High Pancreatic n-3 Fatty Acids Prevent STZ-Induced Diabetes in Fat-1 Mice: Inflammatory Pathway Inhibition

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OBJECTIVE—Because of confounding factors, the effects of dietary n-3 polyunsaturated fatty acids (PUFA) on type 1 diabetes remain to be clarified. We therefore evaluated whether fat-1 transgenic mice, a well-controlled experimental model endogenously synthesizing n-3 PUFA, were protected against streptozotocin (STZ)-induced diabetes. We then aimed to elucidate the in vivo response at the pancreatic level.

RESEARCH DESIGN AND METHODS—β-Cell destruction was produced by multiple low-doses STZ (MLD-STZ). Blood glucose level, plasma insulin level, and plasma lipid analysis were then performed. Pancreatic mRNA expression of cytokines, the monocyte chemotactic protein, and GLUT2 were evaluated as well as pancreatic nuclear factor (NF-κB) protein expression. Insulin and cleaved caspase-3 immunostaining and lipidomic analysis were performed in the pancreas.

RESULTS—STZ-induced fat-1 mice did not develop hyperglycemia compared with wild-type mice, and β-cell destruction was prevented as evidenced by lack of histological pancreatic damage or reduced insulin level. The prevention of β-cell destruction was associated with no proinflammatory cytokine induction (tumor necrosis factor-α, interleukin-1β, inducible nitric oxide synthase) in the pancreas, a decreased NF-κB, and increased iκB protein expression. In the fat-1–treated mice, proinflammatory arachidonic–derived mediators as prostaaglandin E2 and 12-hydroxyeicosatetraenoic acid were decreased and the anti-inflammatory lipoxin A4 was detected. Moreover, the 18-hydroxyeicosapentaenoic acid, precursor of the anti-inflammatory resolvin E1, was highly increased.

CONCLUSIONS—Collectively, these findings indicate that fat-1 mice were protected against MLD-STZ–induced diabetes and pointed out for the first time in vivo the beneficial effects of n-3 PUFA at the pancreatic level, on each step of the development of the pathology—inflammation, β-cell damage—through cytokine response and lipid mediator production. Diabetes 60:1090–1099, 2011

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are generated from EPA and DHA (20–23). These newly discovered mediators, termed resolvins and protectins, are involved in the resolution of inflammation and have been shown to inhibit NF-kB activity (20).

Very recently, an in vitro study using two cellular models evaluated the direct impact of n-3 PUFA on the function and viability of pancreatic β-cells (23). The authors showed a strong resistance to the destruction of the cells treated by cytokines by stable cellular production of n-3 PUFAs in nfat-1–transfected β-cells. The in vivo relevance of such conclusions remains to be explored as well as the efficiency of high pancreatic n-3 PUFA in alleviating insulin-dependent diabetes.

Transgenic fat-1 mice carry a C. Elegans gene, fat-1, encoding an n-3 fatty acid desaturase catalyzing the conversion of n-6 to n-3 PUFA (24). There is a remarkable difference in the n-6–to–n-3 PUFA ratio in tissues from wild-type (WT) versus fat-1 transgenic mice (20 to 50 and close to 1, respectively) fed diets high in n-6 and low in n-3 PUFA (24). A single diet can therefore be used to generate mice with different fatty acid profiles (high and low n-6–to–n-3 ratios) eliminating potential confounding dietary factors and allowing in vivo investigation on the role of n-6–to–n-3 ratio in the destruction of pancreatic β-cells.

Thereby, we used fat-1 transgenic mice to determine whether endogenously synthesized n-3 PUFA could be β-cell protective in MLD-STZ, and we then evaluated the mechanisms involved at the pancreatic level in such diabetes prevention.

