neered to ubiquitously express the *fat-1* n-3 fatty acid desaturase from *C. elegans* (10). This enzyme, not found in mammals, efficiently converts endogenous n-6 to n-3 fatty acids such that, in *fat-1* mice fed a diet extremely rich in n-6 and deficient in n-3, the tissue n-6:n-3 ratio is ~1:1 compared with ~50:1 in wild-type animals. The *fat-1* transgenic mouse therefore represents the ideal model to study the effects of n-3 fatty acids in an environment that is not confronted by dietary issues.

Herein we show that HF feeding wild-type mice results in diminished n-3 docosanoid resolution mediator synthesis in muscle and adipose tissue and impaired resolution. Transgenic restoration of n-3 fatty acids in HF-fed *fat-1* mice improved resolution capacity and prevented the development of obesity-linked inflammation and insulin resistance. These data uncover a new role for pro-resolving lipid mediators in the counter-regulation of obesity-linked inflammation and its associated metabolic complications.

RESEARCH DESIGN AND METHODS

Hemizygous *fat-1*(+/−) mice (10) were bred with wild-type littermates at the Laval University hospital research center. Six-week-old male mice were fed standard laboratory chow (diet-2018, Harlan Teklad) or HF diets (diet-9302, 55% Kcal from fat, Harlan Teklad) for 8 weeks. Insulin tolerance tests (ITTs) and glucose tolerance tests (GTTs) were performed in week 7 in 6-h fasted mice as previously described (11), and mice were killed in week 8. At sacrifice, a cohort of mice were used for the air-pouch resolution assay. Remaining mice were injected via tail vein with either insulin (3.8 U/kg) or saline 5 min prior to being killed. Tissues were rapidly excised and snap-frozen in liquid nitrogen. Sections of liver and epididymal adipose were placed in 4% paraformaldehyde. Animal procedures were approved and carried out in accordance with the Laval University and Canadian Councils for Animal Care.

Lipidomics. Fatty acid composition of phospholipid fractions was analyzed by gas chromatography as per ref (12). Briefly, lipids were extracted along with internal standards (C:15, Avanti Polar Lipids, Alabaster, AL, USA) in a chloroform–methanol mixture (2:1, by volume). Extracted lipids were then weighed and dissolved in a chloroform–methanol mixture (3:1, by volume). Polar lipids (phospholipids, i.e., phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, and sphingomyelin) were separated by thin-layer chromatography (Silica Gel H, 250 μm, Analtech Inc, Newark, DE, USA) using an isopropyl-ether–acetic acid mixture (96:4, by volume). Fractions were then recovered in individual glass tubes, and direct transesterification was performed by adding acetyl chloride. Fatty acid methyl esters of phospholipids were analyzed by gas chromatography using Hewlett-Packard 5,890, series II (Hewlett-Packard, Toronto, Canada) equipped with a fused silica column (DB23; 30 m, 0.25 mm internal diameter, 0.25 μm film, Agilent Technologies, Mississauga, Canada), helium as carrier gas, a split ratio of 1:72, a flow of 0.72 ml min^{-1}, and a coupled flame ionization detector. The fatty acid methyl esters (FAMEs) were identified by comparison with retention times of a Supelco 37-component FAME mix (Supelco Inc., Bellefonte, PA, USA) and by using one internal standard (C:15, Avanti Polar Lipids, Alabaster, AL, USA).

For liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS), lipid mediators were extracted in the presence of deuterated internal standard (1 ng LTB4-6,7,14,15; Biomol) by solid-phase extraction using Sep-Pak C18 cartridges (Waters). A triple quadrupole linear ion trap mass spectrometer (4000Q-TRAP; Applied Biosystems) equipped with an Acquity ultraperformance LC BEH C18 column (1.7 μm, 1.0 × 150 mm; Waters) was used. MS/MS analyses were conducted in negative ion mode, and eicosanoids/docosanoids were identified by multiple reaction monitoring using transitions for 17-HDoHE (343 > 245m/z), 18-HEPE (317 > 215m/z), PD1 (359 > 153m/z), and RvE1 (349 > 195m/z). Calibration curves (1–1000 pg) and LC retention times for each compound were established with synthetic standards.

In vivo resolution assay. The air pouch resolution assay was performed as described by Levy et al. (13). Dorsal air pouches were raised and maintained 6 and 3 days prior to the assay via subcutaneous injection of sterile air (5 and 3 ml, respectively). On the day of the experiment, 10 ng of recombinant murine tumor necrosis factor α (TNFα) (R&D Systems Inc.) in 100-μl sterile PBS was injected into the pouch. At 0, 4, and 6.5 h after injection, mice were killed and pouches were washed two times with 1 ml of sterile PBS to collect infiltrating polymorphonuclear leukocytes (PMNs). PMNs were then enumerated. Sterile PBS was used as a control for TNFα and did not stimulate PMN infiltration into the pouch (data not shown).

Histology. Adipose and liver sections were embedded and mounted and hematoxylin and eosin staining of liver was performed by the University Laval microscopy facility. Immunohistochemistry detection of F4/80+ cells was performed as previously described (14).

