Resistin Knockout Mice Exhibit Impaired Adipocyte Glucose-Dependent Insulinotropic Polypeptide Receptor (GIPR) Expression

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Glucose-dependent insulinotropic polypeptide (GIP) is an incretin hormone that also plays a regulatory role in fat metabolism. In 3T3-L1 cells, resistin was demonstrated to be a key mediator of GIP stimulation of lipoprotein lipase (LPL) activity, involving activation of protein kinase B (PKB) and reduced phosphorylation of liver kinase B1 (LKB1) and AMP-activated protein kinase (AMPK). The current study was initiated to determine whether resistin has additional roles in GIP-regulated adipocyte functions. Analysis of primary adipocytes isolated from *Retn*$^{-/-}$, *Retn*$^{+/-}$, and *Retn*$^{+/+}$ mice found that GIP stimulated the PKB/LKB1/AMPK/LPL pathway and fatty acid uptake only in *Retn*$^{+/+}$ adipocytes, suggesting that GIP signaling and/or GIP responsiveness were compromised in *Retn*$^{+/-}$ and *Retn*$^{-/-}$ adipocytes. GIP receptor (GIPR) protein and mRNA were decreased in *Retn*$^{+/-}$ and *Retn*$^{-/-}$ adipocytes, but resistin treatment rescued LPL responsiveness to GIP. In addition, genes encoding tumor necrosis factor (TNF), TNF receptor 2 (TNFR2), and the signaling proteins stress-activated protein kinase (SAPK)/Jun NH_2-terminal kinase (JNK), were downregulated, and phosphorylated levels of SAPK/JNK/c-Jun were decreased in *Retn*$^{-/-}$ mice. Chromatin immunoprecipitation assays were used to identify a 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-response element (TRE-III) responsible for c-Jun–mediated transcriptional activation of *Gipr*. Blunted GIP responsiveness in *Retn*$^{+/-}$ and *Retn*$^{-/-}$ adipocytes was therefore largely due to the greatly reduced GIPR expression associated with decreased c-Jun–mediated transcriptional activation of *Gipr*. ***Diabetes*** **62:471–477, 2013**

The incretin hormones, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1, affect β-cell secretion, survival, and proliferation (1,2). Because incretin mimetics and inhibitors of the incretin-degrading enzyme, dipeptidyl peptidase-IV, are used as type 2 diabetes therapeutics (3), there is considerable interest in actions of incretins on other target tissues (4). There exists strong evidence that GIP plays a regulatory role in fat metabolism (5–10). We previously demonstrated that insulin antagonizes lipolytic actions of GIP (5), whereas stimulatory effects of GIP on adipocyte lipoprotein lipase (LPL) were insulin-dependent (6,7). Unexpectedly, GIP actions on LPL were found to be mediated through regulation of resistin secretion and activation of a pathway involving increased phosphorylation of protein kinase B (PKB) and reduced phosphorylation of liver kinase B (LKB1) and AMP-activated protein kinase (AMPK) (8).

Controversies exist concerning the physiological functions of resistin (11–13). Although this adipokine has consistently been shown to induce insulin resistance in liver and skeletal muscle (11,13,14), its role in adipose tissue is unclear. Resistin promoted 3T3-L1 cell differentiation to adipocytes (15). However, whereas fat-specific overexpression impaired re-esterification of fatty acids to triglycerides (16), treating mouse adipose explants with human resistin (FIZZ3) increased lipolysis and re-esterification (17). In view of resistin's involvement in GIP-mediated LPL activation (8), we examined resistin knockout (*Retn*$^{-/-}$) mice to assess its involvement in further adipocyte actions and identified a central role in regulating GIP receptor expression.

RESEARCH DESIGN AND METHODS

Animals. *Retn*$^{+/-}$ mice were provided by Dr. Mitchell Lazar, University of Pennsylvania School of Medicine. Breeding and genotyping were performed at the University of British Columbia (UBC). Animal experiments were conducted in accordance with guidelines of the UBC Committee on Animal Care and Canadian Council on Animal Care.

LPL enzyme activity and fatty acid uptake assay. Primary adipocytes were isolated (18) from male *Retn*$^{+/+}$, *Retn*$^{+/-}$, and *Retn*$^{-/-}$ mice (10–12 weeks old) and plated using Matrigel (BD Biosciences). After GIP plus insulin treatment, adipocytes were recovered and LPL activity was measured, as described (6). For the fatty acid uptake assay (19), adipocytes were incubated with 4,4-difluoro-5-methyl-4-bora-3a, 4a-diaza-s-indacene-3-dodecanoic acid (C_1-BODIPY500/510-C_{12}, Molecular Probes) and sorted on a BD LSRII flow cytometer.

Quantitative PCR and RT^2 Profiler Arrays. Total RNA was extracted using an RNeasy Mini Kit (Qiagen) and *Gipr* expression measured, as described previously (10). RT^2 Profiler PCR Arrays were performed to quantify mRNAs of multiple genes, including housekeeping genes, according to the manufacturer's protocol.