**RESEARCH DESIGN AND METHODS**

**Animals and diets.** Transgenic fat-1 mice were generated as described previously (24) and backcrossed onto a C57BL6/J background. We used male fat-1 transgenic mice and nontransgenic littermate controls (14 weeks old, 20–25 g) since male and female mice have different susceptibilities to STZ (25). The presence of the fat-1 gene in each mouse was confirmed both by genotyping and tail fatty acid analysis profiles. Transgenic and WT animals were maintained on a 10% safflower oil diet (INRA, Jouy-en-Josas, France) ad libitum and housed in temperature- and humidity-controlled conditions with a 12-h light/pattern of the

**Lipidomic analysis.** Lipidomic analyses were performed according to Massoudi et al. (22). All pancreatic tissue was homogenized in ice-cold methanol and diluted with ice-cold water to 15% (vol/vol). Internal standards (40 ng PGB2-d4 and 80 ng 12-HETE-d8) (Cayman Chemicals, Ann Arbor, MI) were added to each sample. These suspensions were centrifuged; the clear supernatant added to pH 3 and immediately spli

**Tail, pancreas, and plasma fatty acid composition.** The fatty acid composition in tail (to perform the phenotyping), pancreatic tissue, and plasma was measured using gas chromatography.

**Blood collection and plasma insulin assays.** Before mice were killed, 800 μL of blood were collected from each mouse anesthetized by Isoflurane (TREM, Bordeaux, France) from the retro-orbital vein. Plasma was snap-frozen in liquid nitrogen and stored at −80°C until plasma insulin concentration determination was performed using a mouse ELISA kit (Abcs SA, Paris, France).

**Histological and immunohistochemical analysis.** Immunohistochemistry was performed on formalin-fixed, paraform-embedded pancreas sections stained with hematoxylin and eosin. Sections were deparaffinized and dehydrated in a graded series of ethanol washes. Tissue sections were subjected to heat-induced epitope retrieval (0.1 M citrate buffer boiled in a 600-watt microwave), then cooled at room temperature for 20 min. After washing in PBS (pH 7.4), endogenous peroxidase was quenched using a 3% solution (v/v) of hydrogen peroxide and PBS. After blocking nonspecific staining with PBS3%/BSA, sections were incubated with primary antibodies (overnight at 4°C for both cleaved caspase-3 and insulin antibodies). Sections were then incubated with secondary antibody, washed again in PBS, and incubated for 20 min with horseradish peroxidase-3-amino-9-ethylcarbazole (Aldrich, Saint-Quentin Fallavier, France). Slides were counterstained with hematoxylin. Antibodies for insulin (C27C9) and cleaved-caspase-3 (Asp 175) were obtained from Cell Signaling Technology (Ozyme, Saint-Quentin-en-Yvelines, France) and used at a concentration of 1/800 and 1/100, respectively.

The islet size from STZ-induced and untreated animals has been compared using the following procedure: images of islets were acquired on the Cell Station of CellMap Plateform (IFR100, Dijon, France). Briefly, this station is made of an inverted motorized microscope (Axiovert 200M, Carl Zeiss, Le Pecq, France) equipped with an Axiocam. Image analysis was done using Axiovision software. More precisely, islets were surrounded using polygonal lasso and surfaces were recorded. Only islets bigger than 20,000 μm² were counted.

**Western blot analysis.** Pancreatic tissue (30 mg) was homogenized in Triton protein lysis buffer (20 mM Tris, 150 mM NaCl, 200 μM EDTA, 200 μM EGTA, 1% Triton X-100) containing protease and phosphatase inhibitors (Sigma). Proteins (40 μg) were separated by 10% SDS-PAGE and electrobblotted to Protran nitrocellulose membranes (Whatman, Dassel, Germany). After blocking nonspecific binding in Tris-buffered saline and Tween 20 (TBS) (0.1% Tween-20 in TBS), blots were probed overnight at 4°C with primary antibody against NF-κB p65 and IκBα (Cell Signalling, Oxford, UK) and β-actin (Sigma–Aldrich, Saint-Quentin Fallavier, France) at a concentration of 1/200, 1/800, and 1/5,000, respectively, washed in T-TBS, incubated 1 h at room temperature with horseradish peroxidase-conjugated goat anti-rabbit IgG for NF-κB p65 and goat anti mouse IgG for IκBα and β-actin (Jackson ImmunoResearch Lab, West Grove, PA).