Western blotting. Immunoblotting was performed in gastrocnemius muscle and liver as previously described (15). Fifty ug of protein was loaded onto a 7.5% acrylamide gel, subjected to SDS-PAGE, and then transferred onto nitrocellulose membranes. Membranes were then blocked and probed with the appropriate antibodies. Antibodies for p-AKT ser473, p-JNK thr183/tyr185, and Total JNK were obtained from Cell Signaling Technology (MA, USA). Antibodies for total AKT and iNOS were from Santa Cruz Biotechnology (CA, USA) and BD Transduction Laboratories (Canada), respectively.

Analytical methods. Plasma insulin levels were assessed by radioimmunoassay (Linco, MI, USA). Chemokines and cytokines were quantified in 25 μl of adipose tissue lysates (50 ug of protein in PBS containing 1% NP-40) using a MILLIPLEX MAP kit (Millipore).

Statistical Analysis. LC-MS/MS data were analyzed using Student t test; air-pouch, ITT, and GTT data were analyzed using two-way ANOVA. For all other data, one-way ANOVA was used. Bonferonni was the post hoc test. Results were considered significant when $P < 0.05$.

RESULTS

HF feeding reduces n-3 availability for resolution mediator synthesis. We first examined the effect of HF feeding on n-3 bioavailability in metabolic tissues. The HF diet mimicked Western diets in terms of n-3 content with an n-6:n-3 ratio of ~18–1. After 8 weeks, HF-fed wild-type mice displayed an elevated long-chain n-6:n-3 ratio in skeletal muscle, liver, and adipose tissue membranes compared with their chow-fed counterparts (Fig. 1*A*). Importantly, transgenic expression of the *fat-1* n-3 fatty acid desaturase that converts endogenous n-6 to n-3 fatty acids restored the membrane long-chain n-6:n-3 ratio of HF-fed *fat-1* mice to levels comparable to chow-fed mice (Fig. 1*A*).

Using LC-MS/MS to detect n-3 lipid oxygenation products, we found evidence of both docosanoid and eicosanoid biosynthetic activity in metabolic tissues of HF-fed mice. 17-HDoHE and 18-HEPE, hydroxy-metabolites of docosahexaenoic and eicosapentaenoic acids and biosynthetic markers of PD1 and RvE1, respectively, were readily detected in muscle, liver, and adipose tissue (Fig. 1*B* and *C*). Interestingly, the docosanoid biosynthetic route appeared to have greater flux in these tissues, because 17-HDoHE was present in significantly higher concentrations than 18-HEPE and PD1 was readily detected in all tissues whereas RvE1 was under the detection limit. Compared with HF-fed wild-type mice, HF-fed *fat-1* mice displayed increased flux through the docosanoid biosynthetic route in muscle and adipose tissues but not in liver (Fig. 1*D*). Indeed, 17-HDoHE was increased by ~215% in muscle and 138% in adipose tissue whereas PD1 was increased by ~176% in muscle and 201% in adipose tissue of *fat-1* mice compared with wild-type mice ($P < 0.05$). These data suggest that *fat-1* mice display increased n-3 bioavailability for pro-resolution mediator synthesis in these two key metabolic tissues.

HF feeding impairs resolution. We hypothesized that the HF diet-induced deficit in n-3 resolution mediator synthesis would impact endogenous counterregulation of inflammation in wild-type mice. To test this, we subjected mice to a dorsal air-pouch TNFα challenge, an established model of self-resolving inflammation (13). The injection of 10 ng of TNFα into the air pouch stimulated an influx of PMNs that peaked at 4 h and resolved completely 6.5 h postchallenge. In contrast to their chow-fed counterparts,