Coimmunoprecipitation and Western blot analysis. Protein samples were immunoprecipitated using Dynabead protein A or protein G (Invitrogen) and c-Jun or phospho-serine/threonine antibody (Cell Signaling; ECM Biosciences). Precipitated products or protein samples were resolved by SDS-PAGE and probed with the antibodies indicated in the Figure legends.

Chromatin immunoprecipitation (ChIP). c-Jun was immunoprecipitated from optimally sheared chromatin, thus preserving specific protein/DNA complexes of *Retn*$^{+/+}$ and *Retn*$^{-/-}$ adipocytes, using Dynabead protein G and c-Jun antibody. Bound DNA was quantified by SYBR green PCR using oligonucleotides flanking 12-*O*-tetradecanoylphorbol-13-acetate (TPA) response element (TRE)-I, TRE-II, or TRE-III of the mouse *Gipr* promoter.

Statistical analysis. Significance was tested using ANOVA with the Newman-Keuls post hoc test or Student *t* test ($P < 0.05$), as indicated in the Figure legends.

RESULTS

GIP regulates a PKB/LKB1/AMPK/LPL pathway and resistin secretion in *Retn*$^{+/+}$ adipocytes but not in *Retn*$^{+/-}$ or *Retn*$^{-/-}$ adipocytes. Mating of heterozygous *Retn*$^{+/-}$ mice resulted in expected *Retn*$^{+/+}$, *Retn*$^{+/-}$, and

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Retn$^{-/-}$ genotypes (14). The mice did not differ significantly in body weight, glucose tolerance, or epididymal white adipose tissue (eWAT) adipocyte size (Supplementary Fig. 1*A*–*E*). Immunoreactive resistin was undetectable in *Retn*$^{-/-}$ mice plasma and eWAT extracts, whereas plasma and fat levels in *Retn*$^{+/-}$ mice were <25% of *Retn*$^{+/+}$ levels (Supplementary Fig. 1*F* and *G*).

To investigate the role of resistin in GIP-mediated increases in LPL activity, responses of *Retn*$^{+/+}$, *Retn*$^{+/-}$, and *Retn*$^{-/-}$ adipocytes to GIP were examined. Incubation of *Retn*$^{+/+}$ adipocytes with GIP plus insulin (1 nmol/L) resulted in concentration-dependent stimulation of LPL activity (Fig. 1*A*), increased levels of phospho-PKB (Ser473), and decreased levels of phospho-LKB1 (Ser428) and phospho-AMPK (Thr172), but was without effect on *Retn*$^{+/-}$ or *Retn*$^{-/-}$ adipocytes (Supplementary Fig. 2*A*–*C*). In addition, treatment of *Retn*$^{+/+}$ adipocytes with GIP plus insulin (1 nmol/L) resulted in significantly increased fatty acid uptake, but was without effect on *Retn*$^{+/-}$ or *Retn*$^{-/-}$ adipocytes (Fig. 1*B*). The complete lack of *Retn*$^{+/-}$ adipocyte responses was surprising and suggested a critical resistin secretion level was required for GIP-mediated stimulation of LPL activity and/or adipocyte responsiveness to GIP was compromised.

The possibility that resistin secretion from *Retn*$^{+/-}$ adipocytes was reduced to ineffective levels for activating the PKB/LKB1/AMPK pathway was first tested. Incubation of *Retn*$^{+/+}$ adipocytes with GIP plus insulin (1 nmol/L) for 2 h resulted in concentration-dependent stimulation of resistin secretion (Supplementary Fig. 3*A*). Basal secretion of resistin by *Retn*$^{+/-}$ adipocytes was significantly reduced, and secretion by *Retn*$^{-/-}$ adipocytes was undetectable. Resistin secretion by *Retn*$^{+/-}$ adipocytes was unresponsive to GIP plus insulin (1 nmol/L). In addition, insulin alone had no effect on resistin secretion by *Retn*$^{+/+}$ adipocytes (Supplementary Fig. 3*B*). These results indicated that the inability of GIP to activate the PKB/LKB1/AMPK/LPL pathway in *Retn*$^{+/-}$ adipocytes involved severely blunted resistin secretion.

The GIP receptor (GIPR) is downregulated in *Retn*$^{+/-}$ and *Retn*$^{-/-}$ adipocytes. To examine the involvement of altered receptor expression, GIPR protein and mRNA levels were determined, and *Retn*$^{+/-}$ and *Retn*$^{-/-}$ adipocytes demonstrated severe downregulation (Fig. 1*C* and *D*). We next examined whether resistin acted as a direct regulator of GIPR expression. Treatment of *Retn*$^{+/+}$ adipocytes with resistin (100 nmol/L) resulted in a delayed increase in GIPR expression, after 72-h incubation (Fig. 2*A*), indicating chronic effects on expression, and 72-h treatment with resistin rescued GIP-induced LPL activity in *Retn*$^{+/-}$ and *Retn*$^{-/-}$ adipocytes (Fig. 2*B*).