**Cell culture and treatments.** Human T cells were isolated from healthy donors by negative selection using the Miltenyi Biotec MACS kit (Miltenyi Biotec, Germany). The purity of the T cell preparation was confirmed by high-speed flow cytometry and was typically >98%. Cells were cultured in RPMI supplemented with 10% FBS, 2 mM L-glutamine, penicillin, and streptomycin.

**Flow cytometry.** T-cell activation was determined by measuring surface expression of CD40 and CD80 using APC-conjugated antibodies (BD Biosciences, San Jose, CA) and analyzed by flow cytometry.

**Multiplex cytokine arrays.** Human cytokine protein arrays were performed using the Human TH1/TH2/TH17/TH17 multiplex kit (R&D Systems) as described in detail previously (35). The cytokine expression in the supernatants of cultured T cells was quantified using a Hoechst 33342 dye (Sigma-Aldrich) for the nucleic acid content and PicoGreen dye (Invitrogen) for the protein content. The signals were then measured using a Molecular Dynamics Storm 860 scanner (Molecular Dynamics, Palo Alto, CA). The cytokine expression was expressed as the area under the curve.
Laboratories, West Grove, PA). Detection was performed using the enhanced chemiluminescence (ECL) Western blotting analysis procedure (ECL Plus, Amersham, Freiburg, Germany).

Statistical analysis. Results were expressed as the arithmetical mean and SE (mean ± SE) for each group. To determine significant differences, means ± SE were analyzed using ANOVA and the Newman-Keuls test. Statistical significance in the pancreas major fatty acid composition, total n-6, total n-3, and the n-6-to-n-3 ratio between WT and fat-1 transgenic mice was determined using a Student t test.

RESULTS
MLD-STZ–induced fat-1 transgenic mice do not develop hyperglycemia. No significant body weight change was observed in WT and fat-1 mice after STZ treatment. One week after the last injection of STZ, STZ-induced mice started to develop hyperglycemia, which persisted for the entire observation period (25 days). As shown Fig. 1, glycemia increased in all the WT STZ-induced mice throughout the study period; in contrast, glycemia in all the fat-1 STZ-induced mice remained unchanged and was identical to that in WT and fat-1 citrate-treated mice. In the WT STZ-induced group, the mean blood glucose concentration was 320 mg/dL, with no body weight change, whereas in the STZ-induced fat-1 mice and the control citrate-treated mice this mean was 130 mg/dL.

n-3 enrichment protects fat-1 transgenic mice from MLD-STZ–induced β-cell damage. STZ induced severe degenerative and necrotic changes and islet shrinkage in WT mice compared with the citrate; in contrast, no histological changes were observed in STZ-induced fat-1 mice compared with the WT or fat-1 citrate-treated mice (Fig. 2A).

The effect of STZ administration on pancreatic GLUT2 gene expression was also assessed, since STZ is taken up via GLUT2. GLUT2 mRNA expression was decreased in STZ-induced WT mice compared with citrate-treated animals (Fig. 2B).

β-Cell insulin level was assessed by immunostaining pancreatic tissue, and pictures of representative islets are shown (Fig. 2C). Pancreas sections from citrate-treated WT and fat-1 mice showed islets with normal insulin content of insulin-expressing cells. As expected, diabetic STZ-induced WT mice showed lower insulin content and increased islet destruction. In contrast, STZ-induced fat-1 mice showed large islets with normal insulin content similar to the one observed in vehicle-treated animals. The number of islets bigger than 20,000 μm² was decreased by 70% in WT STZ-induced mice compared with WT citrate-treated animals. The percentage of islets bigger than 20,000 μm² was much higher in citrate-treated fat-1 mice compared with WT (+120%) and decreased by 33% when fat-1 mice were given STZ. Nevertheless, this percentage remained higher in fat-1 STZ-induced mice than in WT citrate-treated animals.

