Leptin- or Troglitazone-induced Lipopenia Protects Islets from Interleukin 1β Cytotoxicity

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Abstract

Interleukin 1β (IL-1β)–induced β cell cytotoxicity has been implicated in the autoimmune cytotoxicity of insulin-dependent diabetes mellitus. These cytotoxic effects may be mediated by nitric oxide (NO). Since long-chain fatty acids (FFA), like IL-1β, upregulate inducible nitric oxide synthase and enhance NO generation in islets, it seemed possible that islets might be protected from IL-1β–induced damage by lowering their lipid content. We found that IL-1β–induced NO production varied directly and islet cell viability inversely with islet triglyceride (TG) content. Fat-laden islets of obese rats were most vulnerable to IL-1β, while moderately fat-depleted islets of food-restricted normal rats were less vulnerable than those of free-feeding normal rats. Severely lipopenic islets of rats made chronically hyperleptinemic by adenoviral leptin gene transfer resisted IL-1β cytotoxicity even at 300 pg/ml, the maximal concentration. Troglitazone lowered islet TG in cultured islets from both normal rats and obese, leptin-resistant rats and reduced NO production and enhanced cell survival. We conclude that measures that lower islet TG content protect against IL-1β–induced NO production and cytotoxicity. Leptin or troglitazone could provide in vivo protection against insulin-dependent diabetes mellitus. (*J. Clin. Invest.* 1997. 100:1750–1754.) Key words: diabetes • obesity • interleukin 1β • troglitazone • leptin

Introduction

Interleukin 1β (IL-1β) is cytotoxic to pancreatic β cells (1, 2). This has been attributed to upregulation of inducible nitric oxide synthase (iNOS)[1] and increased production of nitric oxide (NO) (3), which kills islet cells (4, 5). For these reasons IL-1β has been implicated in the autoimmune destruction of insulin-dependent diabetes mellitus (1, 6). In fact, iNOS is expressed in the insulitis of BB rats (7) and iNOS inhibitors protect against insulin-dependent diabetes mellitus (8, 9), providing compelling support for this hypothesis.

We have demonstrated that, like IL-1β, long-chain fatty acids (FFA) also upregulate iNOS expression and enhance NO generation in rat islets (10). This suggested that the lipid content of islets might influence the cytotoxic effects of IL-1β and that islet tissue lipopenia might protect β cells against NO-mediated cytotoxicity. In a study designed to test this possibility, we demonstrate a striking relationship between islet triglyceride (TG) content and IL-1β–mediated NO production and cytotoxicity; we further show that leptin and troglitazone, agents that lower islet TG content (11, 12), reduce IL-1β–induced NO production and cytotoxicity. The results suggest that agents that decrease islet lipid content might be useful in the prevention of autoimmune diabetes.

Methods

Animals. Obese homozygous (*fa*/*fa*) Zucker Diabetic Fatty (ZDF)-drt rats and lean wild-type (+/+) ZDF littermates were bred in our laboratory from [ZDF/Drt-fa (F10)] rats obtained from Dr. R. Peterson (University of Indiana School of Medicine, Indianapolis, IN). 7-wk-old male rats were used in all experiments. Genotypes were determined as described by Phillips et al. (13).

Hyperleptinemic rat model. Recombinant adenovirus containing either the rat leptin cDNA (AdCMV-leptin) or the bacterial β-galactosidase gene (AdCMV-β-Gal) was prepared as previously described in complete detail (14). 2 ml of AdCMV-leptin or AdCMV-β-Gal containing a total of 10^{12} pfu was infused into homozygous (+/+) ZDF rats. Animals were studied in individual metabolic cages, and food intake and body weight were measured daily. Blood samples were collected from the tail vein 7 d after adenovirus infusion. Plasma leptin was assayed using the Linco leptin assay kit (Linco Research Immunoassay, St. Charles, MO).

Islet isolation and culture. Pancreatic islets were isolated according to the method of Naber et al. (15) and maintained in suspension culture as previously described (10). The culture medium consisted of RPMI 1640 supplemented with 10% FBS and 2% BSA (fraction V; Miles Inc., Kankakee, IL). Recombinant human IL-1β (Sigma Chemical Co., St. Louis, MO) in a concentration of 0, 10, 100, or 300 pg/ml was added either with or without 1 mM long-chain fatty acids (oleate/palmitate, 2:1, sodium salt; Sigma Chemical Co.).

TG measurements. Islet TG content was measured by the Sigma TG kit (GPO-Trinder) as described previously (16).