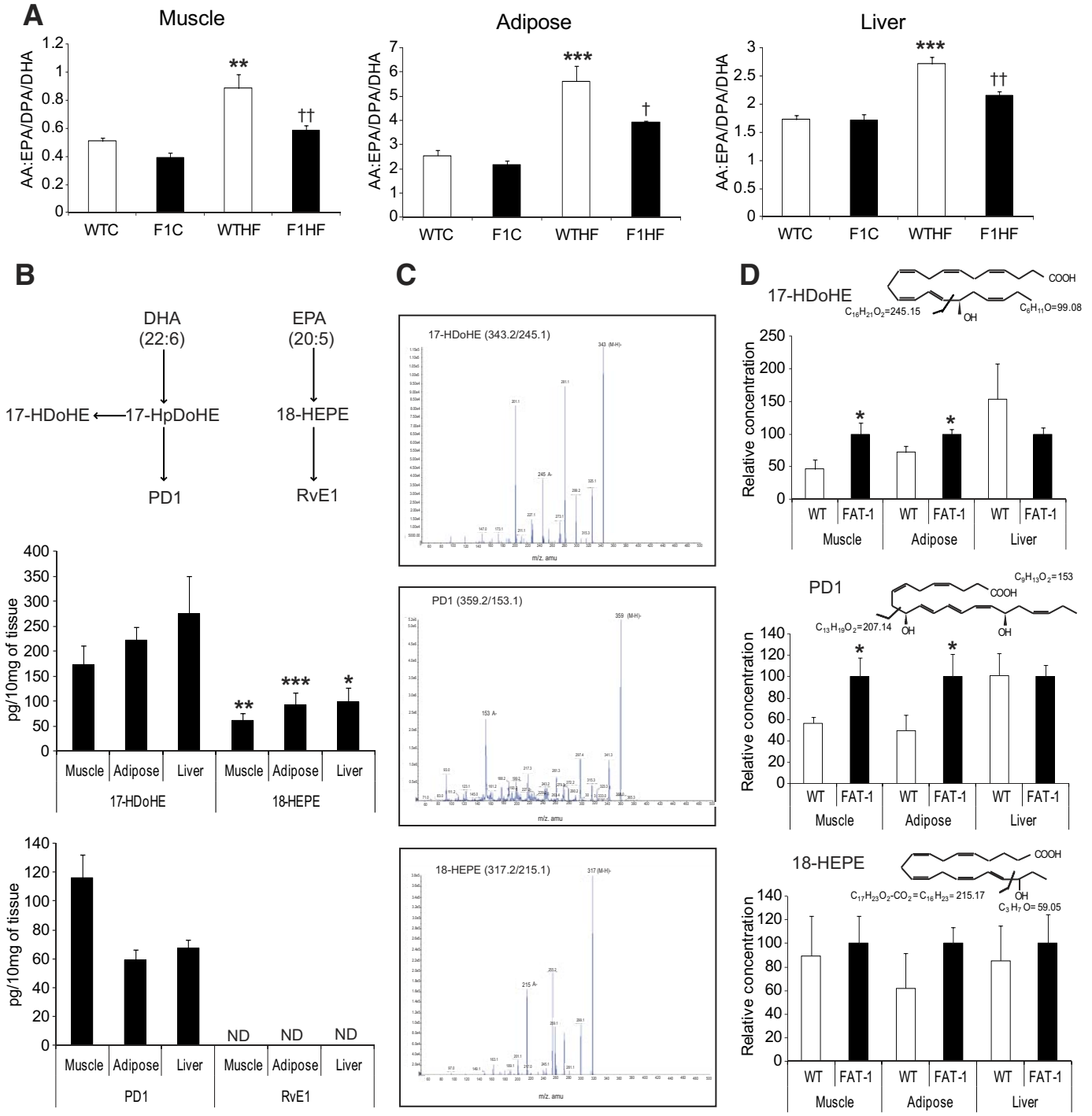

FIG. 1. HF feeding reduces n-3 availability for resolution mediator synthesis. *A*: 8 weeks of HF feeding raised but *fat-1* (F1) transgenesis restored the long-chain n-6:n-3 ratio in membrane phospholipids of muscle, liver, and epididymal adipose tissue. C, standard laboratory chow; AA, arachidonic acid (20:4 n-6); EPA, eicosapentaenoic acid (20:5 n-3); DPA, docosapentaenoic acid (22:5 n-3); DHA, docosahexaenoic acid (22:6 n-3). Data are mean ± SEM (n = 3). **P < 0.01 versus WTC; ***P < 0.001 versus WTC; †P < 0.05 versus WTHF; ††P < 0.01 versus WTHF. *B*: Comparison of n-3 docosanoid and eicosanoid biosynthetic pathways by LC-MS/MS in muscle, liver, and epididymal adipose tissue of HF-fed mice revealed that the docosanoid biosynthetic pathway has greater flux in metabolic tissues. Above left schematic diagram of docosanoid biosynthetic pathway showing the biosynthetic marker 17-HDoHE and PD1 (10*R*,17*S*-dihydroxydocosa-4*Z*,7*Z*,11*E*,13*E*,15*Z*,19*Z*-hexaenoic acid [28]) as well as the immediate PD1 precursor 17-HpDoHE. At right, the eicosanoid pathway showing 18-HEPE and RvE1 (5*S*,12*R*,18*R*-trihydroxy-6*Z*,8*E*,10*E*,14*Z*,16*E*-EPA [29]). ND indicates not detected. Data are mean ± SEM (n = 9–14). *P < 0.05, **P < 0.01, ***P < 0.001 versus 17-HDoHE. *C*: Representative LC-MS/MS spectra for 17-HDoHE, PD1, and 18-HEPE; retention times were 22.6, 18.2, and 20.6 min, respectively. *D*: Comparison of n-3 docosanoid and eicosanoid biosynthetic pathway activity by LC-MS/MS in muscle, liver, and epididymal adipose tissue of HF-fed wild-type and F1 mice reveals increased levels of docosanoid resolution mediator synthesis in muscle and adipose tissue of F1 mice compared with wild-type mice. Data are mean ± SEM (n = 6–10). *P < 0.05 versus WTHF. (A high-quality color representation of this figure is available in the online issue.)

HF-fed wild-type mice only resolved ~65% of infiltrating PMN by this time (Fig. 2*A* and *B*). Restoration of n-3 in HF-fed *fat-1* mice was sufficient to completely recover the deficit in resolution capacity ($P < 0.001$). This is the first evidence that HF-diet-induced n-3 deficiency can impede the normal resolution of inflammation.