On day 25, the plasma insulin level (Fig. 2C) was dramatically decreased in WT STZ-induced mice, whereas this

FIG. 1. n-3 fatty acid enrichment prevents animals from MLD-STZ–induced hyperglycemia. Blood glucose level was measured in nonfasted WT and transgenic animals given STZ or STZ-vehicle as control (n = 4). Results are presented as a mean ± SE. Differences were analyzed by the Newman-Keuls test. Means assigned a superscript letters (a, b, c) were statistically different at P < 0.05. WC, citrate-treated WT mice; WS, STZ-induced WT mice; FC, citrate-treated fat-1 mice; FS, STZ-induced fat-1 mice.

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was prevented in fat-1 mice. Meanwhile, the plasma insulin level was significantly higher in citrate-treated transgenic mice compared with WT; however, when fat-1 mice were given STZ, the insulin level decreased to that of WT citrate-treated mice.

To further determine whether fat-1 mice were protected against MLD-STZ-induced pancreatic damage, the presence of apoptotic β-cells in pancreas sections was assessed by immunostaining for cleaved caspase-3, and pictures of representative islets are shown (Fig. 2D). Islets from STZ-induced WT mice showed a marked increase in apoptotic β-cells compared with islets of citrate-treated mice (Fig. 2D). However, STZ-induced fat-1 mice showed that the levels of apoptotic cells were identical to those of citrate-treated fat-1 mice and were even lower than in sections from citrate-treated WT mice.

Pancreas n-3 fatty acid enrichment inhibits proinflammatory cytokines, NF-κB protein expression and increases IκBα protein expression in MLD-STZ-induced fat-1 transgenic mice. A preliminary time-course study (days 1, 3, and 9 after the last STZ injection) has been performed to estimate the initiation and development of the inflammatory process and β-cell damage. Our results show that TNF-α mRNA expression was increased from day 3 and remained highly expressed at day 9 in the WT STZ-induced mice, when no expression of this proinflammatory cytokine was observed in the fat-1 animals, whatever the treatment (Fig. 3A). Moreover, in parallel to the induction of TNF-α expression, we observed an islet damage progression (Fig. 3B).

NF-κB activation plays a critical role in proinflammatory cytokine expression. We, therefore, examined pancreatic
expression of the NF-κB p65 protein to determine the underlying mechanisms involved in inhibition of STZ-induced inflammatory responses by fat-1 transgenic mice. Our results show (Fig. 4A) that the expression of the NF-κB p65 subunit was greatly increased in STZ-induced WT mice but not in STZ-induced fat-1 mice, suggesting that the endogenous n-3 fatty acid enrichment in the latter inhibited induction of NF-κB p65. Moreover, IκB protein expression, the NF-κB repressor, was highly repressed in WT STZ-induced mice but not in STZ-induced fat-1 mice.

In view of the preliminary results shown in Fig. 3A and in order to gain insight into the mechanisms underlying the protection of fat-1 mice from STZ-induced diabetes, we next examined at day 3 pancreatic mRNA expression of the inflammatory cytokines TNF-α/IL-1β, the expression of the chemokine MCP-1, and iNOS. As shown in Fig. 4B, the fat-1 mice showed a significant reduction in TNF-α, IL-1β, and iNOS mRNA expression compared with WT mice. Although MCP-1 gene expression was significantly increased in STZ-induced WT mice, it remained quite unchanged in STZ-induced fat-1 mice, as compared with citrate-treated groups.