Nitrite determination. 250 μl of culture medium was incubated with an equal volume of the Griess reagent (1% sulfanilamide in 0.1 mol/liter HCl and 0.1% naphthyl ethylenediamine dihydrochloride) for 10 min at room temperature and NO was determined as nitrite from the absorbance at 550 nm using sodium nitrite as standard (10, 17).

Cell viability assay. Islet viability was measured using the XTT dye-reduction assay kit (Boehringer Mannheim, Indianapolis, IN) (18). The assay is based on the cleavage of XTT (sodium 3-[1-(phenylamino-carbonyl)-3,4-tetrazolium]-bis (4-methoxy-6-nitro) benzene sulfonic acid hydrate) to form formazan by mitochondrial respiration

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1. *Abbreviations used in this paper:* AdCMV-leptin, adenovirus containing the rat leptin cDNA; AdCMV-β-Gal, adenovirus containing the bacterial β-galactosidase gene; iNOS, inducible nitric oxide synthase; NO, nitric oxide; TG, triglyceride; ZDF, Zucker Diabetic Fatty.

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Table I. TG Content of 24-h–cultured Pancreatic Islets Isolated from AdCMV-β-Gal and AdCMV-leptin Infused Lean ZDF Rats, Pair-fed Controls and Obese fa/fa ZDF Rats

	Lean +/+ ZDF			Obese *fa/fa* ZDF
	AdCMV-β-Gal	AdCMV-leptin	Pair-fed	Intact
TG (ng/islet)	18.9±1.1 (12)	2.3±0.4*‡ (12)	13.0±1.5 (12)	70.3±6.3 (4)

Values are expressed as the mean±SEM. Numbers of experiments are in parentheses. *$P < 0.001$ vs. AdCMV-β-Gal, ‡$P < 0.001$ vs. pair-fed.

in viable cells (18). After each 24-h treatment, islets were incubated with XTT labeling mixture (final concentration: XTT, 0.3 mg/ml; *N*-methyl dibenzopyradine methyl sulfate, 8.3 μM) for 4 h at 37°C, and the absorbance was measured at 492 nm and 690 nm. The values were expressed as the percentages of the values observed in the absence of IL-1β.

Statistical analysis. Values are expressed as the mean±SEM. Statistical analysis was performed by two-tailed unpaired Student's *t* test or two-way ANOVA.

Results

Effect of islet TG content on IL-1β–induced nitrite production and cell viability. To determine the influence of islet TG content on IL-1β–induced NO production and cytotoxicity we measured islet nitrite accumulation and cell viability in rats with a wide range of tissue fat. At one extreme we used 7-wk-old obese male ZDF rats (*fa/fa*) with fat-laden islets (16, 19); at the other extreme we used the most stringent method of lipid depletion, induction in normal lean rats of leptin overexpression by AdCMV-leptin infusion (12, 14). The phenotype is extreme tissue lipopenia and the disappearance of visible fat. A less profound reduction in islet fat content in vivo was produced by pair-feeding of intact rats to the hyperleptinemic rats (12). The TG content of islets isolated from these groups of rats and from free-feeding control rats infused with AdCMV-β-Gal are indicated in Table I.

In islets of free-feeding AdCMV-β-Gal–infused controls with normal TG content, nitrite production at 300 pg/ml of IL-1β reached 56 pmol/islet per 24 h (Fig. 1 *A*), and cell viability declined to < 60% of the control value (Fig. 1 *B*). In TG-depleted islets of hyperleptinemic rats, by contrast, baseline nitrite accumulation was only ~ 12 pmol/islet per 24 h and the addition of 300 pg/ml of IL-1β failed to raise it significantly above this level (Fig. 1 *A*); viability remained above 80% of the control value (Fig. 1 *B*). In pair-fed rats with a 50% reduction in islet TG content, nitrite rose significantly in the presence of IL-1β ($P < 0.001$) but remained at ~ 40 pmol/islet per 24 h, significantly below the nitrite value of 56 pmol/islet per 24 h in free-feeding β-Gal controls ($P < 0.001$) (Fig. 1 *A*); however, viability was not significantly different from the latter (Fig. 1 *B*).

In the fat-laden islets of obese homozygous (*fa/fa*) ZDF rats, nitrite accumulation, which averaged 68±6.8 pmol/islet per 24 h in the absence of IL-1β (Fig. 1 *A*), reached a peak level of 147±7.8 pmol/islet per 24 h at the 300 pg/ml concentration of IL-1β, 3.6 times the nitrite level of the lean β-Gal controls ($P < 0.001$) (Fig. 1 *A*); viability was 40%, significantly below that of islets of lean β-Gal controls ($P < 0.001$) (Fig. 1 *B*).