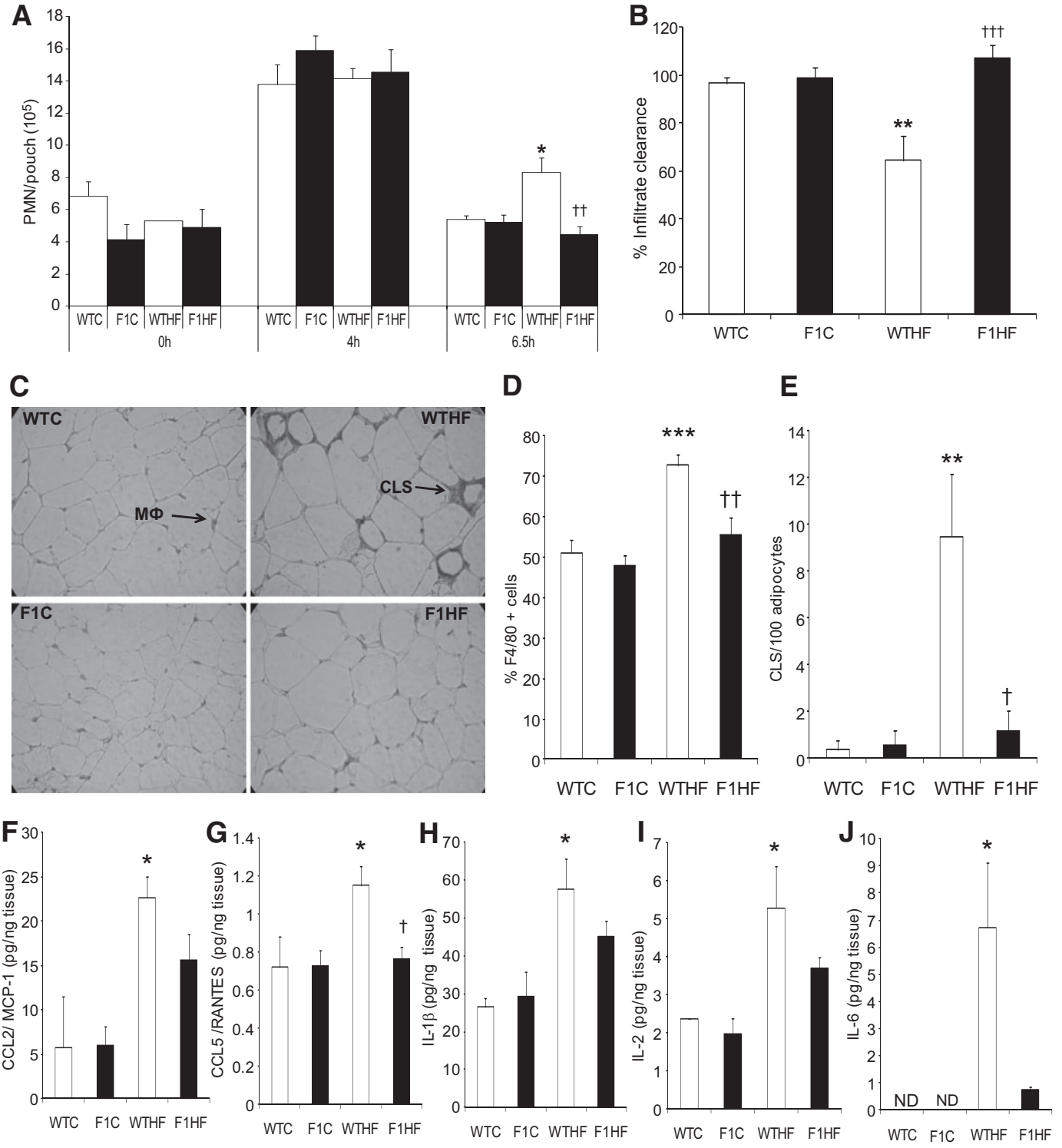

FIG. 2. Transgenic restoration of n-3 resolution mediators reestablishes resolution capacity and prevents adipose inflammation in HF-fed mice. *A*: Clearance of inflammatory PMN infiltrates in dorsal air pouches of HF-fed mice is impaired during the resolution phase of the in vivo resolution assay (n = 5–6). *B*: Percent infiltrate clearance 6.5 h after TNFα injection was reduced by ~35% in obese HF-fed wild-type mice; HF-fed F1 transgenic mice displayed normal infiltrate clearance (n = 5–6). *C*: Representative image of F4/80 immunohistochemistry in epididymal adipose tissue show mass accumulation of macrophage in HF-fed wild-type mice that is prevented in HF-fed transgenic mice. MØ, macrophage. *D*: Percent F4/80+ cells in epididymal adipose tissue (n = 4–6). *E*: Macrophages present in adipose HF-fed wild-type mice formed multiple CLS; the formation of these inflammatory macrophage aggregates was greatly reduced in HF-fed F1 mice (number of CLS per 100 adipocytes). *F–J*: Chemokine and cytokine expression in epididymal adipose tissue was elevated by HF compared with chow feeding in wild-type mice, but these factors were not significantly raised by HF feeding in F1 mice (n = 5–10). All data are mean ± SEM, *P < 0.05, **P < 0.01, ***P < 0.001 versus WTC; †P < 0.05, ††P < 0.01, †††P < 0.001 versus WTHF.