Intriguingly, GIPR protein was also downregulated in brown adipose tissue and the stromal vascular fraction of *Retn*$^{+/-}$ and *Retn*$^{-/-}$ mice (Supplementary Fig. 4*A* and *B*), whereas no significant differences were noted in skeletal muscle or pancreatic islet GIPR protein expression among the groups (Supplementary Fig 4*C* and *D*). In addition, treatment of *Retn*$^{+/+}$ islets with resistin (100 nmol/L) had no effect on GIPR expression (Supplementary Fig. 4*E*), implying that regulation was tissue-specific.

Adipocyte *Gipr* expression is regulated via a c-Jun–mediated pathway. With a view to identifying proteins involved in regulating the adipocyte *Gipr*, a PCR array was used to screen a panel of 84 genes associated with obesity, insulin resistance, and early onset of diabetes (RT^2 Profiler) in eWAT from *Retn*$^{+/+}$ and *Retn*$^{-/-}$ mice. This

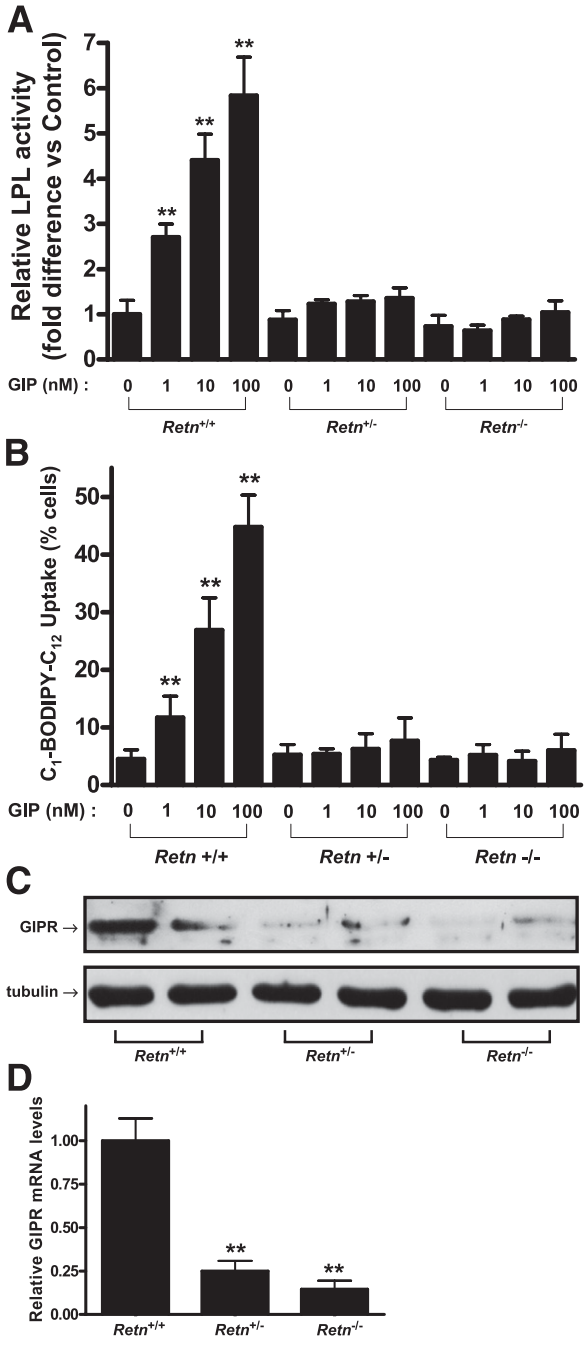

FIG. 1. GIPR expression is downregulated in *Retn*$^{+/-}$ and *Retn*$^{-/-}$ adipocytes. *A*: GIP-mediated LPL activity in *Retn*$^{+/+}$, *Retn*$^{+/-}$, and *Retn*$^{-/-}$ adipocytes. Primary adipocytes were isolated from eWAT of *Retn*$^{+/+}$, *Retn*$^{+/-}$, and *Retn*$^{-/-}$ mice and plated using Matrigel. Adipocytes were treated with the indicated concentrations of GIP (nmol/L) for 24 h in the presence of insulin (1 nmol/L), and LPL activity was determined. ***P* < 0.05 vs. control. *B*: GIP-mediated fatty acid uptake. Primary adipocytes were treated as described in *A*, and incubated with C_1-BODIPY500/510-C_{12} (10 μmol/L) for 1 h. After treatment, cells were recovered using BD recovery solution, and C_1-BODIPY500/510-C_{12} uptake was determined by flow cytometry. Significance was tested using ANOVA with Newman-Keuls post hoc test. ***P* < 0.05 vs. control. *C*: GIPR protein expression in *Retn*$^{+/+}$, *Retn*$^{+/-}$, and *Retn*$^{-/-}$ adipocytes. Primary adipocytes were isolated from eWAT of *Retn*$^{+/+}$, *Retn*$^{+/-}$, and *Retn*$^{-/-}$ mice. Protein extracts were isolated and Western blot analyses performed with antibodies against GIPR and β-tubulin. *D*: *Gipr* mRNA expression in *Retn*$^{+/+}$, *Retn*$^{+/-}$, and *Retn*$^{-/-}$ adipocytes. Real-time RT-PCR was performed on extracts to quantify *Gipr* mRNA levels and is shown as the fold difference vs. *Retn*$^{+/+}$ adipocytes normalized to 18S rRNA expression levels. Significance was tested using ANOVA with Newman-Keuls post hoc test. ***P* < 0.05 vs. control or *Retn*$^{+/+}$ adipocytes. Data represent the mean ± SEM.