Effect of FFA on IL-1β–induced nitrite production and cytotoxicity in islets. To compare the in vitro effects of FFA on IL-1β–induced nitrite production in islets with varying esterification capacities we isolated islets from normal rats and from obese ZDF rats, which have been shown to have a high esterification capacity (19); we cultured them with or without 1 mM FFA and examined IL-1β–induced nitrite production and cytotoxicity (Fig. 2). In the absence of IL-1β, FFA raised nitrite accumulation in islets from lean rats to 33±5.2 pmol/islet per 24 h, close to the peak values induced by the cytokine in the absence of FFA; FFA plus IL-1β increased nitrite production to ~ 80 pmol/islet per 24 h (Fig. 2 *A*). In the islets from obese rats cultured in FFA without IL-1β, nitrite accumulation exceeded the peak induced in islets of lean rats by FFA plus 300 pg/ml of IL-1β; in islets of obese rats FFA plus 300 pg/ml of IL-1β increased the cumulative nitrite level to ~ 300 pmol/islet per 24 h (Fig. 2 *A*). This was significantly above the response of lean controls (291±23.0 vs. 77±4.9 pmol/islet per 24 h, $P < 0.001$). In the presence of 300 pg/ml of IL-1β, 1 mM FFA reduced viability of normal islets to ~ 45%, about the same as islets from obese rats without the FFA. Viability of islets of obese rats declined below 30% when 1 mM FFA and 300 pg/ml of IL-1β were both present in the culture medium (Fig. 2 *B*).

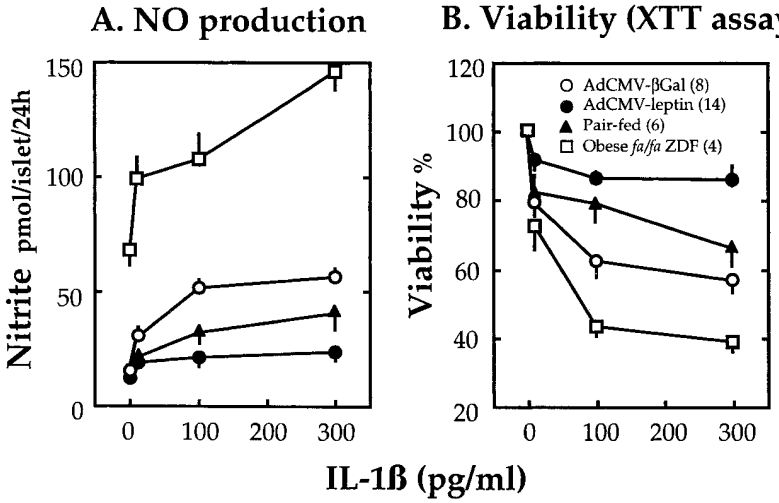

Figure 1. Effect of TG content on (*A*) IL-1β–induced nitrite production and (*B*) cell viability in islets isolated from AdCMV-leptin–induced wild-type (+/+) ZDF rats, the pair-fed controls, free-feeding AdCMV-β-Gal controls, and obese *fa/fa* ZDF. Islets were isolated 7 d after virus infusion and cultured for 24 h with recombinant human IL-1β at the indicated concentrations. Data are expressed as the mean±SEM. Viability is expressed as the percentage of the values observed in the absence of IL-1β. Numbers of experiments are in parentheses.