Transgenic restoration of n-3 prevents adipose inflammation. To determine what role the HF diet-induced deficit in n-3 docosanoid mediators plays in obesity-linked inflammation, we examined macrophage accrual in adipose tissue. Because PD1 directs tissue phagocyte flux in inflammatory exudates (16), we hypothesized that trans-

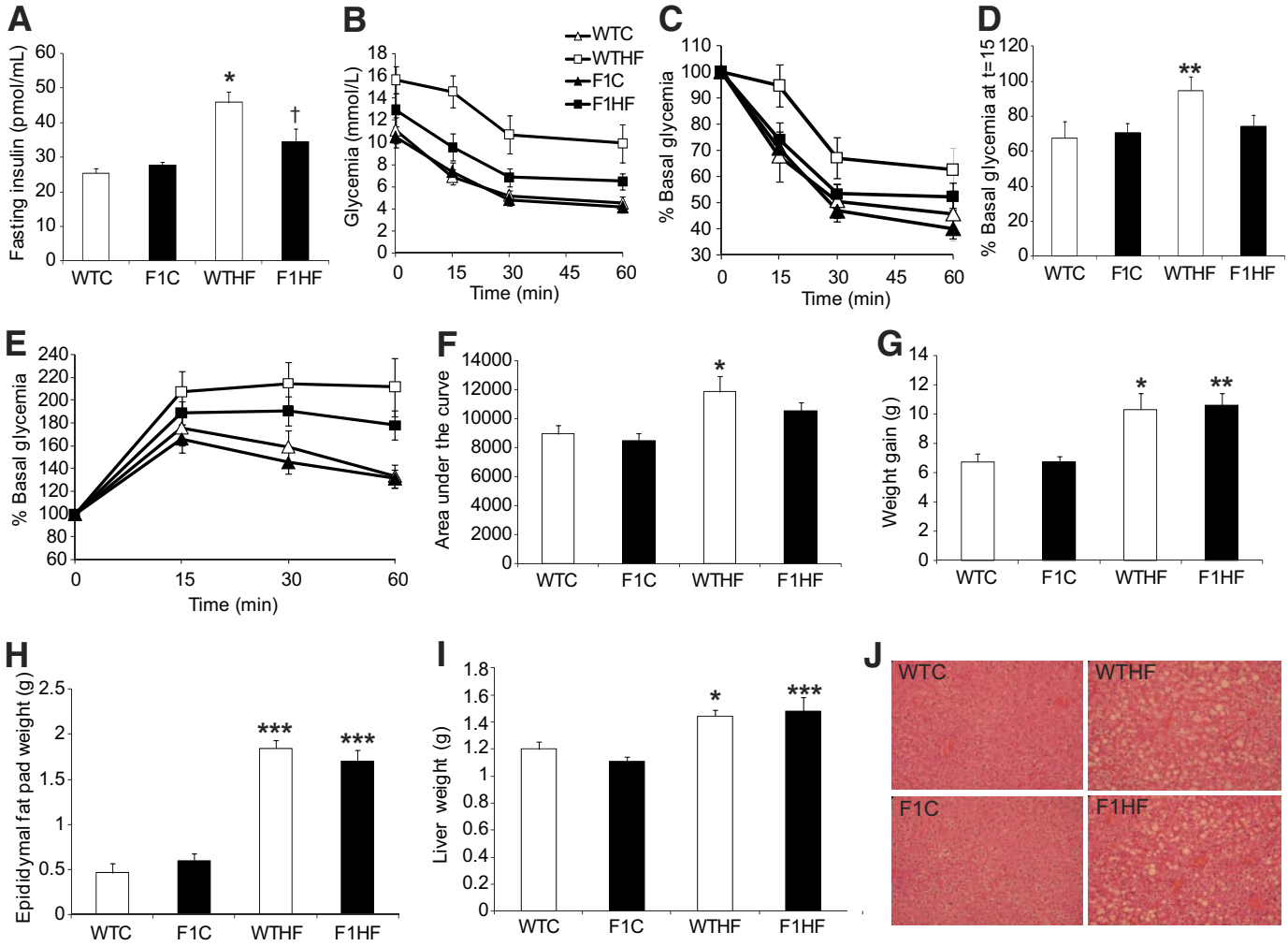

FIG. 3. Transgenic restoration of long-chain n-3 PUFA protects against obesity-linked insulin resistance and glucose intolerance. *A*: HF-diet–induced elevation of fasting plasma insulin was prevented by transgenic restoration of n-3 derived resolution mediators (n = 4–9). *B*: Glycemic excursion from 1.5 U/kg i.p. ITT was normalized in HF-fed F1 mice (n = 8–12). *C*: Glycemic excursion expressed as percent basal glycemia. *D*: Percent basal glycemia at T = 15 min after insulin injection. *E*: Glycemic excursion from 1g/kg i.p. GTT expressed as percent basal glycemia (n = 7–11), and (*F*) area under the curve from GTT show that HF-fed F1 mice are partially protected from glucose intolerance. *G*: HF-fed F1 mice develop similar obesity to wild-type mice. Weight gain (n = 16–20). *H*: Epididymal fat pad weight (n = 9–14). *I*: Liver weight (n = 9–14). *J*: Representative hematoxylin and eosin stained liver sections showing similar accumulation of fat vesicles in both WTHF and F1HF mice. All data are mean ± SEM, ND not detected, *P < 0.05, **P < 0.01, ***P < 0.001 versus respective chow-fed control; †P < 0.05 versus WTHF. (A high-quality color representation of this figure is available in the online issue.)