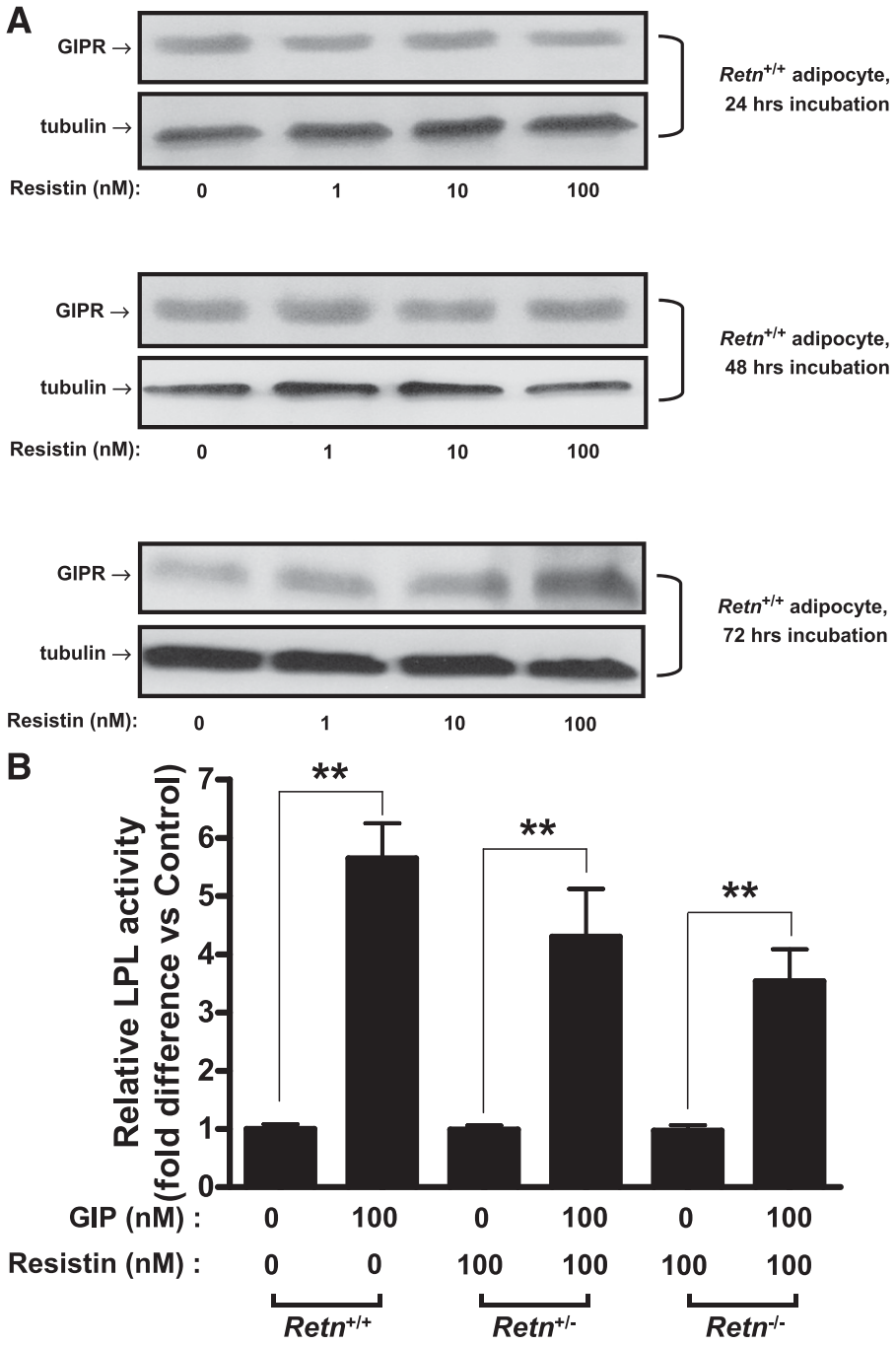

FIG. 2. Resistin increases GIPR expression in *Retn*$^{+/+}$ adipocytes and rescues GIP responsiveness of *Retn*$^{+/-}$ and *Retn*$^{-/-}$ adipocytes. *A*: Effect of resistin on GIPR expression in *Retn*$^{+/+}$ adipocytes. Primary adipocytes were isolated from *Retn*$^{+/+}$ mice, plated using Matrigel, and treated with resistin for the indicated periods of time. Protein extracts were isolated and Western blot analyses performed with antibodies against GIPR and β-actin. *B*: Effect of resistin on GIP-mediated LPL activation in *Retn*$^{+/-}$ and *Retn*$^{-/-}$ adipocytes. Primary adipocytes were isolated from *Retn*$^{+/-}$ and *Retn*$^{-/-}$ mice and plated using Matrigel. Adipocytes were treated with resistin for 72 h, further treated with GIP in the presence of insulin (1 nmol/L) for 24 h, and LPL activity was determined. Significance was tested using ANOVA with Newman-Keuls post hoc test. **$P < 0.05$ vs. control or indicated group. Data represent the mean ± SEM.