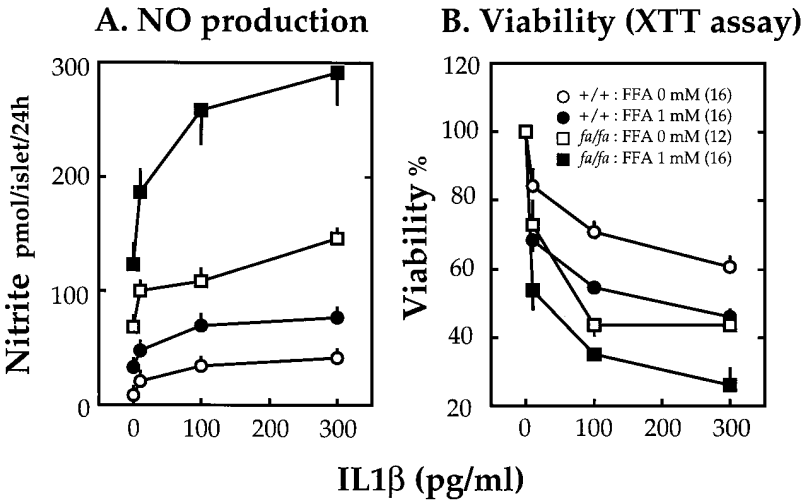

Figure 2. Effect of 1 mM FFA on (*A*) nitrite production and (*B*) cell viability in lean wild-type (+/+) ZDF rats and obese (*fa*/*fa*) ZDF rats. Islets isolated from intact rats were cultured with recombinant human IL-1β at the indicated concentrations, either with or without 1 mM FFA mixture (oleate/palmitate, 2:1) for 24 h. Data are expressed as the mean±SEM. Viability is expressed as the percentage of the values observed in the absence of IL-1β. Numbers of experiments are in parentheses.

Effect of troglitazone on IL-1β–mediated nitrite production and cytotoxicity in islets. Troglitazone has been shown to reduce tissue TG content of liver (11), while leptin depletes TG in islets (12). To determine if this thiazolidinedione also depletes TG in islets, we measured the TG content of islets from normal lean rats cultured for 24 h in the presence of 10 μM troglitazone. TG declined by 42% and, when 1 mM FFA was present, TG accumulation was prevented by troglitazone (Fig. 3 *A*). Thus like leptin (8), troglitazone reduces endogenous TG of islets and prevents the formation of new TG from FFA (12). Troglitazone reduced by 47% the rate of nitrite accumulation in response to IL-1β alone and abolished the enhancing effect of FFA on IL-1β–induced nitrite production (Fig. 3 *B*). In the absence of FFA, troglitazone improved viability to over 90% despite the presence of 300 pg/ml of IL-1β; in the presence of FFA plus IL-1β viability exceeded 75%, which was still significantly above the controls ($P < 0.001$) (Fig. 3 *C*).

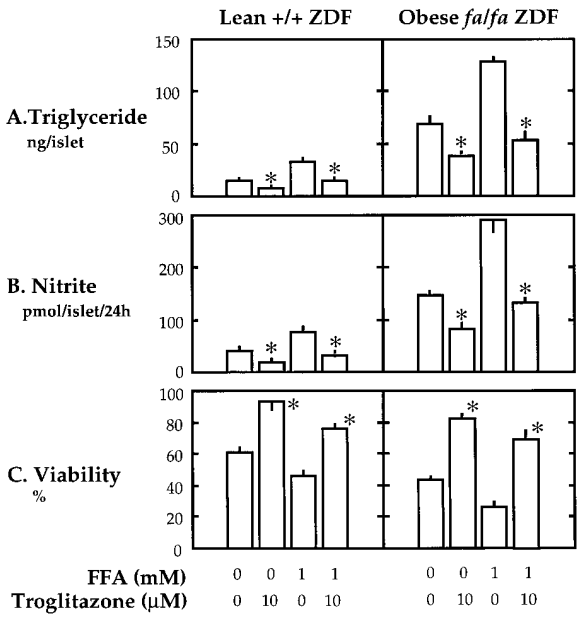

Figure 3. Effect of troglitazone on (*A*) islet TG content, (*B*) nitrite production, and (*C*) islet cell viability. Islets isolated from wild-type (+/+) ZDF rats and obese (*fa*/*fa*) ZDF rats were cultured for 24 h with 300 pg/ml IL-1β, with or without FFA and with or without troglitazone. Data are expressed as the mean±SEM of three experiments. Viability is expressed as the percentage of the values observed at IL-1β 0 pg/ml. Statistical significance is indicated as $^*P < 0.001$ vs. troglitazone 0 pg/ml.

Because of their mutated leptin receptor (13, 20), leptin has no effect on TG content of islets from obese *fa*/*fa* ZDF rats (12); however, troglitazone significantly reduced the TG content (Fig. 3 *A*). Nitrite production declined (Fig. 3 *B*), and viability was significantly improved above the values observed in the absence of troglitazone ($P < 0.001$) (Fig. 3 *C*).

Relationship between TG content and IL-1β–mediated nitrite production and cytotoxicity in islets. The relationship between tissue TG content and IL-1β action was further scrutinized by combining all available in vivo and in vitro data in the various groups to compare islet fat content with IL-1β–induced nitrite production. A remarkably strong correlation was apparent ($R^2 = 0.972$, $P < 0.001$) (Fig. 4).