genic restoration of this n-3 resolution pathway would be sufficient to limit adipose macrophage accumulation in HF-fed mice. Immunohistochemical staining for F4/80+ cells revealed that HF-fed wild-type mice have abundant accumulation of macrophages in adipose tissue compared with their chow-fed counterparts (Fig. 2*C* and *D*). Furthermore, many of the F4/80+ cells in HF-fed wild-type fat clearly formed inflammatory crown-like structures (CLSs) around adipocytes (Fig. 2*C–E*), a hallmark of obesity-linked inflammation (14). In line with the improved resolution capacity, macrophage accrual and CLS formation were entirely prevented in adipose tissue of HF-fed transgenic mice.

To further characterize the impact of n-3 resolution mediators on obesity-linked inflammation, we also examined adipose chemokine and cytokine expression. We detected elevated concentrations of the proinflammatory chemokines CCL2/MCP-1 and CCL5/RANTES alongside the cytokines, IL-1β, IL-2, and IL-6, in HF-fed wild-type mice (Fig. 2*F–J*). Importantly, these key inflammatory factors were not significantly raised by HF feeding in adipose of *fat-1* mice.

Transgenic restoration of n-3 protects against obesity-linked insulin resistance and glucose intolerance. We next characterized whole-body insulin sensitivity to determine whether transgenic restoration of n-3 also prevents the development of obesity-linked insulin resistance. Insulin sensitivity was markedly reduced in HF-fed wild-type mice, as illustrated by elevated fasting insulin levels and diminished glucose excursion during the ITT (Fig. 3*A–D*). Conversely, *fat-1* mice were protected from HF-diet-induced insulin resistance because both fasting insulin values and ITT curves were similar to those observed for chow-fed mice.

Fat-1 mice were also partially protected from HF-diet-induced glucose intolerance (Fig. 3*E* and *F*). The area under the glucose tolerance curves of HF-fed wild-type mice was increased compared with their chow-fed counterparts (Fig. 3*F*); however, this parameter was not significantly different between HF-fed *fat-1* mice and their

chow-fed controls. Importantly, the improved metabolic phenotype of HF-fed *fat-1* mice was not related to changes in food intake (data not shown), body weight gain (Fig. 3*G*), or adiposity (Fig. 3*H*). We also found no changes in hepatic lipid accretion as determined by liver weight (Fig. 3*I*) and histological examination of liver sections, which showed similar accumulation of fat vesicles in both HF-fed wild-type and *fat-1* mice (Fig. 3*J*). Fasting plasma free fatty acids were not significantly influenced by 8 weeks of HF feeding or transgenic restoration of n-3 in our study (data not shown). Although not statistically significant, circulating adiponectin tended to be reduced by ~30% in HF-fed wild-type mice compared with their chow-fed counterparts (2,150 ± 638 ng/ml vs. 3,345 ± 768 ng/ml, respectively); this was not the case in HF-fed *fat-1* mice, which displayed circulating levels of adiponectin that were comparable to chow-fed mice (3,420 ± 565 ng/ml).

To understand the mechanism underlying the improved metabolic phenotype of HF-fed *fat-1* mice, we examined insulin signaling to Akt in muscle and liver. As expected, insulin stimulation induced robust phosphorylation of Akt on ser473 in muscle and liver of chow-fed wild-type mice, but this response was impaired in their HF-fed counterparts (Fig. 4*A*–*B*). Remarkably, this defect was normalized in both muscle and liver of HF-fed *fat-1* mice, despite clear accumulation of ectopic lipid in the latter tissue.

We next examined whether the improved insulin action in metabolic tissues of HF-fed *fat-1* mice resulted from decreased inflammatory signaling. As expected, HF feeding wild-type mice led to robust phosphorylation of JNK on thr183/tyr185 in both muscle and liver; however, this was not the case for HF-fed *fat-1* mice in either tissue (Fig. 4*C* and *D*). HF feeding also resulted in significant iNOS induction in muscle of wild-type mice but not in HF-fed *fat-1* mice (Fig. 4*E*). These data suggest that prevention of HF-diet-induced n-3 deficiency and the maintenance of resolution capacity protects from the development of obesity-linked insulin resistance not only by limiting inflammation in the expanding adipose tissue but also by inhibiting two key inflammatory mediators of insulin resistance, JNK (17) and iNOS (11), in muscle and liver.