revealed large reductions in *Retn*$^{-/-}$ fat expression of genes coding for proteins involved in signaling (ectonucleotide pyrophosphatase/phosphodiesterase 1 [*Enpp1*]), intercellular adhesion (intracellular adhesion molecule [*Icam1*]), GLUT-4 trafficking (syntaxin 4a [*Stx4a*]), and glyceroneogenesis (soluble phosphoenolpyruvate carboxykinase 1 [*Pck1*]) (Fig. 3*A*). Of particular interest, genes encoding tumor necrosis factor (TNF [*Tnf*]), tumor necrosis factor receptor 2 (TNFR2 [*Tnfrsf1b*]), and the downstream signaling protein(s) stress-activated protein kinase (SAPK)/Jun NH_2-terminal kinase (JNK) (*Mapk8*), were decreased in *Retn*$^{-/-}$ fat tissue. In contrast, *Gsk3b* (glycogen synthase kinase 3 [GSK3]-β) expression, a negative regulator of c-Jun (20) was increased in *Retn*$^{-/-}$ fat (Fig. 3*B*). Levels of phospho-SAPK/JNK (Thr183/Tyr185) and phospho-c-Jun, were also decreased (Fig. 3*C* and *D*). Together, these results suggested that decreased adipocyte GIPR expression involved reduced signaling via TNF activation of SAPK/JNK and c-Jun.