Discussion

Earlier studies from our group have demonstrated that lipid overload in islets of obese ZDF rats impairs β cell function and ultimately reduces the β cell population by at least 50% (21). These lipotoxic effects have been attributed to FFA-mediated induction of iNOS and NO-mediated cytotoxicity (10). Because iNOS expression and nitrite production are upregulated in both immunogenic (1, 3–9) and adipogenic (10) diabetes, and because agents that inhibit iNOS expression or nitrite production in vitro and in vivo, such as nicotinamide and aminoguanidine, prevent β cell abnormalities and hyperglycemia in both forms of diabetes (8–10), we examined the possibility that islet TG content might influence the cytotoxic potency of IL-1β, a cytokine implicated in the pathogenesis of autoimmune diabetes (1–9). This premise is consistent with a recent report that obesity increases sensitivity to endotoxin-induced liver injury (22). If so, lowering of lipid content to subnormal levels might confer a measure of protection against such damage.

We therefore studied four groups of rats with widely varying islet TG content. Islets of obese ZDF rats were fat laden, while those of leptin-overexpressing rats were fat depleted.

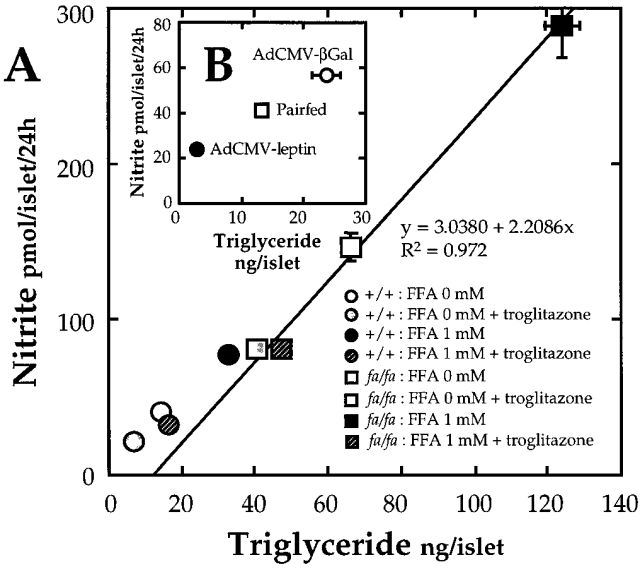

Figure 4. (*A*) Correlation between islet TG content and IL-1β–induced NO production in islets isolated from wild-type (+/+) and obese (*fa*/*fa*) ZDF rats and cultured for 24 h with 300 pg/ml IL-1β, with or without FFA and with or without troglitazone; in these experiments variations in TG content were the result of in vitro manipulations during the 24-h culture period. (*B*) Values in islets isolated from AdCMV-leptin–infused rats, pair-fed controls, and AdCMV-β-Gal controls are shown in the inset; in these experiments the differences in TG content were the result of in vivo manipulations carried out before islet isolation (compare with Table I). Data are expressed as the mean±SEM of four or more experiments.

Normal rats pair-fed to the hyperleptinemic rats exhibited an islet TG content intermediate between the hyperleptinemic group and normal controls. We observed a remarkable relationship between TG content of islets, NO generation, and cell viability. NO production was minimal in islets of the hyperleptinemic rats and highest in those of the obese. Viability was maximal in the islets of hyperleptinemics and minimal in those of the obese rats. These in vivo effects were duplicated in vitro in cultured islets subjected to maneuvers that altered islet TG content. FFA increased islet TG content and NO production rose and viability declined. Troglitazone reduced TG content and NO production declined and viability improved. This was observed even in the islets of the obese ZDF rats, which are resistant to the lipopenic action of leptin because of the mutation in their leptin receptor (13, 20).

If an orally administered agent such as troglitazone lowers islet TG content in humans as it did in the rat islets of this study, one could then consider assessing its coadministration with nicotinamide for the prevention of autoimmune diabetes without the use of immunosuppression. The striking reduction in nitrite production and improvement in cell viability associated with a decrease in islet TG content from normal to subnormal is consistent with the usefulness of this strategy. In addition, the leptin-like effects of troglitazone in lowering TG content provide a new mechanism for understanding its effectiveness in the treatment of adipogenic non–insulin-dependent diabetes (23).

The mechanism by which increased TG content induces iNOS expression is entirely unknown. However, previously we have noted that high levels of FFA induce certain enzymes, among them iNOS (10). In addition, we have speculated previously that intracellular FFA are required for formation of diacyl glycerol and ceramide (19), both of which are believed to participate in the IL-1β signal cascade (1, 3, 7, 8). Perhaps leptin-mediated depletion of TG in cells reduces diacyl glycerol and ceramide formation and thus attenuates IL-1β–induced cytotoxicity.

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