DISCUSSION

In the present study, we took advantage of *fat-1* mice to investigate the role of endogenous n-3 derived resolution mediators in key metabolic tissues in obesity. We found that the biosynthetic flux of the n-3 docosanoid resolution pathway in muscle and adipose tissue is dependent on long-chain n-3 PUFA bioavailability and that the pro-resolving lipid mediator, PD1, is lacking in normal mice chronically fed a typical Western diet (n-6:n-3 ratio ~18–1). HF-fed obese mice exhibited an impaired capacity to resolve an acute inflammatory response to TNFα and showed abundant macrophage infiltration in adipose tissue that was linked to heightened chemokine and cytokine expression, a hallmark of obesity-linked inflammation (18,19). Remarkably, restoration of PD1 via *fat-1* transgenesis improved global resolution capacity and prevented adipose macrophage accrual in HF-fed *fat-1* mice. Accordingly, the expression levels of five key proinflammatory chemokines/cytokines were not found to be significantly elevated in adipose tissue of HF-fed *fat-1* mice compared their chow-fed controls. To the best of our knowledge, this is the first demonstration that endogenous biosynthesis of n-3 derived resolution mediators is associated with obesity-linked inflammation in metabolic tissues.

This is also the first report on the regulation of inflammation and insulin sensitivity in an animal model of obesity in which n-3 status has been enhanced without confounding effects of dietary manipulation. Indeed, previous studies have documented that the anti-inflammatory and metabolic effects of dietary n-3 supplementation were associated with concomitant reductions in either food intake, body weight gain, adiposity, or liver fat accretion (9,20,21). However, we report herein that transgenic-based elevation of long-chain n-3 PUFAs protects from obesity-linked insulin resistance without altering food intake, weight gain, or lipid deposition in adipose tissue or liver. We propose instead that endogenous long-chain n-3 PUFAs exert their protective effect through the actions of their lipid oxygenation products, which resolve inflammation and limit macrophage accrual in the expanding adipose tissue of obese mice.

The lack of effect of *fat-1* expression on hepatic lipid accretion seems at odds with the recent report that dietary supplementation of long-chain n-3 PUFAs reversed hepatic steatosis in genetically obese *ob/ob* mice possibly through the action of the EPA-derived eicosanoid RvE1 (8). However, this may be due in part to the different animal models used (i.e., diet-induced versus genetic-based obesity) and the fact that we readily detected the docosanoid PD1 but not the eicosanoid RvE1 in the liver of our HF-fed *fat-1* mice. Although we saw no effect of *fat-1* transgenesis on resolution mediator synthesis in liver, our data suggests that transgenic restoration of n-3 fatty acids dissociates insulin resistance from hepatic lipid deposition, and this is likely due to inhibition of inflammatory signaling, as revealed by prevention of JNK activation in liver of HF-fed *fat-1* mice. This anti-inflammatory effect in liver may be the result of reduced inflammatory crosstalk from adipose tissue with fewer recruited macrophages or may represent the local actions of another class of bioactive n-3 metabolite such as the newly discovered maresins (22) or EFOX (23), which should be the focus of future investigations.

Our data showing protection from obesity-linked insulin resistance in HF-fed *fat-1* mice in which the levels of long-chain n-3 PUFAs have been restored are in line with epidemiological studies in humans, which showed that native populations traditionally consuming high levels of long-chain n-3 PUFAs display a lower prevalence of type 2 diabetes (24,25). Interestingly, data from another clinical study suggests that the positive influence of long-chain n-3 PUFA supplementation on insulin sensitivity is greater in obese populations that display an inflammatory phenotype (26). These data lend support to our findings, which indicate that the anti-inflammatory actions of n-3 derived resolution mediators in metabolic tissues are key to the positive impact of long-chain n-3 PUFAs on insulin sensitivity. It will be interesting in future studies to examine whether direct administration of purified n-3 derived resolution mediators is sufficient to prevent the development of insulin resistance in obese animals and which mediators carry the greatest antidiabetic potential.

Although our work represents the first study into the effect of endogenously enhancing tissue n-3 content on insulin sensitivity in the context of obesity, it is noteworthy that another group has recently developed an adipose-specific *fat-1* transgenic line (AP-3 mice) to expressly study the influence of adipose n-3 content on weight gain, insulin sensitivity, and glucose tolerance in lean mice (27).