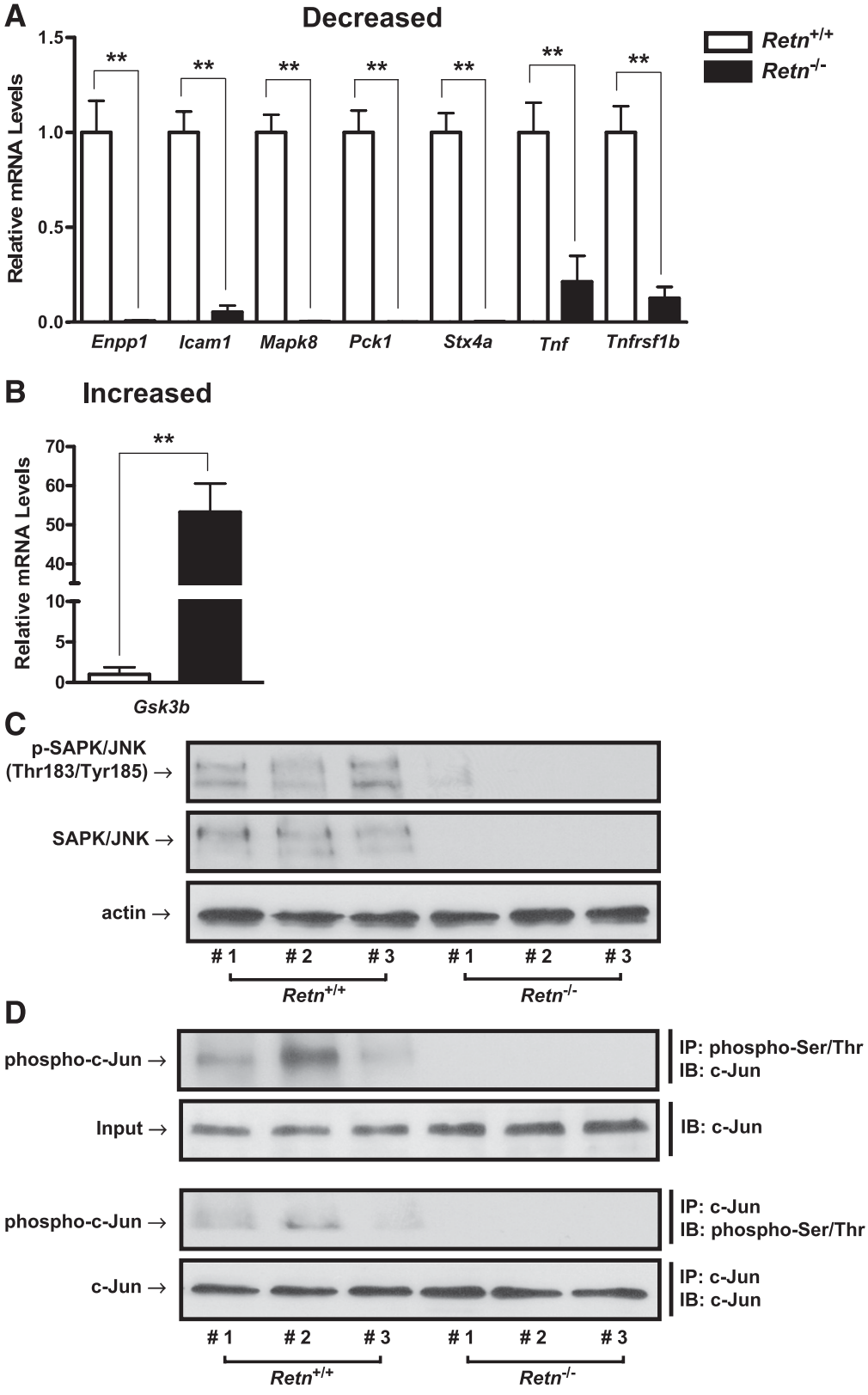

FIG. 3. JNK/c-Jun expression is downregulated in *Retn*$^{-/-}$ adipocytes. *A* and *B*: Gene expression profiles. Total RNA was extracted from eWAT of *Retn*$^{+/+}$ and *Retn*$^{-/-}$ mice, and RT^2 Profiler PCR Arrays were performed to simultaneously quantify mRNA expression levels of multiple genes. Genes changed in *Retn*$^{+/+}$ adipocytes: *Enpp1* (ectonucleotide pyrophosphatase/phosphodiesterase 1), *Icam1* (intercellular adhesion molecule 1), *Pck1* (phosphoenolpyruvate carboxykinase 1 [soluble]), *Stx4a* (syntaxin 4a), *Mapk8* (SAPK/JNK), *Tnf* (TNF), *Tnfrsf1b* (TNFR2) (*A*) and *Gsk3b* (glycogen synthase kinase 3 [GSK3]-β) (*B*). *C* and *D*: JNK/c-Jun expression in *Retn*$^{+/+}$ and *Retn*$^{-/-}$ adipocytes. Primary adipocytes were isolated from eWAT of *Retn*$^{+/+}$ and *Retn*$^{-/-}$ mice and protein extracts prepared. Western blot analyses were performed with antibodies against phospho-SAPK/JNK (Thr183/Tyr185), SAPK/JNK, and β-actin. Protein extracts were immunoprecipitated (IP) with phospho-serine (Ser)/threonine (Thr) or c-Jun, followed by immunoblotting (IB) for c-Jun or phospho-Ser/Thr. Input represents one-tenth of protein extracts used in assay. Western blots are representative of $n = 3$. All data represent the mean ± SEM. Significance was tested using Student *t* test. ***P* < 0.05 vs. *Retn*$^{+/+}$ adipocytes.