**Pancreas n-3 fatty acid enrichment and formation of PUFA-derived mediators.** As shown in Fig. 5A, the fat-1 gene was well expressed in the pancreatic tissue of transgenic animals. Pancreas total lipid fatty acid compositional analysis revealed high increases in alpha-linolenic acid (18:3 n-3) EPA (20:5 n-3), and docosapentaenoic acid (DPA; 22:5 n-3).
n-3) in fat-1 transgenic mice compared with WT mice, whereas AA (20:4 n-6) was decreased by 97% (Fig. 5B). In
addition, the ratio of n-6 PUFA to the long chain n-3 PUFA was drastically reduced (P < 0.01) in fat-1 pancreatic
tissue (2.6 ± 0.4), compared with WT mice (225.8 ± 6.7). These data indicate that n-3 fatty acid desaturase ex-
pression enriched fat-1 pancreatic tissue in n-3 PUFA at the expense of n-6, giving a more balanced n-6-to-n-3 ratio.

We assessed PUFA-derived bioactive mediators, particularly 12- and 15-LO products from the pancreas (Fig. 5C).
Although no n-3 PUFA-derived mediators were detected in the WT pancreas, some were present in the fat-1 pancreas.
Regarding AA-derived mediators, the proinflammatory PGE2 was decreased by 95% in the transgenic STZ-induced mice and the toxic β-cell LO product, 12-HETE, was reduced by 97% in the fat-1 when compared with the STZ-induced WT mice. Moreover, the anti-inflammatory LXA4, undetected in WT, was detected in the fat-1 STZ-induced mice. Among the EPA-derived mediators, only 18-HEPE, precursor for the biosynthetic pathway of the anti-inflammatory resolvin E1, was detected in STZ-induced fat-1 mice, and its concentration was increased eightfold compared with the WT animals.
**Plasma n-3 fatty acid enrichment and total lipid level.**

Plasma total lipid fatty acid compositional analysis revealed huge increases in EPA and DPA in fat-1 transgenic mice compared with WT mice, whereas AA (20:4 n-6) was highly decreased (Fig. 6A). STZ administration did not affect the fatty acid composition of both WT and fat-1 mice. In addition, the ratio of n-6 PUFA to the n-3 PUFA was drastically reduced (*P* < 0.05; **P** < 0.01 (Student t test); *n* = 11 per group. ND, not detected. * The n-6-to-n-3 ratio is given by (18:2 n-6 + 20:4 n-6 + 22:4 n-6 + 22:5 n-6)/(18:3 n-3 + 20:5 n-3 + 22:5 n-3 + 22:6 n-3). C: Presence of different lipid mediators in pancreas samples of STZ-induced WT (*n* = 4) and fat-1 transgenic mice (*n* = 4). **P** < 0.01 (Student t test).

**DISCUSSION**

These results clearly demonstrate that increasing the pancreatic levels of endogenously synthesized n-3 PUFA prevents MLD-STZ–induced diabetes in fat-1 transgenic mice and that this effect is associated with less activation of markers of the inflammatory response. Furthermore, the protection from type 1 diabetes in fat-1 mice is correlated with the formation of anti-inflammatory derivatives of n-3 fatty acids and with downregulation of NF-κB p65 and proinflammatory cytokine expression in the pancreatic tissue of these animals.

More than alleviating chemically induced diabetes, our results show that endogenous production of n-3 fatty acids in fat-1 transgenic mice totally prevents hyperglycemia (Fig. 1). Animal studies have already suggested that PUFA might reduce the risk of chemically induced diabetes and attenuate the oxidant stress in animal models (30,31). Nevertheless, n-3 feeding is unable to mimic the protection observed in the fat-1 mice (19,32). Indeed, in these reports WT animals fed n-3 did not show similar phenotype than the fat-1 mice, which were totally resistant to MLD-STZ–induced diabetes. This can be explained by the fact that the fat-1 mice present the ideal n-6-to-n-3 ratio of about 1—only achievable in WT animals by consuming foods containing this ratio—without introducing
stringent dietary changes, which is not the case in the above dietary experiments. Additionally, inconsistent and/or controversial outcomes may be the result of confounding dietary factors. Many variables can arise from the diet and feeding procedure, including impurities in the oils used, food storage, and diet duration, all of which can affect the tissue fatty acid profile. In contrast, the genetic approach presented in this study allows us to generate two different plasma and pancreatic fatty acid profiles exhibiting a balanced ratio of n-6-to-n-3 PUFA (Fig. 5B, 6A and B) using a single diet rich in linoleic acid (18:2 n-6) but lacking n-3 fatty acids.