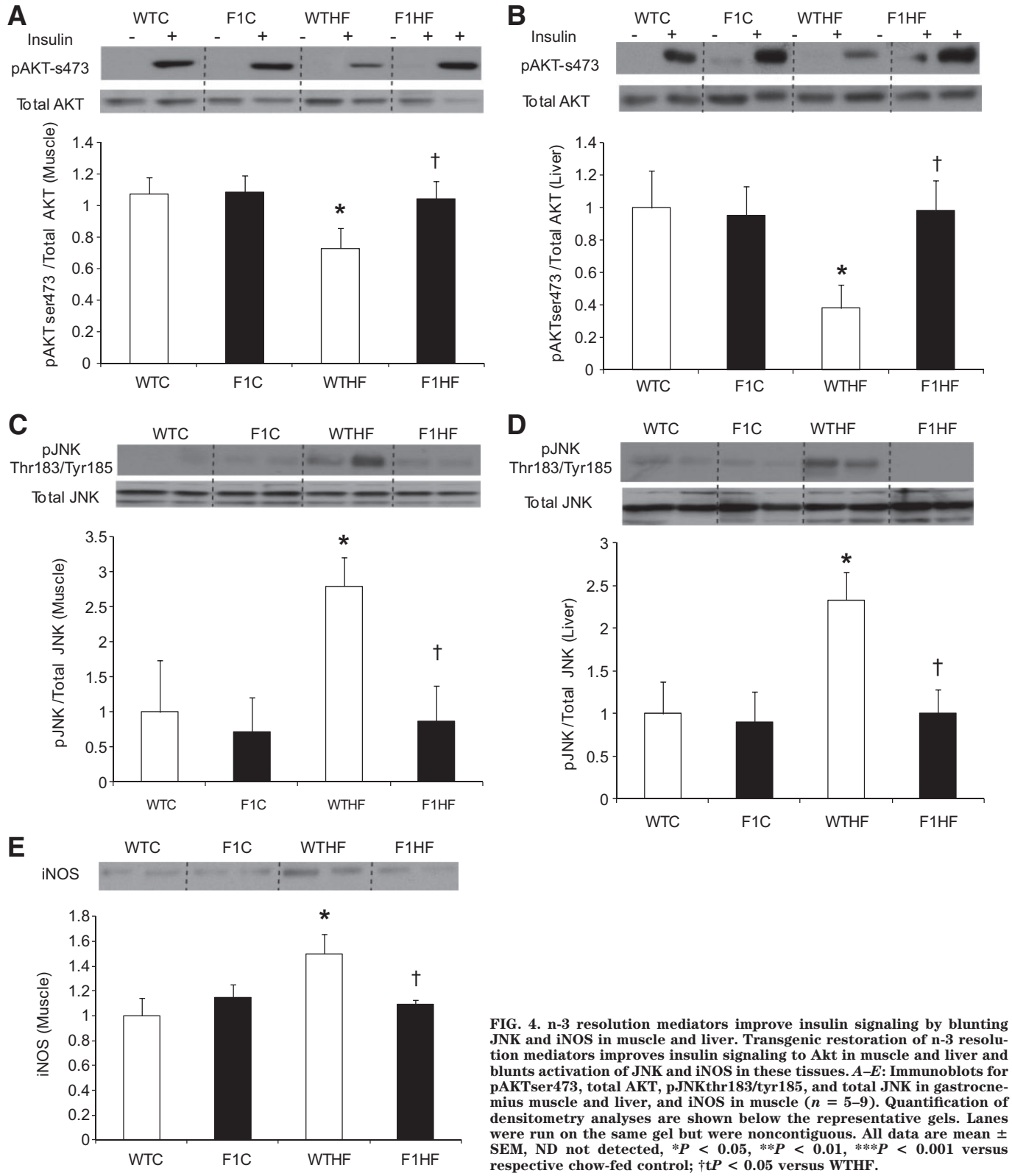

FIG. 4. n-3 resolution mediators improve insulin signaling by blunting JNK and iNOS in muscle and liver. Transgenic restoration of n-3 resolution mediators improves insulin signaling to Akt in muscle and liver and blunts activation of JNK and iNOS in these tissues. *A–E*: Immunoblots for pAKTser473, total AKT, pJNKthr183/tyr185, and total JNK in gastrocnemius muscle and liver, and iNOS in muscle (n = 5–9). Quantification of densitometry analyses are shown below the representative gels. Lanes were run on the same gel but were noncontiguous. All data are mean ± SEM, ND not detected, *P < 0.05, **P < 0.01, ***P < 0.001 versus respective chow-fed control; †tP < 0.05 versus WTHF.

It was found that 3-month-old male, but not female, AP-3 transgenic mice fed a high carbohydrate diet weigh slightly less than their wild-type littermates, although this could not be explained by changes in adiposity. Interestingly, whereas male AP-3 mice were more glucose tolerant than wild-type controls, female AP-3 mice exhibited glucose intolerance as compared with their wild-type littermates. Insulin sensitivity was not affected in either sex. These findings differ from those of our study in which we found that *fat-1* transgenesis had no impact on weight gain or glucose tolerance in lean chow-fed animals. It is of interest that homozygous expression of the *fat-1* transgene in our mice does not influence viability (10), whereas homozygous expression in the AP-3 mice was found to be lethal (27).

In conclusion, we propose that endogenous long-chain

n-3 PUFAs exert their protective effects through their lipid oxygenation products, which reduce macrophage accrual and inflammation in the expanding adipose tissue of obese mice. Our data further suggest that restoring long-chain n-3 PUFAs also prevents obesity-linked insulin resistance by blunting lipid-induced JNK and iNOS activation in muscle and liver. Collectively, our findings unravel a novel mechanism by which endogenous n-3 fatty acids prevent the development of obesity-linked inflammation and insulin resistance. This work supports the use of long-chain n-3 PUFAs for the prevention of insulin resistance and glucose intolerance in obese individuals.

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P.J.W. planned experiments, researched data, and wrote the manuscript. M.A. researched data and reviewed/edited the manuscript. R.T. researched data. J.X.K. helped plan experiments, contributed to discussion, and reviewed/edited the manuscript. A.M. planned and supervised experiments and wrote and edited the manuscript.

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