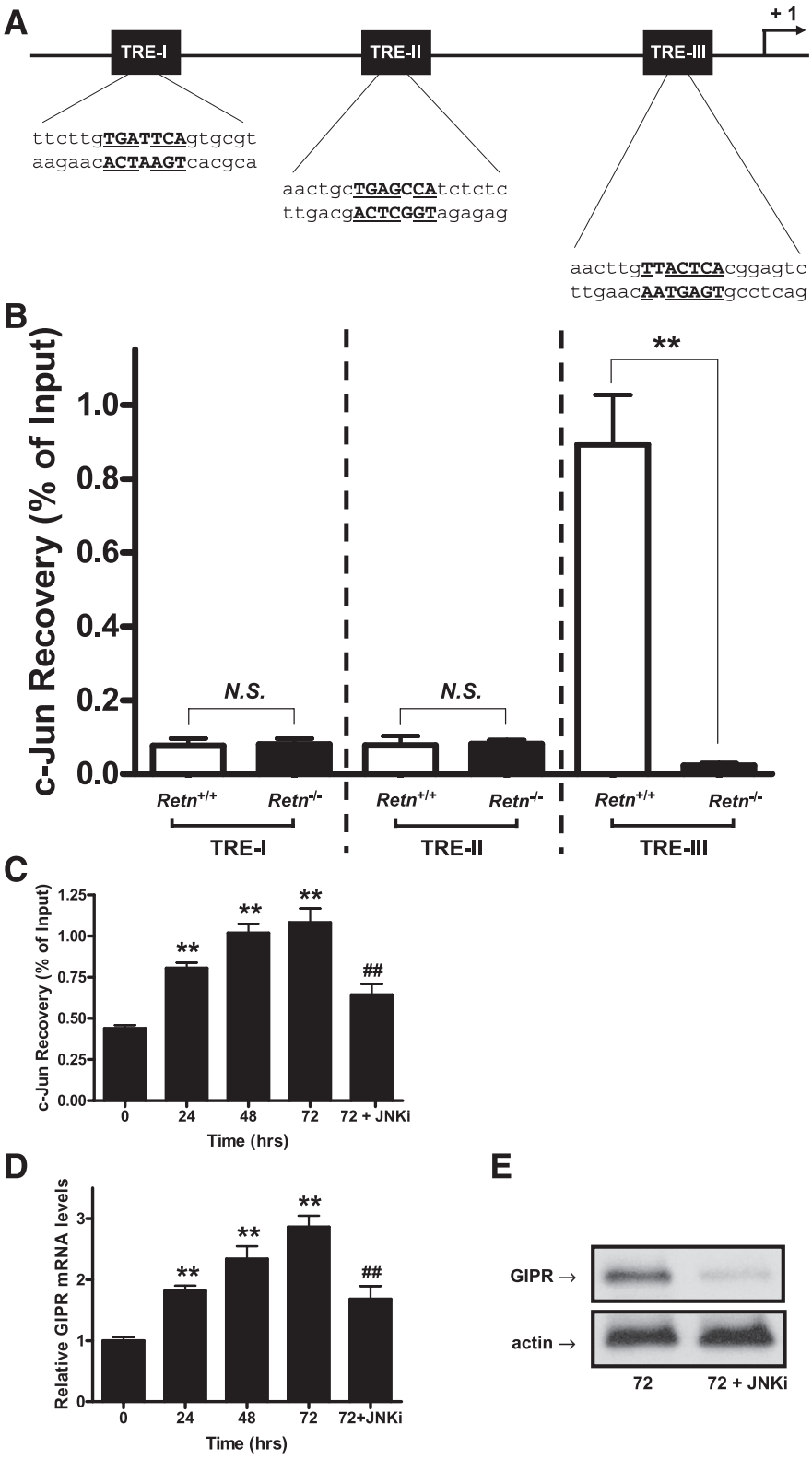

FIG. 4. c-Jun–mediated adipocyte *Gipr* expression. *A* and *B*: c-Jun binds to the TRE-III of *Gipr* promoter in *Retn*$^{+/+}$ adipocytes. *A*: Identification of a putative TRE sequence in the promoter of the mouse *Gipr* gene. The TRE location and the consensus sequence (*bold*) are presented. *B*: Quantitative ChIP in *Retn*$^{+/+}$ and *Retn*$^{-/-}$ adipocytes. Primary adipocytes were isolated from eWAT of *Retn*$^{+/+}$ and *Retn*$^{-/-}$ mice. c-Jun was immunoprecipitated from intact chromatin isolated from *Retn*$^{+/+}$ and *Retn*$^{-/-}$ adipocytes using anti–c-Jun antibody. Precipitated DNA fragments were analyzed by SYBR green real-time PCR using primers flanking the TRE site in the *Gipr* promoter. *C*: Quantitative ChIP in resistin-treated *Retn*$^{+/+}$ adipocytes. Primary adipocytes were isolated from *Retn*$^{+/+}$ mice and treated with resistin (100 nmol/L) for the indicated intervals in the presence or absence of SAPK/JNK inhibitor, JNKi (SP600125, 50 μmol/L). c-Jun was immunoprecipitated as described above, and precipitated DNA fragments were analyzed by SYBR green real-time PCR using primers flanking the TRE-III site. *D*: GIPR mRNA levels in resistin-treated *Retn*$^{+/+}$ adipocytes. *Retn*$^{+/+}$ adipocytes were treated as described in *C*, and real-time RT-PCR was performed to quantify *Gipr* mRNA levels, which are shown as the fold difference vs. control normalized to 18S rRNA expression levels. *E*: Effect of SAPK/JNK inhibitor on GIPR protein levels. *Retn*$^{+/+}$ adipocytes were treated with resistin (100 nmol/L) for 72 h in the presence or absence of JNKi. Protein extracts were isolated and Western blot analyses

In view of the decreased *Gipr* expression and c-Jun phosphorylation in *Retn*$^{-/-}$ fat tissue, we focused on establishing a functional link. Analysis of mouse *Gipr* promoter sequences using MatInspector (Abteling Genetik, Germany) revealed three potential TREs for c-Jun binding, residing between -3583 and -3577, -2936 and -2930, and -298 and -292, that we termed TRE-I, -II, and -III, respectively. TRE-I (TGATTCA), TRE-II (TGAGCCA), and the inverted TRE-III (TGAGTAA) exhibit 85.7% homology to the consensus TRE (TGAG/CTCA) (Fig. 4*A*). To identify functional TRE(s), c-Jun binding to the *Gipr* promoter was determined by measuring TRE occupancy using ChIP assays. Significantly increased c-Jun occupancy in *Retn*$^{+/+}$ over *Retn*$^{-/-}$ adipocytes was detectable only in TRE-III (Fig. 4*B*), indicating it is the functional *cis*-acting element for c-Jun–mediated regulation of the mouse *Gipr* promoter. Resistin (100 nmol/L) treatment of *Retn*$^{+/+}$ adipocytes significantly increased c-Jun occupancy in *Gipr* promoter TRE-III (Fig. 4*C*) and *Gipr* mRNA levels (Fig. 4*D*) by 24 h, with a further increase up to 72 h. c-Jun occupancy and *Gipr* mRNA levels at 72 h after resistin treatment were both greatly reduced by the SAPK/JNK inhibitor (JNKi). Resistin-mediated GIPR protein expression was also greatly attenuated by JNKi (Fig. 4*E*), strongly supporting the involvement of a SAPK/JNK/c-Jun–mediated pathway in the regulation of GIPR expression.