STZ is taken up by pancreatic β-cells via GLUT2, which is expressed at high levels in β-cells. We found that GLUT2 was expressed similarly in fat-1 and in WT mice (Fig. 2B). Moreover, GLUT2 mRNA expression was decreased in STZ-induced WT mice compared with citrate-treated animals, which agree with an alteration of β-cells. Nevertheless, we observed a slight decrease (not statistically different) of GLUT2 mRNA expression in fat-1 STZ-induced mice versus fat-1 controls, consistent with studies reporting that a reduced GLUT2 expression can prevent the diabetogenic action of STZ (33,34). Additionally, STZ itself restricts GLUT2 expression in vivo and in vitro when administered in multiple doses (35).

The dramatic decrease in WT STZ-induced mice plasma insulin level (Fig. 2C) observed in the current study is consistent with the usually described STZ fragmentation of DNA, which induces destruction of the insulin-producing β-cells (mainly by apoptosis), leading to reduced insulin secretion and thus to hyperglycemia (1,25). STZ-induced fat-1 mice showed large islets with no apoptotic β-cells and no decrease in insulin level (Fig. 2C and D). The higher plasma insulin level as well as bigger islets observed in the fat-1 transgenic mice compared with WT agrees with data reported recently on mfat-1 isolated islets (23). These authors explained this phenomenon by reduced level of PGE2, being known as a negative regulator of insulin production. Accordingly, in our study, such reduced PGE2 level is also observed in the fat-1 mice pancreas (Fig. 5C), in relation to higher insulin level. This suggests that the control of inflammation through reduction of n-6 PUFAs can be beneficial on β-cell function.

This demonstrates that n-3 fatty acid enrichment of plasma and the pancreas protects the fat-1 mice from STZ-induced β-cell destruction. We can then conclude that there is a relationship between n-3 PUFA levels and protection from hyperglycemia.

Type 1 diabetes is thought to result from perturbed immune regulation. STZ promotes immune cell invasion of the islet and generally causes pancreatic inflammation known as insulitis (36), generated by cytokines and free radicals. Despite C57Bl/6 J background being sensitive to hyperglycemic action of STZ, it has been reported that this model is resistant to STZ-induced insulitis (25). The present experiment confirms such data, and recent observations on mouse insulinoma 6 cells (data not shown) revealed higher TNF-α expression after STZ administration indicating increased inflammation, independently to immune cell invasion. In the current study, prevention of
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hyperglycemia in fat-1 mice was accompanied by downregulation of NF-κB p65, TNF-α, IL-1β, MCP-1, and iNOS mRNA expression (Fig. 4), suggesting that NF-κB plays an important proapoptotic role in cytokine-induced β-cell destruction. Interestingly, our results also show a higher expression of IkBα in the fat-1 mice than in WT, suggesting that a high n-3 PUFA pancreatic level would be able to overexpress IkBα to prevent NF-κB activation. Recently, Eldor et al. (37) reported that a conditional and specific NF-κB blockade protects pancreatic β-cells from diabeticogenic agents, showing that β-cell–specific activation of NF-κB is key in the progressive loss of β-cells in diabetes.

It is becoming increasingly clear that n-3 PUFA exert their effect on inflammatory gene expression through direct actions on the intracellular signaling pathways that lead to NF-κB activation (38). Our results can be related to a significant decrease in NF-κB activity observed in the colon of fat-1 mice with colitis (21) and colon tumors (39). Moreover, EPA-derived resolvin E1 is able to inhibit TNF-α–induced NF-κB activation (20).