DISCUSSION

We previously demonstrated that GIP stimulated LPL activity in 3T3-L1 cells and human adipocytes through a pathway involving resistin-mediated increases in phosphorylation of PKB and decreases in LKB1 and AMPK phosphorylation (6–8); a similar role was confirmed in *Retn*$^{+/+}$ adipocytes (Fig. 1*A* and Supplementary Fig. 2). However, although resistin content of *Retn*$^{+/-}$ adipocytes was ~25% of that in *Retn*$^{+/+}$ mice, GIP responses were almost completely ablated, suggesting that a critical level of resistin signaling was essential for GIP action and/or that GIPR responsiveness was greatly reduced. Subsequently, GIPR protein and mRNA were both found to be downregulated in *Retn*$^{+/-}$ and *Retn*$^{-/-}$ adipocytes, indicating that attenuated adipocyte GIPR expression was the major contributor to blunted GIP responsiveness (Fig. 1*B* and *C*). In addition, resistin treatment of *Retn*$^{+/+}$ adipocytes for 72 h increased GIPR expression in *Retn*$^{+/+}$ adipocytes (Fig. 2*A*) and rescued GIP responsiveness of LPL activity in *Retn*$^{+/-}$ and *Retn*$^{-/-}$ adipocytes (Fig. 2*B*), supporting a role in its long-term regulation. Although resistin treatment of *Retn*$^{+/+}$ adipocytes resulted in a delayed increase in GIPR protein expression after 72-h incubation (Fig. 2*A*), increases in *Gipr* mRNA (Fig. 4*D*) and c-Jun *Gipr* promoter occupancy (Fig. 4*C*) were detectable after 24-h incubation. These results suggest involvement of complex post-translational regulation in resistin-mediated GIPR protein expression. The receptor is glycosylated, and the islet GIPR was downregulated in response to elevated glucose by a process involving ubiquitination (21), although whether these processes are involved in the delayed expression is unclear.

The PCR array study revealed *Retn*$^{-/-}$ adipose tissue downregulation of genes encoding TNF (*Tnf*), TNFR2 (*Tnfrsf1b*), and the downstream signaling protein SAPK/JNK (*Mapk8*), whereas expression of *Gsk3b* was greatly increased (Fig. 3*A* and *B*). Resistin stimulates TNF-α gene expression in human and mouse macrophages (22), and TNF-α increases resistin protein and mRNA expression via a JNK pathway in human macrophages (23). Because activation of SAPK/JNK/c-Jun by TNF-α is an established pathway for regulating gene transcription (24), we considered it possible that its downregulation may contribute to the reduced *Gipr* expression in *Retn*$^{-/-}$ eWAT. Supporting this, phosphorylated levels of c-Jun and SAPK/JNK (Thr183/Tyr185) were decreased in *Retn*$^{-/-}$ eWAT (Fig. 3*C* and *D*). In addition, using ChIP assays, we established that TRE-III in the mouse *Gipr* promoter was functional and responsible for c-Jun–mediated transcriptional activation, which was downregulated in *Retn*$^{-/-}$ adipocytes (Fig. 4*B*). Treatment of *Retn*$^{+/+}$ adipocytes with a SAPK/JNK inhibitor greatly reduced resistin-induced increases in c-Jun occupancy in TRE-III of the *Gipr* promoter (Fig. 4*C*) and *Gipr* mRNA and protein expression (Fig. 4*D* and *E*), strongly supporting functional involvement of a SAPK/JNK/c-Jun–mediated pathway in GIPR expression regulation. The elevation in *Gsk3b* would also be expected to result in phosphorylation-induced reduction in transcriptional activity of c-Jun (20). These results strongly support involvement of c-Jun in regulating *Gipr* expression; however, other factors could clearly be involved.

Although a strong link between resistin and TNFα has been identified in humans (12), the context in which resistin normally stimulates the TNF/TNFR2 pathway in adipocytes is unclear. TNFR1 and TNFR2 are the primary receptors for soluble and membrane-bound TNF, respectively (24). Most cell types constitutively express TNFR1, whereas TNFR2 expression is regulated by multiple factors. These receptors activate several cellular signaling modules, including SAPK/JNK, and GIP stimulates resistin secretion via activation of SAPK/JNK- and p38 MAPK-mediated pathways (8). Therefore, a complex interplay exists among GIP, resistin, and TNF-α.

In summary, effects of GIP on PKB/LKB1/AMPK/LPL in *Retn*$^{+/+}$ adipocytes were almost completely abolished in *Retn*$^{+/-}$ and *Retn*$^{-/-}$ adipocytes largely due to greatly reduced GIPR expression, associated with downregulated TNFα signaling and c-Jun–mediated transcriptional activation of the *Gipr*. In view of the use of incretin-related compounds for diabetes treatment, it will be important to establish the physiological and pathophysiological significance of the strong interaction between GIP and resistin actions on the adipocyte, especially in humans.

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No potential conflicts of interest relevant to this article were reported.

performed with antibodies against GIPR and β-actin. Western blots are representative of $n = 3$. All data represent the mean ± SEM. Significance was tested using Student *t* test or ANOVA with Newman-Keuls post hoc test. **$P < 0.05$ vs. *Retn*$^{+/+}$ adipocytes or control. ##$P < 0.05$ vs. 72 h *Retn*$^{+/+}$ adipocytes.

S.-J.K. researched data, contributed to discussion, and wrote, reviewed, and edited the manuscript. C.N. researched data. C.H.S.M. contributed to discussion and wrote, reviewed, and edited the manuscript. C.H.S.M. 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.

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