TNF-α and IL-1β play a central role in regulating β-cell destruction in the pancreas (40), and TNF-α and IL-1β inhibitors prevent diabetes in mouse models (41,42). Our findings are consistent with previous studies in animal models and humans, showing that n-3 PUFA decrease TNF-α and IL-6 production (15,43,44), and with recent studies showing a lower TNF-α production in fat-1 mice during inflammation (21,38,45), confirming the function of the n-3 PUFA–derived compounds as anti-inflammatory mediators (20,21).

Both EPA and DHA decrease agonist-induced activation of NF-κB, which might play a role in reducing MCP-1 gene expression (46). We found decreased MCP-1 mRNA expression in transgenic mice (Fig. 4F); we propose that this MCP-1 downregulation is critically involved in the beneficial effects of endogenous n-3 fatty acids, along with downregulation of proinflammatory cytokines. Several of the deleterious effects of cytokines on rodent pancreatic islets are mediated by NO, which is produced by the inducible form of iNOS (47). Our results agree with previous reports showing attenuation of iNOS in an lipopolysaccharide-stimulated macrophages model by n-3 PUFA through NF-κB inhibition (48). Here we show that cytokine-induced NF-κB–mediated iNOS expression was significantly lower in the pancreas of fat-1 transgenic mice compared with WT (Fig. 4G).

The inflammatory process alleviation observed in the current study occurs via mechanisms similar to data recently obtained in vitro on islets, isolated from nfaft-1 mice and then exposed to proinflammatory cytokines (23). Such islets showed a strong resistance to cytokine-caused destruction comparable with the present one. These interesting data observed in vitro needed to be explored in vivo in chemically induced diabetic conditions; our present data evidence that fat-1 expression and its consequent pancreas enrichment in n-3 fatty acids is efficient in deterring diabetes by protecting from the STZ-cellular destruction. Another significant finding of the current study was large differences in the levels of the AA (notably 12-HETE) and EPA–derived mediators in fat-1 mice compared with controls. 12-HETE production has been linked to diabetes (9,49,50). It can activate NF-κB and is directly toxic to β-cells, markedly decreasing insulin secretion and increasing β-cell death (8). These observations can be related to the huge difference in n-6-to-n-3 fatty acid ratio between the transgenic and WT mice (Fig. 5B). Furthermore, the increased production of AA–derived LXA4, in the fat-1 tissue, indicates an overall shift from AA-derived proinflammatory metabolites to an anti-inflammatory and proresolving profile. LXA4 is formed by either transcellular metabolism of AA through two sequential lipoxygenation steps or from 15-HETE esterified in cellular phospholipids (51), a mechanism that has been linked to disease or host defense and that may be preferentially activated in the fat-1 mice. Additionally, EPA can compete with AA as substrate for cyclooxygenase (COX)-2, resulting in reduced levels of PGE2 and increased levels of PGE3. PGE3 was detected, albeit at very low levels, in fat-1 mice pancreatic tissue although it was not found in WT mice. However, the concentration of PGE2 did not reach that of PGE3, suggesting that there is a role for AA-derived lipid mediators that cannot be totally replaced by EPA-derived lipid mediators.

Taken together, our results evidence for the first time that fat-1 expression and its consequent pancreas enrichment in n-3 fatty acid prevents chemically induced diabetes. This prevention occurs by downregulating proinflammatory cytokine gene expression, blocking NF-κB activation, and highly repressing proinflammatory PUFA–derived lipid mediators in the pancreas of fat-1 mice versus WT. If pancreatic n-3 fatty acid enrichment is found to be effective in preventing insulin-dependent diabetes in humans, as was the case in mice in the current study, a nutritional cost-effective intervention could benefit young people affected by this disease, since there is currently no clinically useful preventive measure against developing autoimmune type 1 diabetes.

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